Genomic Predictors of Decitabine Response in AML/MDS

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Genomic Predictors of Decitabine Response in AML/MDS

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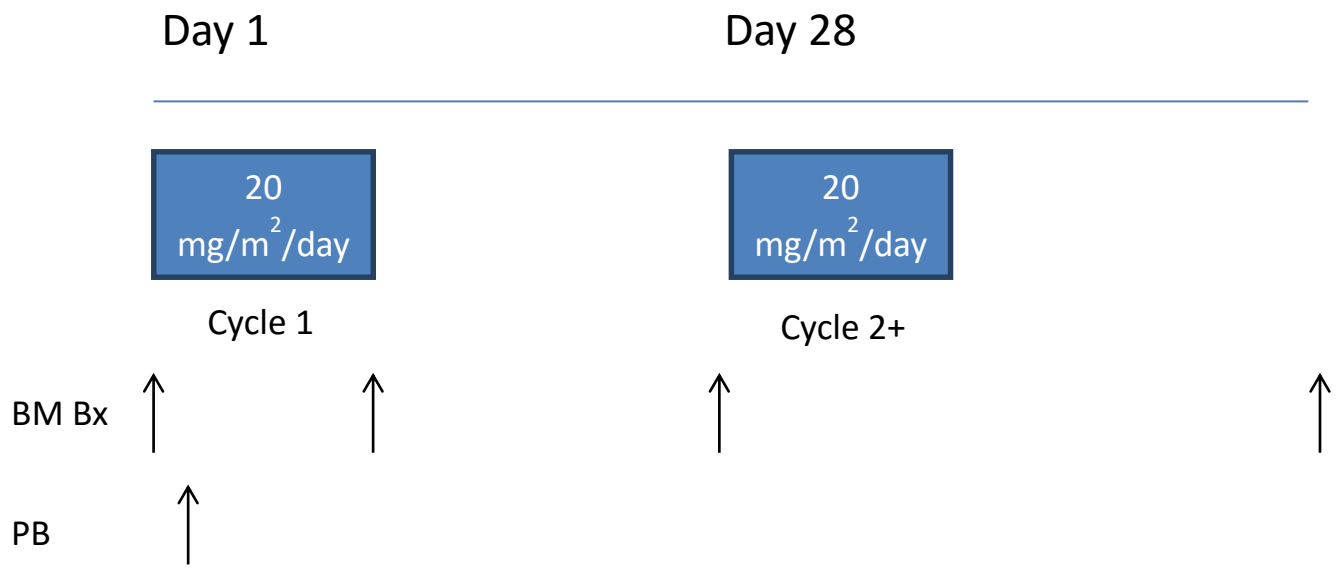
Genomic Predictors of Decitabine Response in AML/MDS
Principal Investigator Signature Page

Principal Investigator:	John Welch, M.D., Ph.D.	
	Signature of Investigator	Date
	Printed Name of Investigator	
	By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IRB/HRPO procedures, the Declaration of Helsinki, ICH Good Clinical Practices guidelines, and the applicable parts of the United States Code of Federal Regulations or local regulations governing the conduct of clinical studies.	

Study Schema:



Patients with progressive disease after two cycles (defined as a clear increase in bone marrow blast count, typically a > 50% increase in bone marrow blasts over baseline) should be removed from study.

Patients with stable disease may continue on study and should undergo a diagnostic bone marrow biopsy at the end of even numbered cycles.

Once AML patients achieve morphologic complete remission with incomplete blood count recovery (CRi) or MDS patients achieve a marrow complete response (Marrow CR) as defined in Section 9.0, decitabine administration may be decreased to 20 mg/m2/day for ten consecutive days every 6 weeks, to limit myelosuppressive toxicity and improve count recovery. After 2 cycles at 6 week intervals, the dose may be reduce to 20 mg/m2/day for five consecutive days in 6 week cycles, at the discretion of the treating physician. After two cycles of five consecutive days of treatment, if the patient meets the definition of CR with complete cytogenetic response as defined in Section 9.0, administration may further be decreased to three consecutive days per cycle at the discretion of the treating physician.

Sample collection for correlative studies:

Bone marrow biopsies should be performed at baseline, at Cycle 1 Day 10 ± 1, and at Day 28 ± 4 of Cycles 1, 2, 4, and 6. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression.

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1.0 INTRODUCTION

1.1 Rationale

Personalized therapy for cancer requires that 1) patient-specific factors (e.g. patient-specific mutations, expression profiles, or pharmacogenomics) influence treatment algorithms, and 2) once therapy is initiated, *in vivo* responsiveness is used to guide further therapy. The modern era of genomics has provided significant insights into cancer pathogenesis by describing patterns of germline and somatic variants, as well as defining disease-specific patterns of gene expression. However, the application and integration of this data with therapeutics remains a difficult task.

[redacted]

AML and MDS are two closely related hematological disorders characterized by ineffective hematopoiesis and malignant expansion of clonal myeloid cells. Both AML and MDS frequently occur in elderly patients and some cases are secondary and/or related to long-term bone marrow damage after radiation or chemotherapy. Current therapy for AML consists of cytotoxic chemotherapy followed by consolidation chemotherapy or stem cell transplantation. Using these approaches, current cure rates may be as high as 60% for patients younger than 60 with good risk cytogenetics (e.g. t(15;17), t(8;21), inv(16)).[4] Outcomes in patients ≥ 60 years old and with relapsed disease remain poor with 2 year survival of only ~25%.[5] Historically, the available treatment for MDS has been symptomatic and has not been shown to be effective in producing sustained improvement in hematopoiesis or in delaying leukemic evolution[6, 7].

Current AML therapy is poorly tolerated in elderly and pre-treated patients due to hematologic and non-hematologic toxicities; following such therapy, patients typically require 3-4 weeks of hospitalization for treatment of neutropenic fevers and for regular transfusions. Even among the relatively healthy patients enrolled in clinical trials, induction mortality in the elderly can be as high as 20-40% following standard induction therapy,[8-11] and moderate to severe non-hematologic toxicity occurs in at least an additional 30% of patients.[9, 12-14] Consequently, many older AML patients are not offered traditional intensive chemotherapy, but receive supportive care alone.[15] More effective and less toxic therapies are needed to provide durable remissions in a significant proportion of elderly AML in populations.

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We and others have recently used whole genome sequencing to identify recurrent, somatic mutations in AML and MDS patients in four genes that are involved in methylcytosine regulation: *DNMT3A, IDH1, IDH2,* and *TET2.*[29-37] Collectively, nearly half of all AML/MDS patients have a mutation in one of these four genes. *DNMT3A* mutations are associated with an adverse outcome in AML patients treated with cytotoxic chemotherapy.[35, 38] However, it is not yet clear whether the presence of mutations in one or more of these genes is associated with response or resistance to decitabine.

In this study, we will treat patients with the current state-of-the-art decitabine schedule.[3] We will perform exome sequencing on all patients and correlate response with mutation status, rate of mutation clearance, expression profile, plasma decitabine levels, and DNA

methylcytosine content reduction. This approach will comprehensively assess genomic and pharmacologic parameters that could clinically predict patient response to decitabine.

In this study, we will combine analysis of patients with AML and MDS. Recent genomic analysis has shown that AML and MDS have shared mutations in many of the same genes, shared patterns of mutation acquisition, and shared patterns of mutation evolution.[39, 40] Furthermore, MDS, like AML, presents with clonal hematopoiesis, even when the morphologic blast count is zero.[36]Because this study will identify and track the rate of clearance of patient specific mutations, and because decitabine has been shown to have efficacy in both AML and MDS patients, [2, 3, 16-19, 41-45] combining analysis of AML and MDS patients is reasonable. Asymptomatic MDS patients are excluded because these patients are typically followed with watchful-waiting rather than with chemotherapy.

1.2 Decitabine and Hypomethylating agents in AML/MDS

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1.2.2 Experience with extended dosing schedules

[redacted]

[redacted]

[redacted]

1.3 Correlative Studies

All correlative studies will be collected under HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Diseases") that all patients will be concurrently enrolled in. The goals of the correlative studies include:

1. To correlate patient-specific mutations with response to decitabine.
   We will perform exome sequencing of each patient's pre-study bone marrow sample and skin sample. We will determine AML/MDS-associated variants found in the bone marrow, but not skin sample. We will correlate the presence of mutations with response (especially mutations in *DNMT3A, IDH1, IDH2,* and *TET2*). This study is powered to identify mutations with >80% sensitivity to predict decitabine response if used as a clinical assay.

2. To correlate the velocity and depth of response with response to decitabine.
   We will assess tumor burden by allele frequency of patient-specific mutations in bone marrow samples collected pre study, Cycle 1 Day 10±1, Cycle 1 Day 28±4, and Cycle 2 Day 28±4. We will assess mutations in at least 24 recurrently mutated genes (including, but not limited to: *FLT3, DNMT3A, NPM1, TET2, WT1, IDH1, IDH2, RUNX1, NRAS, TP53, U2AF1, KRAS, PHF6, MSTP9, PTPN11, KIT, SMC3, PRUNE2, ETV6, CEBPA, SMC1A, ASXL1, STAG2, RAD21*) as well as all patient-specific mutations discovered during exome sequencing. The leukemic allele frequency will be measured using the Illumnia MySeq or HiSeq platform using DNA capture by gene specific biotinylated-oligonucleotides. Patient bone marrow samples will be assessed pre-study, Cycle 1 Day 10 ± 1, Cycle 1 Day 28 ± 4, and Cycle 2 Day 28 ± 4 and compared with patient specific skin samples (normal control). The velocity of response will be calculated as the natural log rate of patient-specific mutation allele frequency clearance. Depth of response will be defined as the lowest patient-specific mutation

allele frequency in a bone marrow sample. The velocity and depth of tumor response will be correlated to:

- Achievement of clinical CR,
- Time to best response,
- Mutational status,
- Time to disease progression.

Because all patient-specific exome-discovered mutations will be assessed a second time in the primary bone marrow sample as well as the Cycle 1 Day 10 ± 1, Cycle 1 Day 28 ± 4, and Cycle 2 Day 28 ± 4 bone marrow samples, this study will also serve to validate all exome-discovered mutations. This is important, because exome sequencing is associated with a 20-50% false-discovery rate depending on type I error tolerated (mutation-calling algorithms are typically set for greater sensitivity than specificity). Thus, many mutations discovered during exome sequencing are incorrect. This second analysis of these mutations will server to exclude these false positive calls.

3. To correlate the bone marrow expression profile from pre-study and Cycle 1 Day 10 ± 1 with response to decitabine.
   We will correlate expression signatures, and changes in expression signatures, with:
   - Achievement of clinical CR,
   - Mutation status.

4. To correlate the extent of bone marrow DNA hypomethylation on Cycle 1 Day 10 ± 1 with response to decitabine.
   We will assess the percent reduction in bone marrow methylcytosine content using LC-MS. Patients will be evaluated pre-study and Cycle 1 Day 10 ± 1. The change in DNA methylcytosine content will be correlated to:
   - Achievement of clinical CR,
   - Change in expression signature.

   This will determine whether inadequate blast exposure to decitabine correlates with drug resistance.

2.0 OBJECTIVES

2.1 Primary Objective

To determine whether patient-specific mutations correlate with clinical overall response to decitabine in patients with AML or MDS.

2.2 Secondary Objectives

- To determine whether 10 consecutive days per cycle of decitabine improves outcomes compared with historical controls treated with 5 consecutive days per cycle of decitabine.

- To determine whether other biomarkers correlate with clinical overall response to decitabine in patients with AML or MDS. These will include:

    1. The velocity and depth of patient-specific mutation clearance;
    2. The bone marrow expression profile and change in expression profile during decitabine treatment;
    3. The decrease in bone marrow methylcytosine content in response to decitabine.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

All of the following:

- Patient must have non-M3 AML or MDS
- An adverse risk karyotype defined by:
  -Complex karyotype by cytogenetics, or
  -Deletion of all or part of chromosome 5, 7, 12, or 17 defined by FISH or cytogenetics, or
  -Somatic TP53 mutation

All of the following:

1. Patient must have an ECOG performance status ≤ 2.
2. Patient must have >10% disease burden measured by cytomorphology, flow cytometry, or cytogenetics.
3. Patient must have peripheral white blood cell count ≤ 50,000/mcl.
4. Patient must have adequate organ function, defined as:
   a. Total bilirubin ≤ 1.5 x ULN
   b. AST/ALT ≤ 2.5 x ULN
   c. Serum creatinine ≤ 2.0 x ULN
5. Patient must have undergone ≤ 2 cycles of prior hypomethylating agent (decitabine or azacitidine).
6. Patient must be enrolled in HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Diseases").
7. Patient must be ≥ 18 years of age.
8. Patient must be able to understand and willing to sign an IRB-approved written informed consent document.

3.2 Exclusion Criteria

1. Patient must not be pregnant or nursing.
2. The patient must not have acute promyelocytic leukemia or t(15;17) observed by FISH.
3. Patient must not have known CNS leukemia.
4. Patient must not have a history of positive HIV serology.
5. Patient must not have a history of positive Hepatitis C serology.
6. Patient must not have undergone a prior allogeneic stem cell transplant.
7. Patient must not have any uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, ongoing or active GVHD, congestive heart failure of NYHA class 3 or 4, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situation that would limit compliance with study requirements.
8. Patient must not have had radiation therapy within 14 days of enrollment.
9. Patient must not have received any chemotherapy within 21 days of enrollment, and any acute treatment-related toxicities must have returned to baseline. Patients

may be receiving hydrea at time of enrollment.

3.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4.0 REGISTRATION PROCEDURES

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility by Washington University
2. Registration of patient in the Siteman Cancer Center database
3. Assignment of unique patient number (UPN)

Once the patient has been entered in the Siteman Cancer Center OnCore database, the WUSM coordinator will forward verification of enrollment and the UPN via email.

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility collecting the information listed below and scanning and emailing it to the research coordinator listed in the *Siteman Cancer Center Clinical Trials Core Protocol Procedures for Secondary Sites* packet at least one business day prior to registering patient:

1. Your name and contact information (telephone number, fax number, and email address)
2. Your site PI's name, the registering MD's name, and your institution name
3. Patient's race, sex, and DOB
4. Three letters (or two letters and a dash) for the patient's initials
5. Currently approved protocol version date
6. Copy of signed consent form (patient name may be blacked out)
7. Planned date of enrollment
8. Completed eligibility checklist, signed and dated by a member of the study team
9. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center OnCore Database

Registrations may be submitted Monday through Friday between 8am and 5pm CT. Urgent late afternoon or early morning enrollments should be planned in advance and coordinated with the Washington University research coordinator. Registration will be confirmed by the research coordinator or his/her delegate by email within one business day. Verification of eligibility and registration should be kept in the patient chart.

All patients at all sites must be registered through the Siteman Cancer Center OnCore database at Washington University.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. Patients

will also be identified by first, middle, and last initials. If the patient has no middle initial, a dash will be used on the case report forms (CRFs). All data will be recorded with this identification number on the appropriate CRFs.

5.0 INVESTIGATIONAL PLAN

5.1 Summary of Study Design

This is an open-label, single-arm, Phase II, biomarker study of patients with AML or MDS who are treated with 10 consecutive days of decitabine. FAB-M3 AML is excluded from this trial, because other well-tolerated options exist for the treatment of FAB-M3 acute promyelocytic leukemia. AML patients who are < 60 years old and have not received cytotoxic induction chemotherapy are also excluded, because cytotoxic therapy offers a curative possibility for these patients. MDS patients without cytopenias are also excluded because these patients can be followed with watchful-waiting.

All patients will undergo bone marrow biopsies pre-study, on Cycle 1 Day 10 ± 1, and on Day 28 ± 4 of Cycles 1, 2, 4, and 6. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression. Patients will provide a skin biopsy sample pre-study. See Section 8.0 for details on correlative studies.

Patients will receive decitabine 20 mg/m^2/day as a 1-hour infusion on consecutive Days 1-10 in 28-day cycles. The dose will be calculated based on actual patient weight at the beginning of each cycle.

Once AML patients achieve morphologic complete remission with incomplete blood count recovery (CRi) or MDS patients achieve a marrow complete response (Marrow CR) as defined in Section 9.0, decitabine administration may be decreased to 20 mg/m2/day for ten consecutive days every 6 weeks, to limit myelosuppressive toxicity and improve count recovery. After 2 cycles at 6 week intervals, the dose may be reduce to 20 mg/m2/day for five consecutive days in 6 week cycles, at the discretion of the treating physician. After two cycles of five consecutive days of treatment, if the patient meets the definition of CR with complete cytogenetic response as defined in Section 9.0, administration may further be decreased to three consecutive days per cycle at the discretion of the treating physician.

It will be a logistical goal of this trial consistently to return preliminary exome results to the treating physician by the end of Cycle 1 and validated results by the end of Cycle 2. However, this logistical goal is not a primary or secondary aim of this study. Given the limited data regarding mutation presence and drug response, we do not anticipate that the results will directly influence therapeutic decisions made by the treating physician. However, the development of comprehensive genomic pipelines and integration of this data into a clinical trial setting will be required before personalized medicine can be realized.

5.2 Study Procedures

5.2.1 Pre-study Procedures

Pre-study procedures must take place no more than 14 days prior to the first dose of decitabine except where noted below.

1. Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics, and FISH.
2. Bone marrow or peripheral blood for flow cytometry
3. Skin biopsy sample (collected under HRPO# 201011766). There is no required time frame for this sample; it may have been collected months or even years prior to the first dose of decitabine.

5.2.2 Baseline Evaluation

Baseline evaluations must take place no more than 7 days prior to the first dose of decitabine.

1. Medical history
2. Physical exam including height, weight, and ECOG performance score.
3. Record transfusion requirements for 8 weeks before first dose of study drug
4. CBC with differential and platelets
5. Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH
6. Urine βHCG pregnancy test if appropriate
7. Clinical pathology should be carefully reviewed to rule-out acute promyelocytic leukemia (M3-AML), and discussion between the pathologist and treating physician should take place if warranted by morphology or immunophenotype.

5.2.3 Day 1 of Each Cycle

1. Physical exam, including weight
2. CBC with differential and platelets
3. Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH

5.2.4 Cycle 1 Days 1-10

Decitabine administration, 20 mg/m^2 per day given as a 1-hour continuous intravenous infusion. The dose will be calculated based on actual patient weight at

the beginning of each cycle. Patients may be premedicated with lorazepam, prochlorperazine, palonosetron, or ondansetron at the treating physician's discretion.

The clinical research coordinator should monitor for final FISH results to confirm that PML/RARA screening is negative.

5.2.5 Cycle 1 Day 10 ± 1

Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics, and FISH.

5.2.6 Weekly

CBC with differential and platelets. After the first cycle, this may be changed to every other week at the discretion of the treating physician.

5.2.7 Every Other Week

Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH. After the first cycle, this may be changed to every month at the discretion of the treating physician.

5.2.8 Cycles 1, 2, 4 and 6 Day 28 ± 4 days

Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include: correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics, and FISH.

Patients who achieve with clinical response (CRi or better) and are receiving cycles every 6 weeks, will undergo bone marrow biopsies on day 42 ± 4.

5.2.9 End-of-Study Procedures

1. Physical exam
2. CBC with differential and platelets.
3. Serum chemistries including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, and LDH.
4. Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include morphologic assessment, cytogenetics, and FISH.

5.2.10 Post-study Follow-up

Patients are to be followed according to standard patient care procedures for 30 days following the date of the last decitabine dose to monitor for toxicity, survival, and response. Thereafter, the investigator or designee will record information on subsequent therapy, disease progression, and/or death every 6 months for 2 years after last dose of study drug or until the patient is lost to follow-up or dies.

5.3 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented in the case report forms.

In the absence of treatment delays due to adverse events, treatment may continue indefinitely or until one of the following criteria applies:

- Documented and confirmed disease progression
- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious non-compliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study

Patients who prematurely discontinue treatment for any reason will be followed as indicated in the study calendar.

5.4 Concomitant Therapy and Supportive Care Guidelines

5.4.1 Chemotherapy

Patients cannot receive any chemotherapy within 21 days of study enrollment and must have recovered from any treatment-related toxicities. However, patients may be receiving hydrea at enrollment and may continue on hydrea through Cycle 2 of decitabine. An indication for hydrea following 2 cycles of decitabine is evidence that the patient is not responding and decitabine should be discontinued. Other than decitabine, patients cannot receive chemotherapy during this trial.

5.4.2 Growth Factors

Erythroid growth factors should not be routinely administered to patients during the study.

Thrombopoietin receptor agonists should not be routinely administered to patients during the study.

Filgrastim (G-CSF) or sargramostim (GM-CSF) may be administered to patients with septic shock or febrile neutropenia at the discretion of the treating physician.

5.4.3 Transfusions

- *Packed red blood cells (RBCs) transfusion*:
  RBC transfusions will be administered at the discretion of the treating physician.

- *Platelet transfusion*:
  Platelet transfusions will be administered at the discretion of the treating physician

5.4.4 Prophylactic Antimicrobial Agents

Antimicrobial agents will be administered at the discretion of the treating physician. Common antimicrobial prophylaxis during cycles 1 and 2 include acyclovir, ciprofloxacin, and fluconazole.

5.4.5 Radiotherapy

Patients cannot receive radiotherapy within 14 days prior to study enrollment or while on study.

6.0 DOSE MODIFICATIONS

A criterion for discontinuation of the study is a delay of > 8 weeks between any two cycles.

6.1 Delay for Cytopenia or Infection

As cytopenias are a defining feature of AML and MDS, their presence alone does not generally warrant dose modifications or delays. Patients will receive four cycles of therapy without adjustment for any cytopenia. Following Cycle 4, dose modifications or delays for cytopenias will be made at the discretion of the investigator.

Patients with febrile neutropenia (temperature ≥ 38.5° with ANC < 1,000/μl) or systemic infections should have treatment held until clinical resolution of the infection.

6.2 Delay for Organ Dysfunction

If renal dysfunction (creatinine ≥ 3 X institutional upper limit of normal) or hepatic dysfunction (bilirubin ≥ 2.5 mg/dl or ALT/AST ≥ 3 X institutional upper limit of normal) occurs, therapy may not resume until the abnormalities have resolved to below the limits stated above. If renal or hepatic dysfunction occurs during a treatment course, the next dose of decitabine should be held, and serum chemistries should be checked twice per week. Decitabine can be resumed at full dose when creatinine, bilirubin, ALT and AST are below the limits listed above.

For grade 3-4 non-hematologic toxicities related to decitabine, decitabine treatment should be delayed until recovery to baseline or to grade ≤ 1. Decitabine may be reintroduced with a 50% dose reduction, or at full dose at the discretion of the investigator.

6.3 Modification in Dose or Schedule for Treatment Response

Once AML patients achieve morphologic complete remission with incomplete blood count recovery (CRi) or MDS patients achieve a marrow complete response (Marrow CR) as defined in Section 9.0, decitabine administration may be decreased to 20 mg/m2/day for ten consecutive days, and the cycles may be administered every 6 weeks to limit myelosuppressive toxicity, at the discretion of the treating physician. After two cycles at six week intervals, the dose may be reduced to 20 mg/m2/day for five consecutive days of treatment in six week intervals. If the patient meets the definition of CR with complete cytogenetic response as defined in Section 9.0, administration may further be decreased to three consecutive days per cycle at the discretion of the treating physician.

7.0 PHARMACEUTICAL INFORMATION

7.1 Decitabine

[redacted]

8.0 CORRELATIVE STUDIES SAMPLE COLLECTIONS

Bone marrow samples will be collected pre-study, on Cycle 1 Day 10 ± 1, and on Day 28 ± 4 of Cycle 1, 2, 4, and 6 for correlative studies. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression. The bone marrow samples will be collected under HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Diseases") that all patients will be concurrently enrolled in.

Skin biopsy sample will be collected as part of the patient's participation in HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Malignancies"). There is no required time frame for this sample, and it may have been collected months or even years prior to the first dose of decitabine.

9.0 EFFICACY, SAFETY AND CORRELATIVE STUDY MEASURES

A secondary endpoint of this study is to estimate morphologic complete remission rate.

9.1 Response Criteria - AML

AML Patients will be assessed for response according to the IWG criteria:[1]

Morphologic complete remission (CR) – Defined as morphologic leukemia-free state, defined as less than 5% blasts in aspirate with marrow spicules and a count of ≥200 nucleated cells and no blasts with Auer rods or any persistence of extramedullary disease. When erythroid precursors constitute less than 50% of bone marrow nucleated cells, the percentage of blasts is based on all nucleated cells; when there are 50% or more erythroid cells, the percentage blasts should be based on the nonerythroid cells. The presence of mild megaloblastoid changes may be permitted if they are thought to be consistent with treatment effect. However, persistence of pretreatment abnormalities (eg, pseudo-Pelger-Hüet cells, ringed sideroblasts, dysplastic megakaryocytes) are not consistent with CR. In addition, ANC >1000/µl and platelet count of ≥100,000/µl. Patient must be independent of transfusions. No duration requirement for this designation.

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

Morphologic complete remission with incomplete blood count recovery (CRi) – Defined as morphologic complete recovery with the exception of neutropenia <1000/µl or thrombocytopenia <100,000/µl.

Partial remission (PR) – Defined as meeting the above criteria for complete remission, with the following exceptions: decrease of ≥50% in the percentage of blast to 5-25% in the bone marrow aspirate. A value of ≤5% blasts in bone marrow with Auer rods present may also be considered a partial remission.

Stable Disease (SD) – Defined as not meeting the definitions of CR, CRi, PR, PD, or recurrence/morphologic relapse.

Progressive disease (PD) – Defined as a clear increase in bone marrow blast count, typically a greater than 50% increase compared with baseline, or new extramedullary disease. Patients with a > 50% increase in peripheral blast count with an increase of the total peripheral white blood cell count to > 10,000/µl should undergo evaluation by bone marrow biopsy to access for progressive disease.

Recurrence/morphologic relapse **-** Defined as relapse following 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. New dysplastic changes is considered relapse.

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.

9.2 Response Criteria - MDS

MDS Patients will be assessed for response according to the IWG criteria:[69]

Complete remission (CR) – Defined as ≤ 5% myeloblasts with normal maturation of all cell lines in the bone marrow and peripheral blood values of Hgb ≥ 11g/dL, Platelets ≥ 100 x 10^9/L, Neutrophils ≥ 1.0 x 10^9/L, and 0% blasts. Persistent dysplasia does not exclude CR but will be noted.

Marrow Complete Response (Marrow CR) – Defined as ≤ 5% myeloblasts in the bone marrow and a decrease by ≥ 50% from pretreatment values, but not meeting the definition of CR above.

Partial Remission (PR) – Defined as meeting the definition of CR above with a decrease of myeloeblasts in the bone marrow by ≥ 50% from pretreatment values, but absolute myeloblasts still >5%.

Stable Disease (SD) – Defined as not meeting the definitions of CR, Marrow CR, PR, SD, PD, or recurrence/morphologic relapse.

Progressive Disease//Relapse (PD) – Defined as ≥ 50% increase in blasts to > 5% blasts (for patients with less than 5% blasts at baseline only), ≥ 50% increase to > 10% blasts (for patients with 5-10% blasts at baseline only), ≥ 50% increase to > 20% blasts (for patients with l0-20% blasts at baseline only), ≥ 50% increase to > 30% blasts (for patients with 20-30% blasts at baseline only) or any of the following: At least 50% decrement from maximum remission/response in granulocytes or platelets, reduction in Hgb by ≥ 2 g/dL, or New or worsened transfusion dependence not related to study drug toxicity. Or for patients with a CR, Marrow CR, or PR as defined above and subsequently development of one of the following: Return to pretreatment bone marrow blast percentage, decrement of ≥ 50% from maximum remission/response levels in granulocytes or platelets, or reduction in Hgb concentration by ≥ 1.5 g/dL or transfusion dependence.

9.2.1 Hematologic Improvement (HI)

Progressive disease as defined above nullifies hematologic improvement.

Erythroid response requires all of the following (only required if pretreatment HgB <11 g/dL):

- Hgb increase by ≥ 1.5 g/dL
- Relevant reduction of units of RBC transfusions by an absolute number of at least 4 RBC transfusions/8 wk compared with the pretreatment transfusion number in the previous 8 wk. Only RBC transfusions given for a Hgb of ≤ 9.0 g/dL pretreatment will count in the RBC transfusion response evaluation

Platelet response requires one of the following (only required if pretreatment platelets < 100 x 10^9/L):

- Absolute increase of ≥ 30 x 10^9/L (for patients starting with > 20 x 10^9/L platelets)
- Increase from < 20 x 10^9/L to > 20 x 10^9/L and absolute increase ≥ 100% (for patients starting with < 20 x 10^9/L)

Neutrophil response requires the following (only required if pretreatment ANC <1.0 x 10^9/L):

- At least 100% increase and an absolute increase > 0.5 x 10^9/L

9.3.1 Cytogenetic Response

Complete Cytogenetic Response – Defined as reversion to a normal karyotype. For this study, reversion of a normal karyotype is defined as no clonal abnormalities detected in a minimum of 20 mitotic cells. Progressive disease as defined above nullifies cytogenetic response.

9.3 Guidelines for Evaluation of Disease

Bone marrow biopsy should be performed on Day 28 ± 4 of cycles 1, 2, 4 and 6 for clinical disease assessment. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression.

9.4 Other Secondary Efficacy Measures

9.4.1 Treatment Failure, Overall Survival and Event-free Survival

Treatment failure is defined as failure to achieve a complete or partial remission following 4 cycles of therapy.

Overall survival is defined as the date of first dose of study drug to the date of death from any cause. Patients still alive at the time of the final analysis will be right-censored.

Event-free survival is defined as the interval from the date of first dose of study drug to date of treatment failure, recurrence, death due to any cause, or loss to follow up.

9.4.2 Duration of Remission and Relapse-free Survival for Patients Having Complete Remission

Duration of complete remission is defined as the interval from the date that complete remission is documented to the date of recurrence, death due to any cause, or loss 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.

9.4.3 Safety

Safety evaluation will include assessments of adverse experiences, medical history, physical examinations, vital signs, and laboratory assessments at baseline and throughout the study period. Local laboratories may be used for this study.

10.0 REGULATORY AND REPORTING REQUIREMENTS

The entities providing oversight of safety and compliance with the protocol require reporting as outlined below.

The Washington University Human Research Protection Office (HRPO) requires that all events meeting the definition of unanticipated problem or serious noncompliance be reported as outlined in Section 10.2.

The FDA requires that all serious and unexpected adverse events be reported as outlined in Section 10.6. In addition, any fatal or life-threatening adverse experiences where there is a reasonable possibility of relationship to study intervention must be reported.

10.1 Definitions

10.1.1 Adverse Events (AEs)

Definition: any unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site: http://ctep.cancer.gov/reporting/ctc.html. Grade 1-2 events will be recorded for the first fifty enrolled patients. Subsequently, only events graded as 3 or greater will be recorded. Fifty patients should be sufficient to estimate the true incidence of any adverse event to within ± 14% with 95% confidence intervals.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website: (http://www.hhs.gov/ohrp/policy/AdvEvntGuid.htm).

10.1.2 Serious Adverse Event (SAE)

Definition: any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions)
- A congenital anomaly/birth defect

- Any other experience which, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

All unexpected SAEs must be reported to the FDA.

10.1.3 Unexpected Adverse Experience

Definition: any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure (or risk information, if an IB is not required or available).

Events that are both serious AND unexpected must be reported to the FDA.

10.1.4 Life-Threatening Adverse Experience

Definition: any adverse drug experience that places the subject (in the view of the investigator) at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.

Life-threatening adverse experiences must be reported to the FDA.

10.1.5 Unanticipated Problems

Definition:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

10.1.6 Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

10.1.7 Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

10.1.8 Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

Local IRB Pre-approval of all protocol exceptions must be obtained prior to the event. For secondary sites, the Washington University PI will issue approval of the exception, but it must also be submitted to the local IRB with documentation of approval forwarded to Washington University. Washington University IRB approval is not required for protocol exceptions occurring at secondary sites.

10.2 Reporting to the Human Research Protection Office (HRPO) at Washington University:

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH or SLCH institution, or that impacts participants or the conduct of the study.
- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within **10 working days** of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification to the PI of the event.

10.3 Reporting to the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The PI is required to notify the QASMC of any unanticipated problem occurring at WU or any BJH or SLCH institution that has been reported to and acknowledged by HRPO as reportable. (Unanticipated problems reported to HRPO and withdrawn during the review process need not be reported to QASMC.)

QASMC must be notified within **10 days** of receipt of IRB acknowledgment via email to a QASMC auditor.

10.4 Reporting Requirements for Secondary Sites

The research team at each secondary site is required to promptly notify the Washington University PI and research coordinator of all reportable events (as described in Section 7.6) within **1 working day** of the occurrence of the event or notification of the secondary site's PI of the event. This notification may take place via email if there is not yet enough information for a formal written report (using either an FDA MedWatch form if required or an institutional SAE reporting form if not). A formal written report must be sent to the Washington University PI and research coordinator within **10 working days** of the occurrence of the event or notification of the secondary site's PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification of the secondary site's PI of the event.

The research team at a secondary site is responsible for following its site's guidelines for reporting applicable events to its site's IRB according to its own institutional guidelines. The research team at Washington University is responsible for reporting all applicable events to the FDA.

10.5 Reporting to Secondary Sites

The Washington University PI (or designee) will notify the research team at each secondary site of all reportable events that have occurred at other sites within **10 working days** of the occurrence of the event or notification of the PI of the event. This includes events that take place both at Washington University and at other secondary sites, if applicable.

10.6 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. **PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC.** It is the responsibility of the Washington University principal investigator to report any unanticipated problem to the FDA as follows:

- Report any unexpected fatal or life-threatening adverse experiences (Section 10.1.4) associated with use of the drug by telephone or fax no later than **7 calendar days** after initial receipt of the information.
- Report any serious, unexpected adverse experiences (Section 10.1.2), as well as results from animal studies that suggest significant clinical risk within **15 calendar days** after initial receipt of this information.

All MedWatch forms will be sent by the investigator or investigator's team to the FDA at the following address or by fax:

Food and Drug Administration

Center for Drug Evaluation and Research
Division of Oncology Drug Products
5901-B Ammendale Rd.
Beltsville, MD 20705-1266
FAX: 1-800-FDA-0178

Secondary sites must submit a completed MedWatch form to the Washington University PI and research coordinator within **4 calendar days** (for fatal or life-threatening adverse experiences) or **11 calendar days** (for serious, unexpected adverse experiences). The Washington University PI will be responsible for submitting all MedWatch forms from secondary sites to the FDA within the timeframes specified above.

10.7 Timeframe for Tracking Reportable Events:

Adverse events will be tracked for 30 days following the last day of study treatment, or until the start of a new chemotherapy/treatment, whichever comes first.

11.0 DATA SAFETY MONITORING PLAN

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Data and Safety Monitoring Committee (DSMC) will meet to review toxicity data at least every 6 months following the activation of the first secondary site. The report will be prepared by the statistician and will be submitted to the Quality Assurance and Safety Monitoring Committee (QASMC). This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual including numbers from participating sites
- Protocol activation date at each participating site
- Average rate of accrual observed in year 1, year 2, and subsequent years at each participating site
- Expected accrual end date and accrual by site
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities at all participating sites
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

Until such a time as the first secondary site activates this protocol, a semi-annual DSM report to be prepared by the study team will be submitted to the QASM Committee beginning 6 months after study activation at Washington University.

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

A DSMC will consist of no fewer than 3 members including 2 clinical investigators and a biostatistician. Like investigators, DSMC members are subject to the Washington University School of Medicine policies regarding standards of conduct. Individuals invited to serve on the DSMC will disclose any potential conflicts of interest to the trial principal investigator and/or appropriate university officials, in accordance with institution policies. Potential conflicts that develop during a trial or a member's tenure on a DSMC must also be disclosed.

Refer to the Washington University Quality Assurance and Data Safety Monitoring Committee Policies and Procedures for full details on the responsibilities of the DSMC at http://www.siteman.wustl.edu/uploadedFiles/Research_Programs/Clinical_Research_Resources/Protocol_Review_and_Monitoring_Committee/QASMCQualityAssurcance.pdf

12.0 AUDITING

Since Washington University is the coordinating center, each site will be audited annually by Siteman Cancer Center personnel (QASMC) unless the outside institution has an auditing mechanism in place and can provide a report. The outside sites will be asked to send copies of all audit materials, including source documentation. The audit notification will be sent to the Washington University Research Patient Coordinator, who will obtain the audit materials from the participating institution.

Notification of an upcoming audit will be sent to the research team one month ahead of the audit. Once accrual numbers are confirmed, and approximately 30 days prior to the audit, a list of the cases selected for review (up to 10 for each site) will be sent to the research team. However, if during the audit the need arises to review cases not initially selected, the research team will be asked to provide the additional charts within two working days.

Additional details regarding the Auditing Policies and Procedures can be found at https://siteman.wustl.edu/wp-content/uploads/2015/QASMC-Policies-and-Procedures-03.31.2015.pdf

13.0 MULTICENTER REGULATORY REQUIREMENTS

Washington University requires that each participating site sends its informed consent document to be reviewed and approved by the Washington University Regulatory Coordinator (or designee) prior to IRB/IEC submission.

Site activation is defined as when the secondary site has received official written documentation from the coordinating center that the site has been approved to begin enrollment. At a minimum, each participating institution must have the following documents on file at Washington University prior to study activation:

- Documentation of IRB approval of the study in the form of a letter or other official document from the participating institution's IRB. This documentation must show which version of the protocol was approved by the IRB.
- Documentation of IRB approval of an informed consent form. The consent must include a statement that data will be shared with Washington University, including the Quality Assurance and Safety Monitoring Committee (QASMC), the DSMC (if applicable), and the Washington University study team.
- Documentation of FWA, signed FDA Form 1572 (if applicable), and the CVs of all participating investigators.
- Protocol signature page signed and dated by the investigator at each participating site.

The coordinating center Principal Investigator (or designee) is responsible for disseminating to the participating sites all study updates, amendments, reportable adverse events, etc. Protocol/consent modifications and IB updates will be forwarded electronically to the secondary sites within 4 weeks of obtaining Washington University IRB approval. Activated secondary sites are expected to submit protocol/consent/IB modifications to their local IRBs within 4 weeks of receipt unless otherwise noted. Upon the secondary sites obtaining local IRB approval, documentation of such shall be sent to the Washington University study team within 2 weeks of receipt of approval.

Documentation of participating sites' IRB approval of annual continuing reviews, protocol amendments or revisions, all SAE reports, and all protocol violations/deviations/exceptions must be kept on file at Washington University.

The investigator or a designee from each institution must participate in a regular conference call to update and inform regarding the progress of the trial.

14.0 STATISTICAL ANALYSIS METHODS AND SAMPLE SIZE

14.1 Analysis Populations

Intent-to-Treat - The intent-to-treat (ITT) population will be defined as all patients who receive at least one dose of decitabine.

Efficacy-Evaluable - The efficacy-evaluable (EE) population will be defined as all patients who receive at least one cycle of treatment.

Safety - The safety population will be defined as all patients with at least one dose of decitabine.

14.2 Statistical Analysis

14.2.1 Descriptive Analyses

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

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.

14.2.2 Primary Endpoint Analysis

The primary end-point of this phase II study is the correlation biomarkers with overall response in AML/MDS patients to decitabine.

We will correlate the presence of patient specific mutations (especially mutations in *DNMT3A, IDH1, IDH2,* and *TET2*) with overall response rates. Mutation rates in these genes and in other patient-specific genes will be correlated with overall response and their respective 95% confidence intervals will be provided.

Statistical considerations. The relationship between AML/MDS-associated mutations and the responsiveness to decitabine will be described by contingency tables and assessed using Chi-square test or Fisher's exact test as appropriate. The differentially expressed genes between responders and non-responders will be determined using the significance analysis of microarray (SAM). Multivariate logistic regression will also be fit to adjust the potential confounding effects of other demographic and clinical characteristics. The resultant critical *P* values will be corrected for multiple testing. Since the Bonferroni correction is known to be too conservative, a permutation analysis (e.g., 10,000 random assignments for

responsive status) will be used to derive an empirical critical *P* value. The new critical p-value is the number of permutation tests which yield a lower p-value than the original test divided by the total number of permutation tests.[61]

A power analysis was performed using Chi-square test for the association between mutation and response to decitabine. The frequency of *DNMT3A, IDH1/2,* and *TET2* mutations in AML and MDS patients are each between 15% and 30% in multiple cohorts.[29-37] **Table 3** shows the minimal detectable differences of response rates between mutant and wild-type cases, assuming an expected overall 45% CR/CRi rate and 80% power. For an average of 20% frequency of a mutated gene, for example, 100 patients will provide 80% power to detect 30% difference at a 2-sided 0.1 alpha error. The purpose of this study is to determine whether any of these mutations result in a clinically significant association that would warrant pre-treatment testing. We believe that a reasonable cut off would be if at least 30% of clinical response could be attributed to the presence of a specific mutation. However, we anticipate that more power could be achieved as we assess patient groups with combined characteristics (e.g. *DNMT3A* mutation or loss of *DNMT3A* expression). In addition, we expect that the majority of the enrolled 125 patients can be used in the analysis (since all of our molecular information will be acquired within 30 days of enrollment, we anticipate a drop-out rate of 20% or less).

Table 3. Detectable mutation associated response rates (80% power, $\alpha = 0.05$)

Total N	% of patients with a given mutation	Detectable response difference	Sensitivity of mutation to predict response at minimum detectable response difference*
100	15%	0.40	80%
	20%	0.35	75%
	30%	0.30	67%
	40%	0.27	61%
	50%	0.26	58%

* Assuming the overall response rate (CR/Cri) of 45%

14.2.3 Secondary Endpoint Analyses

The overall response rate (CR/CRi/PR) and complete response rate (CR/CRi) will be compared with historical controls[2], and the 95% confidence intervals will be provided.

The rate of patient specific mutation clearance after 10, 28, and 56 days of therapy will be determined using an Illumina based platform. We will compare the rate of mutation clearance between the patients who achieve a CR/CRi after 4 cycles and those who do not. We anticipate that we will identify between 6 and 25 exonic SNVs per case (the range obtained with >200 AML samples sequenced to date with whole genome or exome sequencing), and that the expected 95% confidence interval of the mean variant allele frequency will be ±2.56% and ±1.25%,

respectively, if we assume an anticipated standard deviation of 3.2% within the same clone. The change of tumor burden over time and its association with clinical response will be assessed using 2-way ANOVA for repeated measurement data, followed by the ad-hoc multiple comparisons for between-group differences (e.g., responders vs. non-responders) at given time points as well as the between-time differences within each group. The resultant critical *P* values will also be corrected using permutation analysis for multiple testing.

The percent decrease in bone marrow methylcytosine will be calculated on day 10±1. We will compare this reduction with previously published data and between the patients who achieve CR/CRi and those who do not. The correlation of peak plasma levels of decitabine with response will be assessed using 2-sample t-test or Mann-Whitney non-parametric rank-sum test as appropriate. The change of total bone marrow DNA methylcytosine from baseline and its association with both steady-state plasma drug levels and response will be assessed using 2-way ANOVA for repeated measurement data, followed by the ad-hoc multiple comparisons for between-group differences (e.g., responders vs. non-responders) and between-time differences. Similar analyses will be performed for the correlation of expression profiles and SNP analysis with pharmacokinetic data.

Statistical power justification. A power analysis was performed using two-sample t-test for between-group difference and paired t-test for the change over time. For tumor clearance, this will provide > 99% power to detect a 10% difference in the means at two separate time points for clones with 6 or more variants, and > 98% power to detect a 15% difference if there are 3 or more variants per clone with $\alpha = 0.05$ (power will decrease to 91% and 82%, respectively, in cases where the standard deviation is 4.7%, the maximum we have observed thus far). For pharmacologic correlations, we anticipate 85% power to determine a 25 ng/ml difference between the average C_{max} of 45 evaluable responders and 55 evaluable non-responders with an anticipated average C_{max} of 70 ng/ml and a standard deviation of 40 ng/ml within the entire cohort, and with $\alpha = 0.05$.[22] We believe a less significant difference would not be sufficient to warrant application in clinical decision making (i.e. whether to continue therapy for more than 1 cycle). We anticipate 99% power to determine a difference of 20% in the reduction of bone marrow methylcytosine between 45 evaluable responders and 55 evaluable non-responders with an expected standard deviation of 15% and $\alpha = 0.05$.[28, 66-68] The difference in methylcytosine reduction previously observed was 30% between patients achieving CR/PR and those with only hematologic improvement.[28]

Time to relapse and time to death will also be listed for each patient.

14.2.4 Safety Analysis

Extent of Exposure – Duration (number of cycles/infusions) of decitabine doses (cumulative and intensity) will be summarized.

Adverse Events - All adverse events will be coded using NCI CTCAE v. 4.0. 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. In addition, adverse events will be summarized by investigator attribution of relationship to study medication and by grade. Similar summaries will be provided by treatment cycle.

Clinical Laboratory Evaluation – Applicable laboratory parameters will be graded according to NCI CTCAE version 4.0. The incidence of maximum grade (number and percent of patients experiencing the maximum grade during the study) will be summarized for each laboratory parameter. Similar summaries will be provided by treatment cycle. Graphical displays of select hematological parameters will also be provided.

Vital Signs, Physical Findings, and Other Safety Observations – Vital signs, findings from physical examinations, ECOG scores and weights will be presented in listing format.

14.2.5 Description of Planned Subgroup Analyses

The following subgroups (if sufficient numbers of patients) will be explored with respect to the primary and overall survival endpoints.

- Age (60-69 years old vs ≥ 70 years old)
- Cytogenetics
- Patients with AML-associated mutations in *DNMT3A, TET2, IDH1,* or *IDH2* vs those who do not have somatic variants in these genes.

14.3 Sample Size

We anticipate initially enrolling 25 patients at Washington University. Once we establish a routine exome analysis with validation and calculation of the rate of patient-specific mutation clearance, we will enroll an additional 100 patients from at least 2 additional centers.

15.0 DATA SUBMISSION SCHEDULE

Case Report Form	Submission Schedule
Original Consent Form	Prior to registration
Patient Registration Form Disease Diagnosis Form Inclusion Criteria Worksheet Exclusion Criteria Worksheet	Prior to starting treatment
Treatment Record Form	To be maintained throughout a patient's treatment cycle. The complete record is due at completion of treatment.
Bone Marrow Sample Collection Form	To be maintained throughout the course of the study at designated bone marrow sample collections time points (See Appendix 2).
Pathology: Bone Marrow Biopsy	To be completed upon the return of pathology report for each bone marrow biopsy.
Study Calendar Form	To be completed with the date of each time point upon occurrence.
Labs Form	To be completed upon the return of laboratory reports for each study time point (See Appendix 2)
Dose Modifications	To be maintained throughout the course of the study if dose modifications are performed.
Therapy Response Form	To be filled out upon remission (prior to, and/or on therapy)
End of Study Form	Due at the completion of the EOS visit, at the time of death, and/or at the time that the patient is lost to follow-up
Follow Up Form	• 30 days post date of last decitabine dose. • Every 6 months for 2 years after the last dose of study drug.
Record of Adverse Events	At the time of any AE

Any queries generated by Washington University must be responded to within 28 days of receipt by the participating site. The Washington University research team will conduct a regular review of data status at all secondary sites, with appropriate corrective action to be requested as needed.

Appendix 1 ECOG Performance Scale

Activity Status	Description
0	Asymptomatic, fully active, and able to carry on all predisease performance without restrictions.
1	Symptomatic, fully ambulatory but restricted in physically strenuous activity and able to carry out performance of a light or sedentary nature, eg, light housework, office work.
2	Symptomatic, ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours: in bed less than 50% of day.
3	Symptomatic, capable of only limited self-care, confined to bed or chair more than 50% of waking hours, but not bedridden.
4	Completely disabled. Cannot carry on any self-care. Totally bedridden.
5	Dead

Appendix 2 Study Flow Chart

Study Flow Chart

Test	Pre-study[1]	Base-line[2]	All Cycles Day 1	All Cycles Days 1-10[3]	Cycle 1 Day 4 ± 1	Cycle 1 Day 10 ± 1 and 28 ± 4	Every week	Every 2 weeks	Even cycles Day 28±4	End of Study
Informed Consent	X									
Medical History		X								
Physical Exam		X[a]	X[b]							X
Transfusion Requirements[c]		X[c]	Ongoing							
Bone Marrow Aspirate and Biopsy	X[d]					X[d]			X[d]	X[d]
Flow Cytometry[e]	X									
Pregnancy test		X								
CBC with differential and platelets		X	X				X[f]			X
Serum Chemistries[g]		X	X					X[f]		X
Decitabine administration[h]				X						
Adverse Events			Ongoing							
Post-study follow-up										X[i]

[1] To be performed within 28 days of the first dose of decitabine.
[2] To be performed within 7 days of the first dose of decitabine.
[3] Decitabine may be reduced to Days 1-5 or Days 1-3. See Section 6.3 for details.
[a] To include height, weight, and ECOG.
[b] To include weight.
[c] Care should be given to record the frequency of transfusions from 8 weeks prior to first dose of study drug through the duration of the study.
[d] Bone marrow biopsy and aspirate should be performed at baseline, at Cycle 1 Day 10 ± 1, and at Day 28 ± 4 of Cycles 1, 2, 4 and 6 for clinical disease assessment. Patients who achieve with clinical response (CRi or better) and are receiving cycles every 6 weeks, will undergo bone marrow biopsies on day 42 ± 4. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression. Standard bone marrow procurement procedures will be followed for collection of the tissue. To include: correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics and FISH at all time points. A skin biopsy sample (collected under HRPO# 201011766) is required at pre-study, but may have been collected months or even years prior to first dose of decitabine.
[e] On bone marrow or peripheral blood.
[f] After Cycle 1, these labs may be obtained every other week (CBC) or once a month (Chemistries) at the discretion of the treating physician.
[g] Serum chemistries include bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, and LDH.
[h] Decitabine treatment will be 20 mg/m^2 over a 1-hour IV infusion on consecutive Days 1-10 every 28-day cycle. The dose will be calculated based on actual patient weight at the beginning of each cycle. Patients may be premedicated with lorazepam, prochlorperazine, palonosetron, or ondansetron at the treating physician's discretion.
[i] Patients will be followed for 30 days following the date of the last decitabine dose to monitor for toxicity, survival and response. Thereafter, the investigator or designee will record information on subsequent therapy, disease progression, and death, every 6 months for 2 years after first dose of study drug or until the patient is lost to follow-up.

16.0 REFERENCES

1. Cheson, B.D., et al., *Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia.* J Clin Oncol, 2003. **21**(24): p. 4642-9.
2. Cashen, A.F., et al., *Multicenter, phase II study of decitabine for the first-line treatment of older patients with acute myeloid leukemia.* J Clin Oncol, 2010. **28**(4): p. 556-61.
3. Blum, W., et al., *Clinical response and miR-29b predictive significance in older AML patients treated with a 10-day schedule of decitabine.* Proc Natl Acad Sci U S A, 2010. **107**(16): p. 7473-8.
4. Schlenk, R.F., et al., *Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia.* N Engl J Med, 2008. **358**(18): p. 1909-18.
5. Erba, H.P., *Has there been progress in the treatment of older patients with acute myeloid leukemia?* Best Pract Res Clin Haematol, 2010. **23**(4): p. 495-501.
6. Heaney, M.L. and D.W. Golde, *Myelodysplasia.* N Engl J Med, 1999. **340**(21): p. 1649-60.
7. Bennett, J.M., et al., *Proposals for the classification of the myelodysplastic syndromes.* Br J Haematol, 1982. **51**(2): p. 189-99.
8. Kantarjian, H., et al., *Results of intensive chemotherapy in 998 patients age 65 years or older with acute myeloid leukemia or high-risk myelodysplastic syndrome: predictive prognostic models for outcome.* Cancer, 2006. **106**(5): p. 1090-8.
9. Martin, M.G. and C.N. Abboud, *Induction therapy for elderly patients with acute myeloid leukemia.* Blood Rev, 2008. **22**(6): p. 311-20.
10. Bow, E.J., et al., *Therapy of untreated acute myeloid leukemia in the elderly: remission-induction using a non-cytarabine-containing regimen of mitoxantrone plus etoposide.* J Clin Oncol, 1996. **14**(4): p. 1345-52.
11. Goldstone, A.H., et al., *Attempts to improve treatment outcomes in acute myeloid leukemia (AML) in older patients: the results of the United Kingdom Medical Research Council AML11 trial.* Blood, 2001. **98**(5): p. 1302-11.
12. Stone, R.M., *The difficult problem of acute myeloid leukemia in the older adult.* CA Cancer J Clin, 2002. **52**(6): p. 363-71.
13. Liu Yin, J.A., et al., *Mitozantrone and cytosine arabinoside as first-line therapy in elderly patients with acute myeloid leukaemia.* Br J Haematol, 1991. **79**(3): p. 415-20.
14. Stone, R.M., et al., *Granulocyte-macrophage colony-stimulating factor after initial chemotherapy for elderly patients with primary acute myelogenous leukemia. Cancer and Leukemia Group B.* N Engl J Med, 1995. **332**(25): p. 1671-7.
15. Kantarjian, H., et al., *Decitabine improves patient outcomes in myelodysplastic syndromes.* Cancer, 2006. **106**(8): p. 1794-1803.

16. Lubbert, M., et al., *A multicenter phase II trial of Decitabine as first-line treatment of older AML patients judged unfit for induction chemotherapy.* Haematologica, 2011. **97**(3): p. 393-401.
17. Issa, J.P., et al., *Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies.* Blood, 2004. **103**(5): p. 1635-40.
18. Petti, M.C., et al., *Pilot study of 5-aza-2'-deoxycytidine (Decitabine) in the treatment of poor prognosis acute myelogenous leukemia patients: preliminary results.* Leukemia, 1993. **7 Suppl 1**: p. 36-41.
19. Wijermans, P.W., et al., *Continuous infusion of low-dose 5-Aza-2'-deoxycytidine in elderly patients with high-risk myelodysplastic syndrome.* Leukemia, 1997. **11**(1): p. 1-5.
20. Kantarjian, H., et al., *Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study.* Cancer, 2006. **106**(8): p. 1794-803.
21. Wijermans, P.W., et al., *An epigenetic approach to the treatment of advanced MDS; the experience with the DNA demethylating agent 5-aza-2'-deoxycytidine (decitabine) in 177 patients.* Ann Hematol, 2005. **84 Suppl 1**: p. 9-17.
22. Cashen, A.F., et al., *Pharmacokinetics of decitabine administered as a 3-h infusion to patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS).* Cancer Chemother Pharmacol, 2008. **61**(5): p. 759-66.
23. Chabot, G.G., J. Bouchard, and R.L. Momparler, *Kinetics of deamination of 5-aza-2'-deoxycytidine and cytosine arabinoside by human liver cytidine deaminase and its inhibition by 3-deazauridine, thymidine or uracil arabinoside.* Biochem Pharmacol, 1983. **32**(7): p. 1327-8.
24. Momparler, R.L., et al., *Cell cycle effects and cellular pharmacology of 5-aza-2'-deoxycytidine.* Cancer Chemother Pharmacol, 1984. **13**(3): p. 191-4.
25. Raza, A., et al., *Biological significance of cell cycle kinetics in 128 standard risk newly diagnosed patients with acute myelocytic leukemia.* Br J Haematol, 1991. **79**(1): p. 33-9.
26. Brons, P.P., et al., *Proliferation patterns in acute myeloid leukemia: leukemic clonogenic growth and in vivo cell cycle kinetics.* Ann Hematol, 1993. **66**(5): p. 225-33.
27. Raza, A., et al., *Direct relationship between remission duration in acute myeloid leukemia and cell cycle kinetics: a leukemia intergroup study.* Blood, 1990. **76**(11): p. 2191-7.
28. Shen, L., et al., *DNA methylation predicts survival and response to therapy in patients with myelodysplastic syndromes.* J Clin Oncol, 2010. **28**(4): p. 605-13.
29. Tefferi, A., et al., *IDH1 and IDH2 mutation studies in 1473 patients with chronic-, fibrotic- or blast-phase essential thrombocythemia, polycythemia vera or myelofibrosis.* Leukemia, 2010. **24**(7): p. 1302-9.
30. Ley, T.J., et al., *DNMT3A mutations in acute myeloid leukemia.* N Engl J Med, 2010. **363**(25): p. 2424-33.
31. Mardis, E.R., et al., *Recurring mutations found by sequencing an acute myeloid leukemia genome.* N Engl J Med, 2009. **361**(11): p. 1058-66.

32. Abdel-Wahab, O., J. Patel, and R.L. Levine, *Clinical Implications of Novel Mutations in Epigenetic Modifiers in AML.* Hematol Oncol Clin North Am, 2011. **25**(6): p. 1119-33.
33. Reuther, G.W., *Recurring mutations in myeloproliferative neoplasms alter epigenetic regulation of gene expression.* Am J Cancer Res, 2011. **1**(6): p. 752-62.
34. Paschka, P., et al., *IDH1 and IDH2 mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3 internal tandem duplication.* J Clin Oncol, 2010. **28**(22): p. 3636-43.
35. Shen, Y., et al., *Gene mutation patterns and their prognostic impact in a cohort of 1185 patients with acute myeloid leukemia.* Blood, 2011. **118**(20): p. 5593-603.
36. Tefferi, A., et al., *Detection of mutant TET2 in myeloid malignancies other than myeloproliferative neoplasms: CMML, MDS, MDS/MPN and AML.* Leukemia, 2009. **23**(7): p. 1343-5.
37. Walter, M.J., et al., *Recurrent DNMT3A mutations in patients with myelodysplastic syndromes.* Leukemia, 2011. **25**(7): p. 1153-8.
38. Lin, J., et al., *Recurrent DNMT3A R882 Mutations in Chinese Patients with Acute Myeloid Leukemia and Myelodysplastic Syndrome.* PLoS One, 2011. **6**(10): p. e26906.
39. Bejar, R., et al., *Clinical effect of point mutations in myelodysplastic syndromes.* N Engl J Med, 2011. **364**(26): p. 2496-506.
40. Walter, M.J., et al., *Clonal architecture of secondary acute myeloid leukemia.* N Engl J Med, 2012. **366**(12): p. 1090-8.
41. Kantarjian, H.M., et al., *Survival advantage with decitabine versus intensive chemotherapy in patients with higher risk myelodysplastic syndrome: comparison with historical experience.* Cancer, 2007. **109**(6): p. 1133-7.
42. Lubbert, M., et al., *Continued Low-Dose Decitabine (DAC) Is an Active First-Line Treatment in All Cytogenetic Subgroups of Older AML Patients: Results of the FR00331 Multicenter Phase II Study.* ASH Annual Meeting Abstracts, 2007. **110**(11): p. 300-.
43. Blum, W., et al., *Phase I Study of Decitabine Alone or in Combination With Valproic Acid in Acute Myeloid Leukemia.* J Clin Oncol, 2007. **25**(25): p. 3884-3891.
44. Issa, J.-P.J., et al., *Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies.* Blood, 2004. **103**(5): p. 1635-1640.
45. Garcia-Manero, G., et al., *Phase 1/2 study of the combination of 5-aza-2'-deoxycytidine with valproic acid in patients with leukemia.* Blood, 2006. **108**(10): p. 3271-3279.
46. Issa, J.P., S.B. Baylin, and J.G. Herman, *DNA methylation changes in hematologic malignancies: biologic and clinical implications.* Leukemia, 1997. **11 Suppl 1**: p. S7-11.
47. Ganetzky, R.D., et al., *Differences Between Normal and Leukemic Stem Cell-Specific Methylome Indicates Aberrantly Silenced Genes Involved in the*

Pathogenesis of Malignant Evolution. ASH Annual Meeting Abstracts, 2008. **112**(11): p. 599-.

48. Christman, J.K., *5-Azacytidine and 5-aza-2'-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy.* Oncogene, 2002. **21**(35): p. 5483-95.
49. Lubbert, M., *DNA methylation inhibitors in the treatment of leukemias, myelodysplastic syndromes and hemoglobinopathies: clinical results and possible mechanisms of action.* Curr Top Microbiol Immunol, 2000. **249**: p. 135-64.
50. Kantarjian, H., et al., *Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia.* Blood, 2007. **109**(1): p. 52-57.
51. Steensma, D.P., et al., *Multicenter Study of Decitabine Administered Daily for 5 Days Every 4 Weeks to Adults With Myelodysplastic Syndromes: The Alternative Dosing for Outpatient Treatment (ADOPT) Trial.* J Clin Oncol, 2009: p. JCO.2008.19.6550.
52. Cashen, A.F., et al., *Preliminary Results of a Multicenter Phase II Trial of 5-Day Decitabine as Front-Line Therapy for Elderly Patients with Acute Myeloid Leukemia (AML).* ASH Annual Meeting Abstracts, 2008. **112**(11): p. 560-.
53. Jabbour, E., et al., *Evolution of decitabine development: accomplishments, ongoing investigations, and future strategies.* Cancer, 2008. **112**(11): p. 2341-51.
54. Kantarjian, H., et al., *Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia.* Blood, 2007. **109**(1): p. 52-7.
55. Blum, W.G., Klisovic, R., Liu, S., Kefauver, C., Grever, M. R., Schaaf, L., Chan, K., Byrd, J. C., Villalona-Calero, M., Marcucci, G. *Efficacy of a novel schedule of decitabine in previously untreated AML, age 60 or older*. in *ASCO Annual Meeting*. 2009.
56. van Groeningen, C.J., et al., *Phase I and pharmacokinetic study of 5-aza-2'-deoxycytidine (NSC 127716) in cancer patients.* Cancer Res, 1986. **46**(9): p. 4831-6.
57. Rivard, G.E., et al., *Phase I study on 5-aza-2'-deoxycytidine in children with acute leukemia.* Leuk Res, 1981. **5**(6): p. 453-62.
58. Lin, K.T., R.L. Momparler, and G.E. Rivard, *Sample preparation for the determination of 5-aza-2'-deoxycytidine in plasma by high-performance liquid chromatography.* J Chromatogr, 1985. **345**(1): p. 162-7.
59. Momparler, R.L., G.E. Rivard, and M. Gyger, *Clinical trial on 5-aza-2'-deoxycytidine in patients with acute leukemia.* Pharmacol Ther, 1985. **30**(3): p. 277-86.
60. Raska, K., Jurovcik, M., Sormova., Z., *On the metabolism of 5-azacytidine and 5-aza-2'-deoxycytidine in mice.* Coll Czech Chem Commun, 1965. **30**: p. 3001-3005.
61. Momparler, R.L., et al., *Pilot phase I-II study on 5-aza-2'-deoxycytidine (Decitabine) in patients with metastatic lung cancer.* Anticancer Drugs, 1997. **8**(4): p. 358-68.

62. Aparicio, A., et al., *Phase I trial of continuous infusion 5-aza-2'-deoxycytidine.* Cancer Chemother Pharmacol, 2003. **51**(3): p. 231-9.
63. Bryan, J., et al., *Pharmacokinetic evaluation of decitabine for the treatment of leukemia.* Expert Opin Drug Metab Toxicol, 2011. **7**(5): p. 661-72.
64. Scandura, J.M., et al., *Phase 1 study of epigenetic priming with decitabine prior to standard induction chemotherapy for patients with AML.* Blood, 2011. **118**(6): p. 1472-80.
65. Westfall, P.H. and S.A. Young, *Resampling-based multiple testing: examples and methods for p-value adjustment*. 1993, New York: John Wiley & Sons, Inc.
66. Samlowski, W.E., et al., *Evaluation of a 7-day continuous intravenous infusion of decitabine: inhibition of promoter-specific and global genomic DNA methylation.* J Clin Oncol, 2005. **23**(17): p. 3897-905.
67. Blum, W., et al., *Phase I study of decitabine alone or in combination with valproic acid in acute myeloid leukemia.* J Clin Oncol, 2007. **25**(25): p. 3884-91.
68. Yang, A.S., et al., *DNA methylation changes after 5-aza-2'-deoxycytidine therapy in patients with leukemia.* Cancer Res, 2006. **66**(10): p. 5495-503.
69. Cheson, B.D., et al., *Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia.* Blood, 2006. **108**(2): p. 419-25.

Genomic Predictors of Decitabine Response in AML/MDS

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Genomic Predictors of Decitabine Response in AML/MDS

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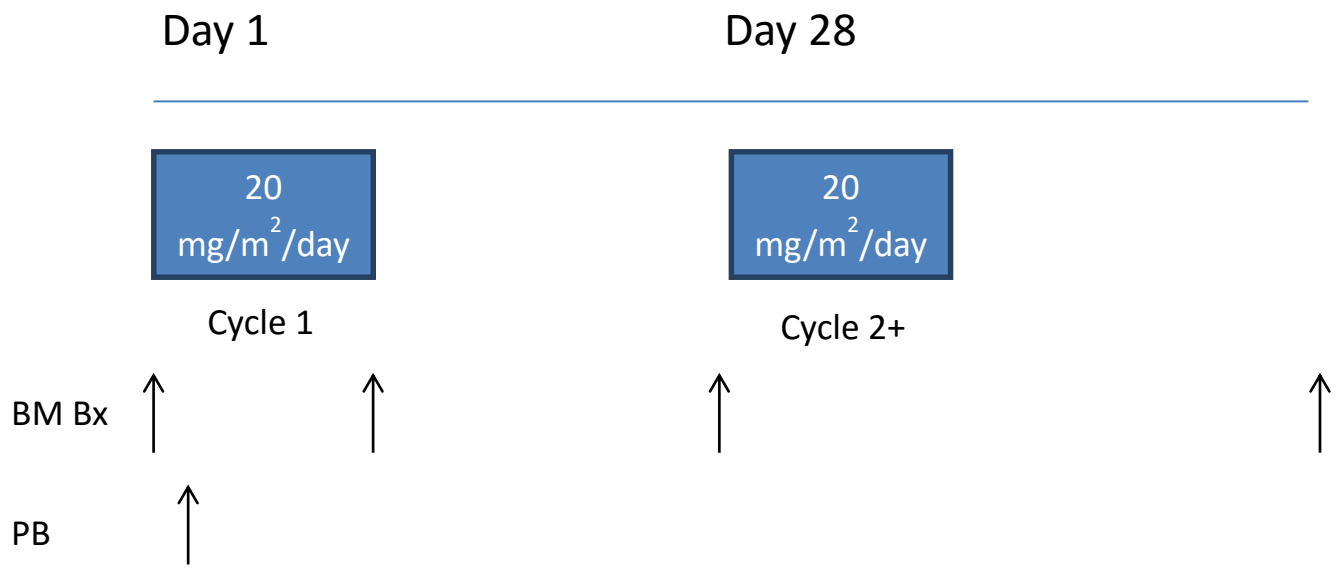
Genomic Predictors of Decitabine Response in AML/MDS
Principal Investigator Signature Page

Principal Investigator:	John Welch, M.D., Ph.D.	
	Signature of Investigator	Date
	Printed Name of Investigator	
	By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IRB/HRPO procedures, the Declaration of Helsinki, ICH Good Clinical Practices guidelines, and the applicable parts of the United States Code of Federal Regulations or local regulations governing the conduct of clinical studies.	

Study Schema:



Patients with progressive disease after two cycles (defined as a clear increase in bone marrow blast count, typically a > 50% increase in bone marrow blasts over baseline) should be removed from study.

Patients with stable disease may continue on study and should undergo a diagnostic bone marrow biopsy at the end of even numbered cycles.

Once AML patients achieve morphologic complete remission with incomplete blood count recovery (CRi) or MDS patients achieve a marrow complete response (Marrow CR) as defined in Section 9.0, decitabine administration may be decreased to 20 mg/m2/day for ten consecutive days every 6 weeks, to limit myelosuppressive toxicity and improve count recovery. After 2 cycles at 6 week intervals, the dose may be reduce to 20 mg/m2/day for five consecutive days in 6 week cycles, at the discretion of the treating physician. After two cycles of five consecutive days of treatment, if the patient meets the definition of CR with complete cytogenetic response as defined in Section 9.0, administration may further be decreased to three consecutive days per cycle at the discretion of the treating physician.

Sample collection for correlative studies:

Bone marrow biopsies should be performed at baseline, at Cycle 1 Day 10 ± 1, and at Day 28 ± 4 of Cycles 1, 2, 4, and 6. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression.

Genomic Predictors of Decitabine Response in AML/MDS

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1.0 INTRODUCTION

1.1 Rationale

Personalized therapy for cancer requires that 1) patient-specific factors (e.g. patient-specific mutations, expression profiles, or pharmacogenomics) influence treatment algorithms, and 2) once therapy is initiated, *in vivo* responsiveness is used to guide further therapy. The modern era of genomics has provided significant insights into cancer pathogenesis by describing patterns of germline and somatic variants, as well as defining disease-specific patterns of gene expression. However, the application and integration of this data with therapeutics remains a difficult task.

[redacted]

AML and MDS are two closely related hematological disorders characterized by ineffective hematopoiesis and malignant expansion of clonal myeloid cells. Both AML and MDS frequently occur in elderly patients and some cases are secondary and/or related to long-term bone marrow damage after radiation or chemotherapy. Current therapy for AML consists of cytotoxic chemotherapy followed by consolidation chemotherapy or stem cell transplantation. Using these approaches, current cure rates may be as high as 60% for patients younger than 60 with good risk cytogenetics (e.g. t(15;17), t(8;21), inv(16)).[4] Outcomes in patients ≥ 60 years old and with relapsed disease remain poor with 2 year survival of only ~25%.[5] Historically, the available treatment for MDS has been symptomatic and has not been shown to be effective in producing sustained improvement in hematopoiesis or in delaying leukemic evolution[6, 7].

Current AML therapy is poorly tolerated in elderly and pre-treated patients due to hematologic and non-hematologic toxicities; following such therapy, patients typically require 3-4 weeks of hospitalization for treatment of neutropenic fevers and for regular transfusions. Even among the relatively healthy patients enrolled in clinical trials, induction mortality in the elderly can be as high as 20-40% following standard induction therapy,[8-11] and moderate to severe non-hematologic toxicity occurs in at least an additional 30% of patients.[9, 12-14] Consequently, many older AML patients are not offered traditional intensive chemotherapy, but receive supportive care alone.[15] More effective and less toxic therapies are needed to provide durable remissions in a significant proportion of elderly AML in populations.

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We and others have recently used whole genome sequencing to identify recurrent, somatic mutations in AML and MDS patients in four genes that are involved in methylcytosine regulation: *DNMT3A, IDH1, IDH2,* and *TET2.*[29-37] Collectively, nearly half of all AML/MDS patients have a mutation in one of these four genes. *DNMT3A* mutations are associated with an adverse outcome in AML patients treated with cytotoxic chemotherapy.[35, 38] However, it is not yet clear whether the presence of mutations in one or more of these genes is associated with response or resistance to decitabine.

In this study, we will treat patients with the current state-of-the-art decitabine schedule.[3] We will perform exome sequencing on all patients and correlate response with mutation status, rate of mutation clearance, expression profile, plasma decitabine levels, and DNA

methylcytosine content reduction. This approach will comprehensively assess genomic and pharmacologic parameters that could clinically predict patient response to decitabine.

In this study, we will combine analysis of patients with AML and MDS. Recent genomic analysis has shown that AML and MDS have shared mutations in many of the same genes, shared patterns of mutation acquisition, and shared patterns of mutation evolution.[39, 40] Furthermore, MDS, like AML, presents with clonal hematopoiesis, even when the morphologic blast count is zero.[36]Because this study will identify and track the rate of clearance of patient specific mutations, and because decitabine has been shown to have efficacy in both AML and MDS patients, [2, 3, 16-19, 41-45] combining analysis of AML and MDS patients is reasonable. Asymptomatic MDS patients are excluded because these patients are typically followed with watchful-waiting rather than with chemotherapy.

1.2 Decitabine and Hypomethylating agents in AML/MDS

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1.2.2 Experience with extended dosing schedules

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1.3 Correlative Studies

All correlative studies will be collected under HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Diseases") that all patients will be concurrently enrolled in. The goals of the correlative studies include:

1. To correlate patient-specific mutations with response to decitabine.
   We will perform exome sequencing of each patient's pre-study bone marrow sample and skin sample. We will determine AML/MDS-associated variants found in the bone marrow, but not skin sample. We will correlate the presence of mutations with response (especially mutations in *DNMT3A, IDH1, IDH2,* and *TET2*). This study is powered to identify mutations with >80% sensitivity to predict decitabine response if used as a clinical assay.

2. To correlate the velocity and depth of response with response to decitabine.
   We will assess tumor burden by allele frequency of patient-specific mutations in bone marrow samples collected pre study, Cycle 1 Day 10±1, Cycle 1 Day 28±4, and Cycle 2 Day 28±4. We will assess mutations in at least 24 recurrently mutated genes (including, but not limited to: *FLT3, DNMT3A, NPM1, TET2, WT1, IDH1, IDH2, RUNX1, NRAS, TP53, U2AF1, KRAS, PHF6, MSTP9, PTPN11, KIT, SMC3, PRUNE2, ETV6, CEBPA, SMC1A, ASXL1, STAG2, RAD21*) as well as all patient-specific mutations discovered during exome sequencing. The leukemic allele frequency will be measured using the Illumnia MySeq or HiSeq platform using DNA capture by gene specific biotinylated-oligonucleotides. Patient bone marrow samples will be assessed pre-study, Cycle 1 Day 10 ± 1, Cycle 1 Day 28 ± 4, and Cycle 2 Day 28 ± 4 and compared with patient specific skin samples (normal control). The velocity of response will be calculated as the natural log rate of patient-specific mutation allele frequency clearance. Depth of response will be defined as the lowest patient-specific mutation

allele frequency in a bone marrow sample. The velocity and depth of tumor response will be correlated to:

- Achievement of clinical CR,
- Time to best response,
- Mutational status,
- Time to disease progression.

Because all patient-specific exome-discovered mutations will be assessed a second time in the primary bone marrow sample as well as the Cycle 1 Day 10 ± 1, Cycle 1 Day 28 ± 4, and Cycle 2 Day 28 ± 4 bone marrow samples, this study will also serve to validate all exome-discovered mutations. This is important, because exome sequencing is associated with a 20-50% false-discovery rate depending on type I error tolerated (mutation-calling algorithms are typically set for greater sensitivity than specificity). Thus, many mutations discovered during exome sequencing are incorrect. This second analysis of these mutations will server to exclude these false positive calls.

3. To correlate the bone marrow expression profile from pre-study and Cycle 1 Day 10 ± 1 with response to decitabine.
   We will correlate expression signatures, and changes in expression signatures, with:
   - Achievement of clinical CR,
   - Mutation status.

4. To correlate the extent of bone marrow DNA hypomethylation on Cycle 1 Day 10 ± 1 with response to decitabine.
   We will assess the percent reduction in bone marrow methylcytosine content using LC-MS. Patients will be evaluated pre-study and Cycle 1 Day 10 ± 1. The change in DNA methylcytosine content will be correlated to:
   - Achievement of clinical CR,
   - Change in expression signature.

   This will determine whether inadequate blast exposure to decitabine correlates with drug resistance.

2.0 OBJECTIVES

2.1 Primary Objective

To determine whether patient-specific mutations correlate with clinical overall response to decitabine in patients with AML or MDS.

2.2 Secondary Objectives

- To determine whether 10 consecutive days per cycle of decitabine improves outcomes compared with historical controls treated with 5 consecutive days per cycle of decitabine.

- To determine whether other biomarkers correlate with clinical overall response to decitabine in patients with AML or MDS. These will include:

    1. The velocity and depth of patient-specific mutation clearance;
    2. The bone marrow expression profile and change in expression profile during decitabine treatment;
    3. The decrease in bone marrow methylcytosine content in response to decitabine.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

All of the following:

- Patient must have non-M3 AML or MDS
- An adverse risk karyotype defined by:
  -Complex karyotype by cytogenetics, or
  -Deletion of all or part of chromosome 5, 7, 12, or 17 defined by FISH or cytogenetics, or
  -Somatic TP53 mutation

All of the following:

1. Patient must have an ECOG performance status ≤ 2.
2. Patient must have >10% disease burden measured by cytomorphology, flow cytometry, or cytogenetics.
3. Patient must have peripheral white blood cell count ≤ 50,000/mcl.
4. Patient must have adequate organ function, defined as:
   a. Total bilirubin ≤ 1.5 x ULN
   b. AST/ALT ≤ 2.5 x ULN
   c. Serum creatinine ≤ 2.0 x ULN
5. Patient must have undergone ≤ 2 cycles of prior hypomethylating agent (decitabine or azacitidine).
6. Patient must be enrolled in HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Diseases").
7. Patient must be ≥ 18 years of age.
8. Patient must be able to understand and willing to sign an IRB-approved written informed consent document.

3.2 Exclusion Criteria

1. Patient must not be pregnant or nursing.
2. The patient must not have acute promyelocytic leukemia or t(15;17) observed by FISH.
3. Patient must not have known CNS leukemia.
4. Patient must not have a history of positive HIV serology.
5. Patient must not have a history of positive Hepatitis C serology.
6. Patient must not have undergone a prior allogeneic stem cell transplant.
7. Patient must not have any uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, ongoing or active GVHD, congestive heart failure of NYHA class 3 or 4, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situation that would limit compliance with study requirements.
8. Patient must not have had radiation therapy within 14 days of enrollment.
9. Patient must not have received any chemotherapy within 21 days of enrollment, and any acute treatment-related toxicities must have returned to baseline. Patients

may be receiving hydrea at time of enrollment.

3.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4.0 REGISTRATION PROCEDURES

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility by Washington University
2. Registration of patient in the Siteman Cancer Center database
3. Assignment of unique patient number (UPN)

Once the patient has been entered in the Siteman Cancer Center OnCore database, the WUSM coordinator will forward verification of enrollment and the UPN via email.

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility collecting the information listed below and scanning and emailing it to the research coordinator listed in the *Siteman Cancer Center Clinical Trials Core Protocol Procedures for Secondary Sites* packet at least one business day prior to registering patient:

1. Your name and contact information (telephone number, fax number, and email address)
2. Your site PI's name, the registering MD's name, and your institution name
3. Patient's race, sex, and DOB
4. Three letters (or two letters and a dash) for the patient's initials
5. Currently approved protocol version date
6. Copy of signed consent form (patient name may be blacked out)
7. Planned date of enrollment
8. Completed eligibility checklist, signed and dated by a member of the study team
9. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center OnCore Database

Registrations may be submitted Monday through Friday between 8am and 5pm CT. Urgent late afternoon or early morning enrollments should be planned in advance and coordinated with the Washington University research coordinator. Registration will be confirmed by the research coordinator or his/her delegate by email within one business day. Verification of eligibility and registration should be kept in the patient chart.

All patients at all sites must be registered through the Siteman Cancer Center OnCore database at Washington University.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. Patients

will also be identified by first, middle, and last initials. If the patient has no middle initial, a dash will be used on the case report forms (CRFs). All data will be recorded with this identification number on the appropriate CRFs.

5.0 INVESTIGATIONAL PLAN

5.1 Summary of Study Design

This is an open-label, single-arm, Phase II, biomarker study of patients with AML or MDS who are treated with 10 consecutive days of decitabine. FAB-M3 AML is excluded from this trial, because other well-tolerated options exist for the treatment of FAB-M3 acute promyelocytic leukemia. AML patients who are < 60 years old and have not received cytotoxic induction chemotherapy are also excluded, because cytotoxic therapy offers a curative possibility for these patients. MDS patients without cytopenias are also excluded because these patients can be followed with watchful-waiting.

All patients will undergo bone marrow biopsies pre-study, on Cycle 1 Day 10 ± 1, and on Day 28 ± 4 of Cycles 1, 2, 4, and 6. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression. Patients will provide a skin biopsy sample pre-study. See Section 8.0 for details on correlative studies.

Patients will receive decitabine 20 mg/m^2/day as a 1-hour infusion on consecutive Days 1-10 in 28-day cycles. The dose will be calculated based on actual patient weight at the beginning of each cycle.

Once AML patients achieve morphologic complete remission with incomplete blood count recovery (CRi) or MDS patients achieve a marrow complete response (Marrow CR) as defined in Section 9.0, decitabine administration may be decreased to 20 mg/m2/day for ten consecutive days every 6 weeks, to limit myelosuppressive toxicity and improve count recovery. After 2 cycles at 6 week intervals, the dose may be reduce to 20 mg/m2/day for five consecutive days in 6 week cycles, at the discretion of the treating physician. After two cycles of five consecutive days of treatment, if the patient meets the definition of CR with complete cytogenetic response as defined in Section 9.0, administration may further be decreased to three consecutive days per cycle at the discretion of the treating physician.

It will be a logistical goal of this trial consistently to return preliminary exome results to the treating physician by the end of Cycle 1 and validated results by the end of Cycle 2. However, this logistical goal is not a primary or secondary aim of this study. Given the limited data regarding mutation presence and drug response, we do not anticipate that the results will directly influence therapeutic decisions made by the treating physician. However, the development of comprehensive genomic pipelines and integration of this data into a clinical trial setting will be required before personalized medicine can be realized.

5.2 Study Procedures

5.2.1 Pre-study Procedures

Pre-study procedures must take place no more than 14 days prior to the first dose of decitabine except where noted below.

1. Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics, and FISH.
2. Bone marrow or peripheral blood for flow cytometry
3. Skin biopsy sample (collected under HRPO# 201011766). There is no required time frame for this sample; it may have been collected months or even years prior to the first dose of decitabine.

5.2.2 Baseline Evaluation

Baseline evaluations must take place no more than 7 days prior to the first dose of decitabine.

1. Medical history
2. Physical exam including height, weight, and ECOG performance score.
3. Record transfusion requirements for 8 weeks before first dose of study drug
4. CBC with differential and platelets
5. Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH
6. Urine βHCG pregnancy test if appropriate
7. Clinical pathology should be carefully reviewed to rule-out acute promyelocytic leukemia (M3-AML), and discussion between the pathologist and treating physician should take place if warranted by morphology or immunophenotype.

5.2.3 Day 1 of Each Cycle

1. Physical exam, including weight
2. CBC with differential and platelets
3. Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH

5.2.4 Cycle 1 Days 1-10

Decitabine administration, 20 mg/m^2 per day given as a 1-hour continuous intravenous infusion. The dose will be calculated based on actual patient weight at

the beginning of each cycle. Patients may be premedicated with lorazepam, prochlorperazine, palonosetron, or ondansetron at the treating physician's discretion.

The clinical research coordinator should monitor for final FISH results to confirm that PML/RARA screening is negative.

5.2.5 Cycle 1 Day 10 ± 1

Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics, and FISH.

5.2.6 Weekly

CBC with differential and platelets. After the first cycle, this may be changed to every other week at the discretion of the treating physician.

5.2.7 Every Other Week

Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH. After the first cycle, this may be changed to every month at the discretion of the treating physician.

5.2.8 Cycles 1, 2, 4 and 6 Day 28 ± 4 days

Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include: correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics, and FISH.

Patients who achieve with clinical response (CRi or better) and are receiving cycles every 6 weeks, will undergo bone marrow biopsies on day 42 ± 4.

5.2.9 End-of-Study Procedures

1. Physical exam
2. CBC with differential and platelets.
3. Serum chemistries including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, and LDH.
4. Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include morphologic assessment, cytogenetics, and FISH.

5.2.10 Post-study Follow-up

Patients are to be followed according to standard patient care procedures for 30 days following the date of the last decitabine dose to monitor for toxicity, survival, and response. Thereafter, the investigator or designee will record information on subsequent therapy, disease progression, and/or death every 6 months for 2 years after last dose of study drug or until the patient is lost to follow-up or dies.

5.3 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented in the case report forms.

In the absence of treatment delays due to adverse events, treatment may continue indefinitely or until one of the following criteria applies:

- Documented and confirmed disease progression
- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious non-compliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study

Patients who prematurely discontinue treatment for any reason will be followed as indicated in the study calendar.

5.4 Concomitant Therapy and Supportive Care Guidelines

5.4.1 Chemotherapy

Patients cannot receive any chemotherapy within 21 days of study enrollment and must have recovered from any treatment-related toxicities. However, patients may be receiving hydrea at enrollment and may continue on hydrea through Cycle 2 of decitabine. An indication for hydrea following 2 cycles of decitabine is evidence that the patient is not responding and decitabine should be discontinued. Other than decitabine, patients cannot receive chemotherapy during this trial.

5.4.2 Growth Factors

Erythroid growth factors should not be routinely administered to patients during the study.

Thrombopoietin receptor agonists should not be routinely administered to patients during the study.

Filgrastim (G-CSF) or sargramostim (GM-CSF) may be administered to patients with septic shock or febrile neutropenia at the discretion of the treating physician.

5.4.3 Transfusions

- *Packed red blood cells (RBCs) transfusion*:
  RBC transfusions will be administered at the discretion of the treating physician.

- *Platelet transfusion*:
  Platelet transfusions will be administered at the discretion of the treating physician

5.4.4 Prophylactic Antimicrobial Agents

Antimicrobial agents will be administered at the discretion of the treating physician. Common antimicrobial prophylaxis during cycles 1 and 2 include acyclovir, ciprofloxacin, and fluconazole.

5.4.5 Radiotherapy

Patients cannot receive radiotherapy within 14 days prior to study enrollment or while on study.

6.0 DOSE MODIFICATIONS

A criterion for discontinuation of the study is a delay of > 8 weeks between any two cycles.

6.1 Delay for Cytopenia or Infection

As cytopenias are a defining feature of AML and MDS, their presence alone does not generally warrant dose modifications or delays. Patients will receive four cycles of therapy without adjustment for any cytopenia. Following Cycle 4, dose modifications or delays for cytopenias will be made at the discretion of the investigator.

Patients with febrile neutropenia (temperature ≥ 38.5° with ANC < 1,000/μl) or systemic infections should have treatment held until clinical resolution of the infection.

6.2 Delay for Organ Dysfunction

If renal dysfunction (creatinine ≥ 3 X institutional upper limit of normal) or hepatic dysfunction (bilirubin ≥ 2.5 mg/dl or ALT/AST ≥ 3 X institutional upper limit of normal) occurs, therapy may not resume until the abnormalities have resolved to below the limits stated above. If renal or hepatic dysfunction occurs during a treatment course, the next dose of decitabine should be held, and serum chemistries should be checked twice per week. Decitabine can be resumed at full dose when creatinine, bilirubin, ALT and AST are below the limits listed above.

For grade 3-4 non-hematologic toxicities related to decitabine, decitabine treatment should be delayed until recovery to baseline or to grade ≤ 1. Decitabine may be reintroduced with a 50% dose reduction, or at full dose at the discretion of the investigator.

6.3 Modification in Dose or Schedule for Treatment Response

Once AML patients achieve morphologic complete remission with incomplete blood count recovery (CRi) or MDS patients achieve a marrow complete response (Marrow CR) as defined in Section 9.0, decitabine administration may be decreased to 20 mg/m2/day for ten consecutive days, and the cycles may be administered every 6 weeks to limit myelosuppressive toxicity, at the discretion of the treating physician. After two cycles at six week intervals, the dose may be reduced to 20 mg/m2/day for five consecutive days of treatment in six week intervals. If the patient meets the definition of CR with complete cytogenetic response as defined in Section 9.0, administration may further be decreased to three consecutive days per cycle at the discretion of the treating physician.

7.0 PHARMACEUTICAL INFORMATION

[redacted]

8.0 CORRELATIVE STUDIES SAMPLE COLLECTIONS

Bone marrow samples will be collected pre-study, on Cycle 1 Day 10 ± 1, and on Day 28 ± 4 of Cycle 1, 2, 4, and 6 for correlative studies. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression. The bone marrow samples will be collected under HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Diseases") that all patients will be concurrently enrolled in.

Skin biopsy sample will be collected as part of the patient's participation in HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Malignancies"). There is no required time frame for this sample, and it may have been collected months or even years prior to the first dose of decitabine.

9.0 EFFICACY, SAFETY AND CORRELATIVE STUDY MEASURES

A secondary endpoint of this study is to estimate morphologic complete remission rate.

9.1 Response Criteria - AML

AML Patients will be assessed for response according to the IWG criteria:[1]

Morphologic complete remission (CR) – Defined as morphologic leukemia-free state, defined as less than 5% blasts in aspirate with marrow spicules and a count of ≥200 nucleated cells and no blasts with Auer rods or any persistence of extramedullary disease. When erythroid precursors constitute less than 50% of bone marrow nucleated cells, the percentage of blasts is based on all nucleated cells; when there are 50% or more erythroid cells, the percentage blasts should be based on the nonerythroid cells. The presence of mild megaloblastoid changes may be permitted if they are thought to be consistent with treatment effect. However, persistence of pretreatment abnormalities (eg, pseudo-Pelger-Hüet cells, ringed sideroblasts, dysplastic megakaryocytes) are not consistent with CR. In addition, ANC >1000/µl and platelet count of ≥100,000/µl. Patient must be independent of transfusions. No duration requirement for this designation.

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

Morphologic complete remission with incomplete blood count recovery (CRi) – Defined as morphologic complete recovery with the exception of neutropenia <1000/µl or thrombocytopenia <100,000/µl.

Partial remission (PR) – Defined as meeting the above criteria for complete remission, with the following exceptions: decrease of ≥50% in the percentage of blast to 5-25% in the bone marrow aspirate. A value of ≤5% blasts in bone marrow with Auer rods present may also be considered a partial remission.

Stable Disease (SD) – Defined as not meeting the definitions of CR, CRi, PR, PD, or recurrence/morphologic relapse.

Progressive disease (PD) – Defined as a clear increase in bone marrow blast count, typically a greater than 50% increase compared with baseline, or new extramedullary disease. Patients with a > 50% increase in peripheral blast count with an increase of the total peripheral white blood cell count to > 10,000/µl should undergo evaluation by bone marrow biopsy to access for progressive disease.

Recurrence/morphologic relapse **-** Defined as relapse following 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. New dysplastic changes is considered relapse.

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.

9.2 Response Criteria - MDS

MDS Patients will be assessed for response according to the IWG criteria:[69]

Complete remission (CR) – Defined as ≤ 5% myeloblasts with normal maturation of all cell lines in the bone marrow and peripheral blood values of Hgb ≥ 11g/dL, Platelets ≥ 100 x 10^9/L, Neutrophils ≥ 1.0 x 10^9/L, and 0% blasts. Persistent dysplasia does not exclude CR but will be noted.

Marrow Complete Response (Marrow CR) – Defined as ≤ 5% myeloblasts in the bone marrow and a decrease by ≥ 50% from pretreatment values, but not meeting the definition of CR above.

Partial Remission (PR) – Defined as meeting the definition of CR above with a decrease of myeloeblasts in the bone marrow by ≥ 50% from pretreatment values, but absolute myeloblasts still >5%.

Stable Disease (SD) – Defined as not meeting the definitions of CR, Marrow CR, PR, SD, PD, or recurrence/morphologic relapse.

Progressive Disease//Relapse (PD) – Defined as ≥ 50% increase in blasts to > 5% blasts (for patients with less than 5% blasts at baseline only), ≥ 50% increase to > 10% blasts (for patients with 5-10% blasts at baseline only), ≥ 50% increase to > 20% blasts (for patients with l0-20% blasts at baseline only), ≥ 50% increase to > 30% blasts (for patients with 20-30% blasts at baseline only) or any of the following: At least 50% decrement from maximum remission/response in granulocytes or platelets, reduction in Hgb by ≥ 2 g/dL, or New or worsened transfusion dependence not related to study drug toxicity. Or for patients with a CR, Marrow CR, or PR as defined above and subsequently development of one of the following: Return to pretreatment bone marrow blast percentage, decrement of ≥ 50% from maximum remission/response levels in granulocytes or platelets, or reduction in Hgb concentration by ≥ 1.5 g/dL or transfusion dependence.

9.2.1 Hematologic Improvement (HI)

Progressive disease as defined above nullifies hematologic improvement.

Erythroid response requires all of the following (only required if pretreatment HgB <11 g/dL):

- Hgb increase by ≥ 1.5 g/dL
- Relevant reduction of units of RBC transfusions by an absolute number of at least 4 RBC transfusions/8 wk compared with the pretreatment transfusion number in the previous 8 wk. Only RBC transfusions given for a Hgb of ≤ 9.0 g/dL pretreatment will count in the RBC transfusion response evaluation

Platelet response requires one of the following (only required if pretreatment platelets < 100 x 10^9/L):

- Absolute increase of ≥ 30 x 10^9/L (for patients starting with > 20 x 10^9/L platelets)
- Increase from < 20 x 10^9/L to > 20 x 10^9/L and absolute increase ≥ 100% (for patients starting with < 20 x 10^9/L)

Neutrophil response requires the following (only required if pretreatment ANC <1.0 x 10^9/L):

- At least 100% increase and an absolute increase > 0.5 x 10^9/L

9.3.1 Cytogenetic Response

Complete Cytogenetic Response – Defined as reversion to a normal karyotype. For this study, reversion of a normal karyotype is defined as no clonal abnormalities detected in a minimum of 20 mitotic cells. Progressive disease as defined above nullifies cytogenetic response.

9.3 Guidelines for Evaluation of Disease

Bone marrow biopsy should be performed on Day 28 ± 4 of cycles 1, 2, 4 and 6 for clinical disease assessment. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression.

9.4 Other Secondary Efficacy Measures

9.4.1 Treatment Failure, Overall Survival and Event-free Survival

Treatment failure is defined as failure to achieve a complete or partial remission following 4 cycles of therapy.

Overall survival is defined as the date of first dose of study drug to the date of death from any cause. Patients still alive at the time of the final analysis will be right-censored.

Event-free survival is defined as the interval from the date of first dose of study drug to date of treatment failure, recurrence, death due to any cause, or loss to follow up.

9.4.2 Duration of Remission and Relapse-free Survival for Patients Having Complete Remission

Duration of complete remission is defined as the interval from the date that complete remission is documented to the date of recurrence, death due to any cause, or loss 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.

9.4.3 Safety

Safety evaluation will include assessments of adverse experiences, medical history, physical examinations, vital signs, and laboratory assessments at baseline and throughout the study period. Local laboratories may be used for this study.

10.0 REGULATORY AND REPORTING REQUIREMENTS

The entities providing oversight of safety and compliance with the protocol require reporting as outlined below.

The Washington University Human Research Protection Office (HRPO) requires that all events meeting the definition of unanticipated problem or serious noncompliance be reported as outlined in Section 10.2.

The FDA requires that all serious and unexpected adverse events be reported as outlined in Section 10.6. In addition, any fatal or life-threatening adverse experiences where there is a reasonable possibility of relationship to study intervention must be reported.

10.1 Definitions

10.1.1 Adverse Events (AEs)

Definition: any unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site: http://ctep.cancer.gov/reporting/ctc.html. Grade 1-2 events will be recorded for the first fifty enrolled patients. Subsequently, only events graded as 3 or greater will be recorded. Fifty patients should be sufficient to estimate the true incidence of any adverse event to within ± 14% with 95% confidence intervals.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website: (http://www.hhs.gov/ohrp/policy/AdvEvntGuid.htm).

10.1.2 Serious Adverse Event (SAE)

Definition: any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions)
- A congenital anomaly/birth defect

- Any other experience which, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

All unexpected SAEs must be reported to the FDA.

10.1.3 Unexpected Adverse Experience

Definition: any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure (or risk information, if an IB is not required or available).

Events that are both serious AND unexpected must be reported to the FDA.

10.1.4 Life-Threatening Adverse Experience

Definition: any adverse drug experience that places the subject (in the view of the investigator) at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.

Life-threatening adverse experiences must be reported to the FDA.

10.1.5 Unanticipated Problems

Definition:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

10.1.6 Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

10.1.7 Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

10.1.8 Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

Local IRB Pre-approval of all protocol exceptions must be obtained prior to the event. For secondary sites, the Washington University PI will issue approval of the exception, but it must also be submitted to the local IRB with documentation of approval forwarded to Washington University. Washington University IRB approval is not required for protocol exceptions occurring at secondary sites.

10.2 Reporting to the Human Research Protection Office (HRPO) at Washington University:

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH or SLCH institution, or that impacts participants or the conduct of the study.
- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within **10 working days** of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification to the PI of the event.

10.3 Reporting to the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The PI is required to notify the QASMC of any unanticipated problem occurring at WU or any BJH or SLCH institution that has been reported to and acknowledged by HRPO as reportable. (Unanticipated problems reported to HRPO and withdrawn during the review process need not be reported to QASMC.)

QASMC must be notified within **10 days** of receipt of IRB acknowledgment via email to a QASMC auditor.

10.4 Reporting Requirements for Secondary Sites

The research team at each secondary site is required to promptly notify the Washington University PI and research coordinator of all reportable events (as described in Section 7.6) within **1 working day** of the occurrence of the event or notification of the secondary site's PI of the event. This notification may take place via email if there is not yet enough information for a formal written report (using either an FDA MedWatch form if required or an institutional SAE reporting form if not). A formal written report must be sent to the Washington University PI and research coordinator within **10 working days** of the occurrence of the event or notification of the secondary site's PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification of the secondary site's PI of the event.

The research team at a secondary site is responsible for following its site's guidelines for reporting applicable events to its site's IRB according to its own institutional guidelines. The research team at Washington University is responsible for reporting all applicable events to the FDA.

10.5 Reporting to Secondary Sites

The Washington University PI (or designee) will notify the research team at each secondary site of all reportable events that have occurred at other sites within **10 working days** of the occurrence of the event or notification of the PI of the event. This includes events that take place both at Washington University and at other secondary sites, if applicable.

10.6 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. **PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC.** It is the responsibility of the Washington University principal investigator to report any unanticipated problem to the FDA as follows:

- Report any unexpected fatal or life-threatening adverse experiences (Section 10.1.4) associated with use of the drug by telephone or fax no later than **7 calendar days** after initial receipt of the information.
- Report any serious, unexpected adverse experiences (Section 10.1.2), as well as results from animal studies that suggest significant clinical risk within **15 calendar days** after initial receipt of this information.

All MedWatch forms will be sent by the investigator or investigator's team to the FDA at the following address or by fax:

Food and Drug Administration

Center for Drug Evaluation and Research
Division of Oncology Drug Products
5901-B Ammendale Rd.
Beltsville, MD 20705-1266
FAX: 1-800-FDA-0178

Secondary sites must submit a completed MedWatch form to the Washington University PI and research coordinator within **4 calendar days** (for fatal or life-threatening adverse experiences) or **11 calendar days** (for serious, unexpected adverse experiences). The Washington University PI will be responsible for submitting all MedWatch forms from secondary sites to the FDA within the timeframes specified above.

10.7 Timeframe for Tracking Reportable Events:

Adverse events will be tracked for 30 days following the last day of study treatment, or until the start of a new chemotherapy/treatment, whichever comes first.

11.0 DATA SAFETY MONITORING PLAN

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Data and Safety Monitoring Committee (DSMC) will meet to review toxicity data at least every 6 months following the activation of the first secondary site. The report will be prepared by the statistician and will be submitted to the Quality Assurance and Safety Monitoring Committee (QASMC). This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual including numbers from participating sites
- Protocol activation date at each participating site
- Average rate of accrual observed in year 1, year 2, and subsequent years at each participating site
- Expected accrual end date and accrual by site
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities at all participating sites
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

Until such a time as the first secondary site activates this protocol, a semi-annual DSM report to be prepared by the study team will be submitted to the QASM Committee beginning 6 months after study activation at Washington University.

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

A DSMC will consist of no fewer than 3 members including 2 clinical investigators and a biostatistician. Like investigators, DSMC members are subject to the Washington University School of Medicine policies regarding standards of conduct. Individuals invited to serve on the DSMC will disclose any potential conflicts of interest to the trial principal investigator and/or appropriate university officials, in accordance with institution policies. Potential conflicts that develop during a trial or a member's tenure on a DSMC must also be disclosed.

Refer to the Washington University Quality Assurance and Data Safety Monitoring Committee Policies and Procedures for full details on the responsibilities of the DSMC at http://www.siteman.wustl.edu/uploadedFiles/Research_Programs/Clinical_Research_Resources/Protocol_Review_and_Monitoring_Committee/QASMCQualityAssurcance.pdf

12.0 AUDITING

Since Washington University is the coordinating center, each site will be audited annually by Siteman Cancer Center personnel (QASMC) unless the outside institution has an auditing mechanism in place and can provide a report. The outside sites will be asked to send copies of all audit materials, including source documentation. The audit notification will be sent to the Washington University Research Patient Coordinator, who will obtain the audit materials from the participating institution.

Notification of an upcoming audit will be sent to the research team one month ahead of the audit. Once accrual numbers are confirmed, and approximately 30 days prior to the audit, a list of the cases selected for review (up to 10 for each site) will be sent to the research team. However, if during the audit the need arises to review cases not initially selected, the research team will be asked to provide the additional charts within two working days.

Additional details regarding the Auditing Policies and Procedures can be found at https://siteman.wustl.edu/wp-content/uploads/2015/QASMC-Policies-and-Procedures-03.31.2015.pdf

13.0 MULTICENTER REGULATORY REQUIREMENTS

Washington University requires that each participating site sends its informed consent document to be reviewed and approved by the Washington University Regulatory Coordinator (or designee) prior to IRB/IEC submission.

Site activation is defined as when the secondary site has received official written documentation from the coordinating center that the site has been approved to begin enrollment. At a minimum, each participating institution must have the following documents on file at Washington University prior to study activation:

- Documentation of IRB approval of the study in the form of a letter or other official document from the participating institution's IRB. This documentation must show which version of the protocol was approved by the IRB.
- Documentation of IRB approval of an informed consent form. The consent must include a statement that data will be shared with Washington University, including the Quality Assurance and Safety Monitoring Committee (QASMC), the DSMC (if applicable), and the Washington University study team.
- Documentation of FWA, signed FDA Form 1572 (if applicable), and the CVs of all participating investigators.
- Protocol signature page signed and dated by the investigator at each participating site.

The coordinating center Principal Investigator (or designee) is responsible for disseminating to the participating sites all study updates, amendments, reportable adverse events, etc. Protocol/consent modifications and IB updates will be forwarded electronically to the secondary sites within 4 weeks of obtaining Washington University IRB approval. Activated secondary sites are expected to submit protocol/consent/IB modifications to their local IRBs within 4 weeks of receipt unless otherwise noted. Upon the secondary sites obtaining local IRB approval, documentation of such shall be sent to the Washington University study team within 2 weeks of receipt of approval.

Documentation of participating sites' IRB approval of annual continuing reviews, protocol amendments or revisions, all SAE reports, and all protocol violations/deviations/exceptions must be kept on file at Washington University.

The investigator or a designee from each institution must participate in a regular conference call to update and inform regarding the progress of the trial.

14.0 STATISTICAL ANALYSIS METHODS AND SAMPLE SIZE

14.1 Analysis Populations

Intent-to-Treat - The intent-to-treat (ITT) population will be defined as all patients who receive at least one dose of decitabine.

Efficacy-Evaluable - The efficacy-evaluable (EE) population will be defined as all patients who receive at least one cycle of treatment.

Safety - The safety population will be defined as all patients with at least one dose of decitabine.

14.2 Statistical Analysis

14.2.1 Descriptive Analyses

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

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.

14.2.2 Primary Endpoint Analysis

The primary end-point of this phase II study is the correlation biomarkers with overall response in AML/MDS patients to decitabine.

We will correlate the presence of patient specific mutations (especially mutations in *DNMT3A, IDH1, IDH2,* and *TET2*) with overall response rates. Mutation rates in these genes and in other patient-specific genes will be correlated with overall response and their respective 95% confidence intervals will be provided.

Statistical considerations. The relationship between AML/MDS-associated mutations and the responsiveness to decitabine will be described by contingency tables and assessed using Chi-square test or Fisher's exact test as appropriate. The differentially expressed genes between responders and non-responders will be determined using the significance analysis of microarray (SAM). Multivariate logistic regression will also be fit to adjust the potential confounding effects of other demographic and clinical characteristics. The resultant critical *P* values will be corrected for multiple testing. Since the Bonferroni correction is known to be too conservative, a permutation analysis (e.g., 10,000 random assignments for

responsive status) will be used to derive an empirical critical *P* value. The new critical p-value is the number of permutation tests which yield a lower p-value than the original test divided by the total number of permutation tests.[61]

A power analysis was performed using Chi-square test for the association between mutation and response to decitabine. The frequency of *DNMT3A, IDH1/2,* and *TET2* mutations in AML and MDS patients are each between 15% and 30% in multiple cohorts.[29-37] **Table 3** shows the minimal detectable differences of response rates between mutant and wild-type cases, assuming an expected overall 45% CR/CRi rate and 80% power. For an average of 20% frequency of a mutated gene, for example, 100 patients will provide 80% power to detect 30% difference at a 2-sided 0.1 alpha error. The purpose of this study is to determine whether any of these mutations result in a clinically significant association that would warrant pre-treatment testing. We believe that a reasonable cut off would be if at least 30% of clinical response could be attributed to the presence of a specific mutation. However, we anticipate that more power could be achieved as we assess patient groups with combined characteristics (e.g. *DNMT3A* mutation or loss of *DNMT3A* expression). In addition, we expect that the majority of the enrolled 125 patients can be used in the analysis (since all of our molecular information will be acquired within 30 days of enrollment, we anticipate a drop-out rate of 20% or less).

Table 3. Detectable mutation associated response rates (80% power, $\alpha = 0.05$)

Total N	% of patients with a given mutation	Detectable response difference	Sensitivity of mutation to predict response at minimum detectable response difference*
100	15%	0.40	80%
	20%	0.35	75%
	30%	0.30	67%
	40%	0.27	61%
	50%	0.26	58%

* Assuming the overall response rate (CR/Cri) of 45%

14.2.3 Secondary Endpoint Analyses

The overall response rate (CR/CRi/PR) and complete response rate (CR/CRi) will be compared with historical controls[2], and the 95% confidence intervals will be provided.

The rate of patient specific mutation clearance after 10, 28, and 56 days of therapy will be determined using an Illumina based platform. We will compare the rate of mutation clearance between the patients who achieve a CR/CRi after 4 cycles and those who do not. We anticipate that we will identify between 6 and 25 exonic SNVs per case (the range obtained with >200 AML samples sequenced to date with whole genome or exome sequencing), and that the expected 95% confidence interval of the mean variant allele frequency will be ±2.56% and ±1.25%,

respectively, if we assume an anticipated standard deviation of 3.2% within the same clone. The change of tumor burden over time and its association with clinical response will be assessed using 2-way ANOVA for repeated measurement data, followed by the ad-hoc multiple comparisons for between-group differences (e.g., responders vs. non-responders) at given time points as well as the between-time differences within each group. The resultant critical *P* values will also be corrected using permutation analysis for multiple testing.

The percent decrease in bone marrow methylcytosine will be calculated on day 10±1. We will compare this reduction with previously published data and between the patients who achieve CR/CRi and those who do not. The correlation of peak plasma levels of decitabine with response will be assessed using 2-sample t-test or Mann-Whitney non-parametric rank-sum test as appropriate. The change of total bone marrow DNA methylcytosine from baseline and its association with both steady-state plasma drug levels and response will be assessed using 2-way ANOVA for repeated measurement data, followed by the ad-hoc multiple comparisons for between-group differences (e.g., responders vs. non-responders) and between-time differences. Similar analyses will be performed for the correlation of expression profiles and SNP analysis with pharmacokinetic data.

Statistical power justification. A power analysis was performed using two-sample t-test for between-group difference and paired t-test for the change over time. For tumor clearance, this will provide > 99% power to detect a 10% difference in the means at two separate time points for clones with 6 or more variants, and > 98% power to detect a 15% difference if there are 3 or more variants per clone with $\alpha = 0.05$ (power will decrease to 91% and 82%, respectively, in cases where the standard deviation is 4.7%, the maximum we have observed thus far). For pharmacologic correlations, we anticipate 85% power to determine a 25 ng/ml difference between the average C_{max} of 45 evaluable responders and 55 evaluable non-responders with an anticipated average C_{max} of 70 ng/ml and a standard deviation of 40 ng/ml within the entire cohort, and with $\alpha = 0.05$.[22] We believe a less significant difference would not be sufficient to warrant application in clinical decision making (i.e. whether to continue therapy for more than 1 cycle). We anticipate 99% power to determine a difference of 20% in the reduction of bone marrow methylcytosine between 45 evaluable responders and 55 evaluable non-responders with an expected standard deviation of 15% and $\alpha = 0.05$.[28, 66-68] The difference in methylcytosine reduction previously observed was 30% between patients achieving CR/PR and those with only hematologic improvement.[28]

Time to relapse and time to death will also be listed for each patient.

14.2.4 Safety Analysis

Extent of Exposure – Duration (number of cycles/infusions) of decitabine doses (cumulative and intensity) will be summarized.

Adverse Events - All adverse events will be coded using NCI CTCAE v. 4.0. 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. In addition, adverse events will be summarized by investigator attribution of relationship to study medication and by grade. Similar summaries will be provided by treatment cycle.

Clinical Laboratory Evaluation – Applicable laboratory parameters will be graded according to NCI CTCAE version 4.0. The incidence of maximum grade (number and percent of patients experiencing the maximum grade during the study) will be summarized for each laboratory parameter. Similar summaries will be provided by treatment cycle. Graphical displays of select hematological parameters will also be provided.

Vital Signs, Physical Findings, and Other Safety Observations – Vital signs, findings from physical examinations, ECOG scores and weights will be presented in listing format.

14.2.5 Description of Planned Subgroup Analyses

The following subgroups (if sufficient numbers of patients) will be explored with respect to the primary and overall survival endpoints.

- Age (60-69 years old vs ≥ 70 years old)
- Cytogenetics
- Patients with AML-associated mutations in *DNMT3A, TET2, IDH1,* or *IDH2* vs those who do not have somatic variants in these genes.

14.3 Sample Size

We anticipate initially enrolling 25 patients at Washington University. Once we establish a routine exome analysis with validation and calculation of the rate of patient-specific mutation clearance, we will enroll an additional 100 patients from at least 2 additional centers.

15.0 DATA SUBMISSION SCHEDULE

Case Report Form	Submission Schedule
Original Consent Form	Prior to registration
Patient Registration Form Disease Diagnosis Form Inclusion Criteria Worksheet Exclusion Criteria Worksheet	Prior to starting treatment
Treatment Record Form	To be maintained throughout a patient's treatment cycle. The complete record is due at completion of treatment.
Bone Marrow Sample Collection Form	To be maintained throughout the course of the study at designated bone marrow sample collections time points (See Appendix 2).
Pathology: Bone Marrow Biopsy	To be completed upon the return of pathology report for each bone marrow biopsy.
Study Calendar Form	To be completed with the date of each time point upon occurrence.
Labs Form	To be completed upon the return of laboratory reports for each study time point (See Appendix 2)
Dose Modifications	To be maintained throughout the course of the study if dose modifications are performed.
Therapy Response Form	To be filled out upon remission (prior to, and/or on therapy)
End of Study Form	Due at the completion of the EOS visit, at the time of death, and/or at the time that the patient is lost to follow-up
Follow Up Form	• 30 days post date of last decitabine dose. • Every 6 months for 2 years after the last dose of study drug.
Record of Adverse Events	At the time of any AE

Any queries generated by Washington University must be responded to within 28 days of receipt by the participating site. The Washington University research team will conduct a regular review of data status at all secondary sites, with appropriate corrective action to be requested as needed.

Appendix 1 ECOG Performance Scale

Activity Status	Description
0	Asymptomatic, fully active, and able to carry on all predisease performance without restrictions.
1	Symptomatic, fully ambulatory but restricted in physically strenuous activity and able to carry out performance of a light or sedentary nature, eg, light housework, office work.
2	Symptomatic, ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours: in bed less than 50% of day.
3	Symptomatic, capable of only limited self-care, confined to bed or chair more than 50% of waking hours, but not bedridden.
4	Completely disabled. Cannot carry on any self-care. Totally bedridden.
5	Dead

Appendix 2 Study Flow Chart

Study Flow Chart

Test	Pre-study[1]	Base-line[2]	All Cycles Day 1	All Cycles Days 1-10[3]	Cycle 1 Day 4 ± 1	Cycle 1 Day 10 ± 1 and 28 ± 4	Every week	Every 2 weeks	Even cycles Day 28±4	End of Study
Informed Consent	X									
Medical History		X								
Physical Exam		X[a]	X[b]							X
Transfusion Requirements[c]		X[c]	Ongoing							
Bone Marrow Aspirate and Biopsy	X[d]					X[d]			X[d]	X[d]
Flow Cytometry[e]	X									
Pregnancy test		X								
CBC with differential and platelets		X	X				X[f]			X
Serum Chemistries[g]		X	X					X[f]		X
Decitabine administration[h]				X						
Adverse Events			Ongoing							
Post-study follow-up										X[i]

[1] To be performed within 14 days of the first dose of decitabine.
[2] To be performed within 7 days of the first dose of decitabine.
[3] Decitabine may be reduced to Days 1-5 or Days 1-3. See Section 6.3 for details.
[a] To include height, weight, and ECOG.
[b] To include weight.
[c] Care should be given to record the frequency of transfusions from 8 weeks prior to first dose of study drug through the duration of the study.
[d] Bone marrow biopsy and aspirate should be performed at baseline, at Cycle 1 Day 10 ± 1, and at Day 28 ± 4 of Cycles 1, 2, 4 and 6 for clinical disease assessment. Patients who achieve with clinical response (CRi or better) and are receiving cycles every 6 weeks, will undergo bone marrow biopsies on day 42 ± 4. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression. Standard bone marrow procurement procedures will be followed for collection of the tissue. To include: correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics and FISH at all time points. A skin biopsy sample (collected under HRPO# 201011766) is required at pre-study, but may have been collected months or even years prior to first dose of decitabine.
[e] On bone marrow or peripheral blood.
[f] After Cycle 1, these labs may be obtained every other week (CBC) or once a month (Chemistries) at the discretion of the treating physician.
[g] Serum chemistries include bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, and LDH.
[h] Decitabine treatment will be 20 mg/m^2 over a 1-hour IV infusion on consecutive Days 1-10 every 28-day cycle. The dose will be calculated based on actual patient weight at the beginning of each cycle. Patients may be premedicated with lorazepam, prochlorperazine, palonosetron, or ondansetron at the treating physician's discretion.
[i] Patients will be followed for 30 days following the date of the last decitabine dose to monitor for toxicity, survival and response. Thereafter, the investigator or designee will record information on subsequent therapy, disease progression, and death, every 6 months for 2 years after first dose of study drug or until the patient is lost to follow-up.

16.0 REFERENCES

1. Cheson, B.D., et al., *Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia.* J Clin Oncol, 2003. **21**(24): p. 4642-9.
2. Cashen, A.F., et al., *Multicenter, phase II study of decitabine for the first-line treatment of older patients with acute myeloid leukemia.* J Clin Oncol, 2010. **28**(4): p. 556-61.
3. Blum, W., et al., *Clinical response and miR-29b predictive significance in older AML patients treated with a 10-day schedule of decitabine.* Proc Natl Acad Sci U S A, 2010. **107**(16): p. 7473-8.
4. Schlenk, R.F., et al., *Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia.* N Engl J Med, 2008. **358**(18): p. 1909-18.
5. Erba, H.P., *Has there been progress in the treatment of older patients with acute myeloid leukemia?* Best Pract Res Clin Haematol, 2010. **23**(4): p. 495-501.
6. Heaney, M.L. and D.W. Golde, *Myelodysplasia.* N Engl J Med, 1999. **340**(21): p. 1649-60.
7. Bennett, J.M., et al., *Proposals for the classification of the myelodysplastic syndromes.* Br J Haematol, 1982. **51**(2): p. 189-99.
8. Kantarjian, H., et al., *Results of intensive chemotherapy in 998 patients age 65 years or older with acute myeloid leukemia or high-risk myelodysplastic syndrome: predictive prognostic models for outcome.* Cancer, 2006. **106**(5): p. 1090-8.
9. Martin, M.G. and C.N. Abboud, *Induction therapy for elderly patients with acute myeloid leukemia.* Blood Rev, 2008. **22**(6): p. 311-20.
10. Bow, E.J., et al., *Therapy of untreated acute myeloid leukemia in the elderly: remission-induction using a non-cytarabine-containing regimen of mitoxantrone plus etoposide.* J Clin Oncol, 1996. **14**(4): p. 1345-52.
11. Goldstone, A.H., et al., *Attempts to improve treatment outcomes in acute myeloid leukemia (AML) in older patients: the results of the United Kingdom Medical Research Council AML11 trial.* Blood, 2001. **98**(5): p. 1302-11.
12. Stone, R.M., *The difficult problem of acute myeloid leukemia in the older adult.* CA Cancer J Clin, 2002. **52**(6): p. 363-71.
13. Liu Yin, J.A., et al., *Mitozantrone and cytosine arabinoside as first-line therapy in elderly patients with acute myeloid leukaemia.* Br J Haematol, 1991. **79**(3): p. 415-20.
14. Stone, R.M., et al., *Granulocyte-macrophage colony-stimulating factor after initial chemotherapy for elderly patients with primary acute myelogenous leukemia. Cancer and Leukemia Group B.* N Engl J Med, 1995. **332**(25): p. 1671-7.
15. Kantarjian, H., et al., *Decitabine improves patient outcomes in myelodysplastic syndromes.* Cancer, 2006. **106**(8): p. 1794-1803.

16. Lubbert, M., et al., *A multicenter phase II trial of Decitabine as first-line treatment of older AML patients judged unfit for induction chemotherapy.* Haematologica, 2011. **97**(3): p. 393-401.
17. Issa, J.P., et al., *Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies.* Blood, 2004. **103**(5): p. 1635-40.
18. Petti, M.C., et al., *Pilot study of 5-aza-2'-deoxycytidine (Decitabine) in the treatment of poor prognosis acute myelogenous leukemia patients: preliminary results.* Leukemia, 1993. **7 Suppl 1**: p. 36-41.
19. Wijermans, P.W., et al., *Continuous infusion of low-dose 5-Aza-2'-deoxycytidine in elderly patients with high-risk myelodysplastic syndrome.* Leukemia, 1997. **11**(1): p. 1-5.
20. Kantarjian, H., et al., *Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study.* Cancer, 2006. **106**(8): p. 1794-803.
21. Wijermans, P.W., et al., *An epigenetic approach to the treatment of advanced MDS; the experience with the DNA demethylating agent 5-aza-2'-deoxycytidine (decitabine) in 177 patients.* Ann Hematol, 2005. **84 Suppl 1**: p. 9-17.
22. Cashen, A.F., et al., *Pharmacokinetics of decitabine administered as a 3-h infusion to patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS).* Cancer Chemother Pharmacol, 2008. **61**(5): p. 759-66.
23. Chabot, G.G., J. Bouchard, and R.L. Momparler, *Kinetics of deamination of 5-aza-2'-deoxycytidine and cytosine arabinoside by human liver cytidine deaminase and its inhibition by 3-deazauridine, thymidine or uracil arabinoside.* Biochem Pharmacol, 1983. **32**(7): p. 1327-8.
24. Momparler, R.L., et al., *Cell cycle effects and cellular pharmacology of 5-aza-2'-deoxycytidine.* Cancer Chemother Pharmacol, 1984. **13**(3): p. 191-4.
25. Raza, A., et al., *Biological significance of cell cycle kinetics in 128 standard risk newly diagnosed patients with acute myelocytic leukemia.* Br J Haematol, 1991. **79**(1): p. 33-9.
26. Brons, P.P., et al., *Proliferation patterns in acute myeloid leukemia: leukemic clonogenic growth and in vivo cell cycle kinetics.* Ann Hematol, 1993. **66**(5): p. 225-33.
27. Raza, A., et al., *Direct relationship between remission duration in acute myeloid leukemia and cell cycle kinetics: a leukemia intergroup study.* Blood, 1990. **76**(11): p. 2191-7.
28. Shen, L., et al., *DNA methylation predicts survival and response to therapy in patients with myelodysplastic syndromes.* J Clin Oncol, 2010. **28**(4): p. 605-13.
29. Tefferi, A., et al., *IDH1 and IDH2 mutation studies in 1473 patients with chronic-, fibrotic- or blast-phase essential thrombocythemia, polycythemia vera or myelofibrosis.* Leukemia, 2010. **24**(7): p. 1302-9.
30. Ley, T.J., et al., *DNMT3A mutations in acute myeloid leukemia.* N Engl J Med, 2010. **363**(25): p. 2424-33.
31. Mardis, E.R., et al., *Recurring mutations found by sequencing an acute myeloid leukemia genome.* N Engl J Med, 2009. **361**(11): p. 1058-66.

32. Abdel-Wahab, O., J. Patel, and R.L. Levine, *Clinical Implications of Novel Mutations in Epigenetic Modifiers in AML.* Hematol Oncol Clin North Am, 2011. **25**(6): p. 1119-33.
33. Reuther, G.W., *Recurring mutations in myeloproliferative neoplasms alter epigenetic regulation of gene expression.* Am J Cancer Res, 2011. **1**(6): p. 752-62.
34. Paschka, P., et al., *IDH1 and IDH2 mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3 internal tandem duplication.* J Clin Oncol, 2010. **28**(22): p. 3636-43.
35. Shen, Y., et al., *Gene mutation patterns and their prognostic impact in a cohort of 1185 patients with acute myeloid leukemia.* Blood, 2011. **118**(20): p. 5593-603.
36. Tefferi, A., et al., *Detection of mutant TET2 in myeloid malignancies other than myeloproliferative neoplasms: CMML, MDS, MDS/MPN and AML.* Leukemia, 2009. **23**(7): p. 1343-5.
37. Walter, M.J., et al., *Recurrent DNMT3A mutations in patients with myelodysplastic syndromes.* Leukemia, 2011. **25**(7): p. 1153-8.
38. Lin, J., et al., *Recurrent DNMT3A R882 Mutations in Chinese Patients with Acute Myeloid Leukemia and Myelodysplastic Syndrome.* PLoS One, 2011. **6**(10): p. e26906.
39. Bejar, R., et al., *Clinical effect of point mutations in myelodysplastic syndromes.* N Engl J Med, 2011. **364**(26): p. 2496-506.
40. Walter, M.J., et al., *Clonal architecture of secondary acute myeloid leukemia.* N Engl J Med, 2012. **366**(12): p. 1090-8.
41. Kantarjian, H.M., et al., *Survival advantage with decitabine versus intensive chemotherapy in patients with higher risk myelodysplastic syndrome: comparison with historical experience.* Cancer, 2007. **109**(6): p. 1133-7.
42. Lubbert, M., et al., *Continued Low-Dose Decitabine (DAC) Is an Active First-Line Treatment in All Cytogenetic Subgroups of Older AML Patients: Results of the FR00331 Multicenter Phase II Study.* ASH Annual Meeting Abstracts, 2007. **110**(11): p. 300-.
43. Blum, W., et al., *Phase I Study of Decitabine Alone or in Combination With Valproic Acid in Acute Myeloid Leukemia.* J Clin Oncol, 2007. **25**(25): p. 3884-3891.
44. Issa, J.-P.J., et al., *Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies.* Blood, 2004. **103**(5): p. 1635-1640.
45. Garcia-Manero, G., et al., *Phase 1/2 study of the combination of 5-aza-2'-deoxycytidine with valproic acid in patients with leukemia.* Blood, 2006. **108**(10): p. 3271-3279.
46. Issa, J.P., S.B. Baylin, and J.G. Herman, *DNA methylation changes in hematologic malignancies: biologic and clinical implications.* Leukemia, 1997. **11 Suppl 1**: p. S7-11.
47. Ganetzky, R.D., et al., *Differences Between Normal and Leukemic Stem Cell-Specific Methylome Indicates Aberrantly Silenced Genes Involved in the*

Pathogenesis of Malignant Evolution. ASH Annual Meeting Abstracts, 2008. **112**(11): p. 599-.

48. Christman, J.K., *5-Azacytidine and 5-aza-2'-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy.* Oncogene, 2002. **21**(35): p. 5483-95.
49. Lubbert, M., *DNA methylation inhibitors in the treatment of leukemias, myelodysplastic syndromes and hemoglobinopathies: clinical results and possible mechanisms of action.* Curr Top Microbiol Immunol, 2000. **249**: p. 135-64.
50. Kantarjian, H., et al., *Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia.* Blood, 2007. **109**(1): p. 52-57.
51. Steensma, D.P., et al., *Multicenter Study of Decitabine Administered Daily for 5 Days Every 4 Weeks to Adults With Myelodysplastic Syndromes: The Alternative Dosing for Outpatient Treatment (ADOPT) Trial.* J Clin Oncol, 2009: p. JCO.2008.19.6550.
52. Cashen, A.F., et al., *Preliminary Results of a Multicenter Phase II Trial of 5-Day Decitabine as Front-Line Therapy for Elderly Patients with Acute Myeloid Leukemia (AML).* ASH Annual Meeting Abstracts, 2008. **112**(11): p. 560-.
53. Jabbour, E., et al., *Evolution of decitabine development: accomplishments, ongoing investigations, and future strategies.* Cancer, 2008. **112**(11): p. 2341-51.
54. Kantarjian, H., et al., *Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia.* Blood, 2007. **109**(1): p. 52-7.
55. Blum, W.G., Klisovic, R., Liu, S., Kefauver, C., Grever, M. R., Schaaf, L., Chan, K., Byrd, J. C., Villalona-Calero, M., Marcucci, G. *Efficacy of a novel schedule of decitabine in previously untreated AML, age 60 or older*. in *ASCO Annual Meeting*. 2009.
56. van Groeningen, C.J., et al., *Phase I and pharmacokinetic study of 5-aza-2'-deoxycytidine (NSC 127716) in cancer patients.* Cancer Res, 1986. **46**(9): p. 4831-6.
57. Rivard, G.E., et al., *Phase I study on 5-aza-2'-deoxycytidine in children with acute leukemia.* Leuk Res, 1981. **5**(6): p. 453-62.
58. Lin, K.T., R.L. Momparler, and G.E. Rivard, *Sample preparation for the determination of 5-aza-2'-deoxycytidine in plasma by high-performance liquid chromatography.* J Chromatogr, 1985. **345**(1): p. 162-7.
59. Momparler, R.L., G.E. Rivard, and M. Gyger, *Clinical trial on 5-aza-2'-deoxycytidine in patients with acute leukemia.* Pharmacol Ther, 1985. **30**(3): p. 277-86.
60. Raska, K., Jurovcik, M., Sormova., Z., *On the metabolism of 5-azacytidine and 5-aza-2'-deoxycytidine in mice.* Coll Czech Chem Commun, 1965. **30**: p. 3001-3005.
61. Momparler, R.L., et al., *Pilot phase I-II study on 5-aza-2'-deoxycytidine (Decitabine) in patients with metastatic lung cancer.* Anticancer Drugs, 1997. **8**(4): p. 358-68.

62. Aparicio, A., et al., *Phase I trial of continuous infusion 5-aza-2'-deoxycytidine.* Cancer Chemother Pharmacol, 2003. **51**(3): p. 231-9.
63. Bryan, J., et al., *Pharmacokinetic evaluation of decitabine for the treatment of leukemia.* Expert Opin Drug Metab Toxicol, 2011. **7**(5): p. 661-72.
64. Scandura, J.M., et al., *Phase 1 study of epigenetic priming with decitabine prior to standard induction chemotherapy for patients with AML.* Blood, 2011. **118**(6): p. 1472-80.
65. Westfall, P.H. and S.A. Young, *Resampling-based multiple testing: examples and methods for p-value adjustment*. 1993, New York: John Wiley & Sons, Inc.
66. Samlowski, W.E., et al., *Evaluation of a 7-day continuous intravenous infusion of decitabine: inhibition of promoter-specific and global genomic DNA methylation.* J Clin Oncol, 2005. **23**(17): p. 3897-905.
67. Blum, W., et al., *Phase I study of decitabine alone or in combination with valproic acid in acute myeloid leukemia.* J Clin Oncol, 2007. **25**(25): p. 3884-91.
68. Yang, A.S., et al., *DNA methylation changes after 5-aza-2'-deoxycytidine therapy in patients with leukemia.* Cancer Res, 2006. **66**(10): p. 5495-503.
69. Cheson, B.D., et al., *Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia.* Blood, 2006. **108**(2): p. 419-25.

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Genomic Predictors of Decitabine Response in AML/MDS

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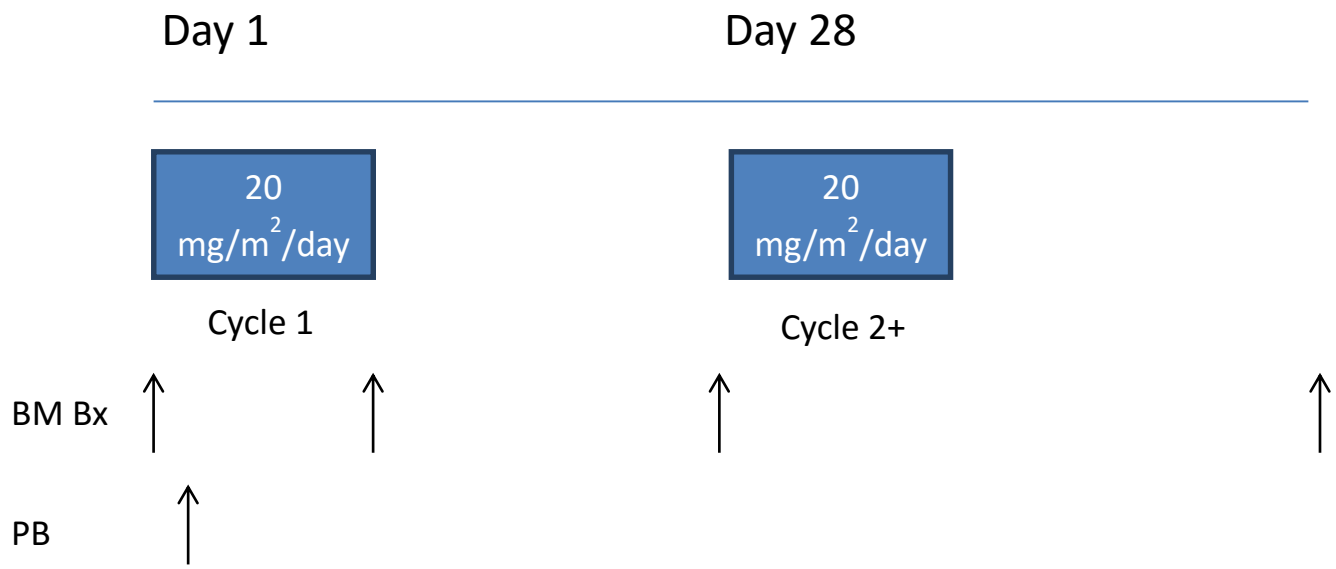
Genomic Predictors of Decitabine Response in AML/MDS
Principal Investigator Signature Page

Principal Investigator:	John Welch, M.D., Ph.D.	
	Signature of Investigator	Date
	Printed Name of Investigator	
	By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IRB/HRPO procedures, the Declaration of Helsinki, ICH Good Clinical Practices guidelines, and the applicable parts of the United States Code of Federal Regulations or local regulations governing the conduct of clinical studies.	

Study Schema:



Patients with progressive disease after two cycles (defined as a clear increase in bone marrow blast count, typically a > 50% increase in bone marrow blasts over baseline) should be removed from study.

Patients with stable disease may continue on study and should undergo a diagnostic bone marrow biopsy at the end of even numbered cycles.

Once AML patients achieve morphologic complete remission with incomplete blood count recovery (CRi) or MDS patients achieve a marrow complete response (Marrow CR) as defined in Section 9.0, decitabine administration may be decreased to 20 mg/m2/day for ten consecutive days every 6 weeks, to limit myelosuppressive toxicity and improve count recovery. After 2 cycles at 6 week intervals, the dose may be reduce to 20 mg/m2/day for five consecutive days in 6 week cycles, at the discretion of the treating physician. After two cycles of five consecutive days of treatment, if the patient meets the definition of CR with complete cytogenetic response as defined in Section 9.0, administration may further be decreased to three consecutive days per cycle at the discretion of the treating physician.

Sample collection for correlative studies:

Bone marrow biopsies should be performed at baseline, at Cycle 1 Day 10 ± 1, and at Day 28 ± 4 of Cycles 1, 2, 4, and 6. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression.

Genomic Predictors of Decitabine Response in AML/MDS

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1.0 INTRODUCTION

1.1 Rationale

Personalized therapy for cancer requires that 1) patient-specific factors (e.g. patient-specific mutations, expression profiles, or pharmacogenomics) influence treatment algorithms, and 2) once therapy is initiated, *in vivo* responsiveness is used to guide further therapy. The modern era of genomics has provided significant insights into cancer pathogenesis by describing patterns of germline and somatic variants, as well as defining disease-specific patterns of gene expression. However, the application and integration of this data with therapeutics remains a difficult task.

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AML and MDS are two closely related hematological disorders characterized by ineffective hematopoiesis and malignant expansion of clonal myeloid cells. Both AML and MDS frequently occur in elderly patients and some cases are secondary and/or related to long-term bone marrow damage after radiation or chemotherapy. Current therapy for AML consists of cytotoxic chemotherapy followed by consolidation chemotherapy or stem cell transplantation. Using these approaches, current cure rates may be as high as 60% for patients younger than 60 with good risk cytogenetics (e.g. t(15;17), t(8;21), inv(16)).[4] Outcomes in patients ≥ 60 years old and with relapsed disease remain poor with 2 year survival of only ~25%.[5] Historically, the available treatment for MDS has been symptomatic and has not been shown to be effective in producing sustained improvement in hematopoiesis or in delaying leukemic evolution[6, 7].

Current AML therapy is poorly tolerated in elderly and pre-treated patients due to hematologic and non-hematologic toxicities; following such therapy, patients typically require 3-4 weeks of hospitalization for treatment of neutropenic fevers and for regular transfusions. Even among the relatively healthy patients enrolled in clinical trials, induction mortality in the elderly can be as high as 20-40% following standard induction therapy,[8-11] and moderate to severe non-hematologic toxicity occurs in at least an additional 30% of patients.[9, 12-14] Consequently, many older AML patients are not offered traditional intensive chemotherapy, but receive supportive care alone.[15] More effective and less toxic therapies are needed to provide durable remissions in a significant proportion of elderly AML in populations.

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We and others have recently used whole genome sequencing to identify recurrent, somatic mutations in AML and MDS patients in four genes that are involved in methylcytosine regulation: *DNMT3A, IDH1, IDH2,* and *TET2.*[29-37] Collectively, nearly half of all AML/MDS patients have a mutation in one of these four genes. *DNMT3A* mutations are associated with an adverse outcome in AML patients treated with cytotoxic chemotherapy.[35, 38] However, it is not yet clear whether the presence of mutations in one or more of these genes is associated with response or resistance to decitabine.

In this study, we will treat patients with the current state-of-the-art decitabine schedule.[3] We will perform exome sequencing on all patients and correlate response with mutation status, rate of mutation clearance, expression profile, plasma decitabine levels, and DNA

methylcytosine content reduction. This approach will comprehensively assess genomic and pharmacologic parameters that could clinically predict patient response to decitabine.

In this study, we will combine analysis of patients with AML and MDS. Recent genomic analysis has shown that AML and MDS have shared mutations in many of the same genes, shared patterns of mutation acquisition, and shared patterns of mutation evolution.[39, 40] Furthermore, MDS, like AML, presents with clonal hematopoiesis, even when the morphologic blast count is zero.[36]Because this study will identify and track the rate of clearance of patient specific mutations, and because decitabine has been shown to have efficacy in both AML and MDS patients, [2, 3, 16-19, 41-45] combining analysis of AML and MDS patients is reasonable. Asymptomatic MDS patients are excluded because these patients are typically followed with watchful-waiting rather than with chemotherapy.

1.2 Decitabine and Hypomethylating agents in AML/MDS

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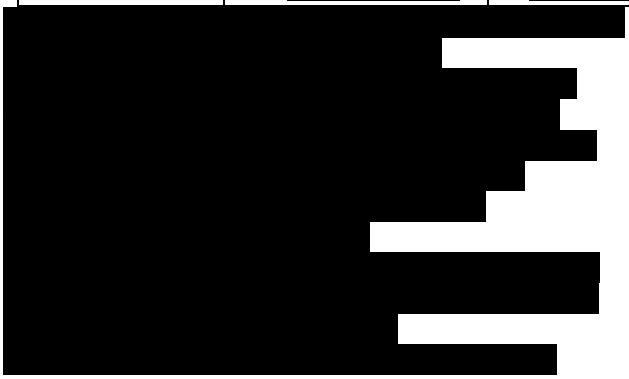

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1.3 Correlative Studies

All correlative studies will be collected under HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Diseases") that all patients will be concurrently enrolled in. The goals of the correlative studies include:

1. To correlate patient-specific mutations with response to decitabine.
   We will perform exome sequencing of each patient's pre-study bone marrow sample and skin sample. We will determine AML/MDS-associated variants found in the bone marrow, but not skin sample. We will correlate the presence of mutations with response (especially mutations in *DNMT3A, IDH1, IDH2,* and *TET2*). This study is powered to identify mutations with >80% sensitivity to predict decitabine response if used as a clinical assay.

2. To correlate the velocity and depth of response with response to decitabine.
   We will assess tumor burden by allele frequency of patient-specific mutations in bone marrow samples collected pre study, Cycle 1 Day 10±1, Cycle 1 Day 28±4, and Cycle 2 Day 28±4. We will assess mutations in at least 24 recurrently mutated genes (including, but not limited to: *FLT3, DNMT3A, NPM1, TET2, WT1, IDH1, IDH2, RUNX1, NRAS, TP53, U2AF1, KRAS, PHF6, MSTP9, PTPN11, KIT, SMC3, PRUNE2, ETV6, CEBPA, SMC1A, ASXL1, STAG2, RAD21*) as well as all patient-specific mutations discovered during exome sequencing. The leukemic allele frequency will be measured using the Illumnia MySeq or HiSeq platform using DNA capture by gene specific biotinylated-oligonucleotides. Patient bone marrow samples will be assessed pre-study, Cycle 1 Day 10 ± 1, Cycle 1 Day 28 ± 4, and Cycle 2 Day 28 ± 4 and compared with patient specific skin samples (normal control). The velocity of response will be calculated as the natural log rate of patient-specific mutation allele frequency clearance. Depth of response will be defined as the lowest patient-specific mutation

allele frequency in a bone marrow sample. The velocity and depth of tumor response will be correlated to:

- Achievement of clinical CR,
- Time to best response,
- Mutational status,
- Time to disease progression.

Because all patient-specific exome-discovered mutations will be assessed a second time in the primary bone marrow sample as well as the Cycle 1 Day 10 ± 1, Cycle 1 Day 28 ± 4, and Cycle 2 Day 28 ± 4 bone marrow samples, this study will also serve to validate all exome-discovered mutations. This is important, because exome sequencing is associated with a 20-50% false-discovery rate depending on type I error tolerated (mutation-calling algorithms are typically set for greater sensitivity than specificity). Thus, many mutations discovered during exome sequencing are incorrect. This second analysis of these mutations will server to exclude these false positive calls.

3. To correlate the bone marrow expression profile from pre-study and Cycle 1 Day 10 ± 1 with response to decitabine.
   We will correlate expression signatures, and changes in expression signatures, with:
   - Achievement of clinical CR,
   - Mutation status.

4. To correlate the extent of bone marrow DNA hypomethylation on Cycle 1 Day 10 ± 1 with response to decitabine.
   We will assess the percent reduction in bone marrow methylcytosine content using LC-MS. Patients will be evaluated pre-study and Cycle 1 Day 10 ± 1. The change in DNA methylcytosine content will be correlated to:
   - Achievement of clinical CR,
   - Change in expression signature.

   This will determine whether inadequate blast exposure to decitabine correlates with drug resistance.

2.0 OBJECTIVES

2.1 Primary Objective

To determine whether patient-specific mutations correlate with clinical overall response to decitabine in patients with AML or MDS.

2.2 Secondary Objectives

- To determine whether 10 consecutive days per cycle of decitabine improves outcomes compared with historical controls treated with 5 consecutive days per cycle of decitabine.

- To determine whether other biomarkers correlate with clinical overall response to decitabine in patients with AML or MDS. These will include:

    1. The velocity and depth of patient-specific mutation clearance;
    2. The bone marrow expression profile and change in expression profile during decitabine treatment;
    3. The decrease in bone marrow methylcytosine content in response to decitabine.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

One of the following:

- Patient must have non-M3 AML and be ≥ 60 years of age OR
- non-M3 AML with relapsed disease OR
- Symptomatic MDS with one of the following:
  - □ Symptomatic anemia with either hemoglobin < 10.0 g/dL or requiring RBC transfusion
  - □ Thrombocytopenia with a history of two or more platelet counts < 50,000/mcL or a significant hemorrhage requiring platelet transfusions
  - □ Neutropenia with two or more ANC < 1,000/mcL

All of the following:

1. Patient must have an ECOG performance status ≤ 2.
2. Patient must have >10% disease burden measured by cytomorphology, flow cytometry, or cytogenetics.
3. Patient must have peripheral white blood cell count ≤ 50,000/mcl.
4. Patient must have adequate organ function, defined as:
   a. Total bilirubin ≤ 1.5 x ULN
   b. AST/ALT ≤ 2.5 x ULN
   c. Serum creatinine ≤ 2.0 x ULN
5. Patient must have undergone ≤ 2 cycles of prior hypomethylating agent (decitabine or azacitidine).
6. Patient must be enrolled in HRPO# 201011766 (“Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Diseases”).
7. Patient must be ≥ 18 years of age.
8. Patient must be able to understand and willing to sign an IRB-approved written informed consent document.

3.2 Exclusion Criteria

1. Patient must not be pregnant or nursing.
2. The patient must not have acute promyelocytic leukemia or t(15;17) observed by FISH.
3. Patient must not have known CNS leukemia.
4. Patient must not have a history of positive HIV serology.
5. Patient must not have a history of positive Hepatitis C serology.
6. Patient must not have undergone a prior allogeneic stem cell transplant.
7. Patient must not have any uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, ongoing or active GVHD, congestive heart failure of NYHA class 3 or 4, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situation that would limit compliance with study requirements.
8. Patient must not have had radiation therapy within 14 days of enrollment.

9. Patient must not have received any chemotherapy within 21 days of enrollment, and any acute treatment-related toxicities must have returned to baseline. Patients may be receiving hydrea at time of enrollment.

3.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4.0 REGISTRATION PROCEDURES

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility by Washington University
2. Registration of patient in the Siteman Cancer Center database
3. Assignment of unique patient number (UPN)

Once the patient has been entered in the Siteman Cancer Center OnCore database, the WUSM coordinator will forward verification of enrollment and the UPN via email.

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility collecting the information listed below and scanning and emailing it to the research coordinator listed in the *Siteman Cancer Center Clinical Trials Core Protocol Procedures for Secondary Sites* packet at least one business day prior to registering patient:

1. Your name and contact information (telephone number, fax number, and email address)
2. Your site PI's name, the registering MD's name, and your institution name
3. Patient's race, sex, and DOB
4. Three letters (or two letters and a dash) for the patient's initials
5. Currently approved protocol version date
6. Copy of signed consent form (patient name may be blacked out)
7. Planned date of enrollment
8. Completed eligibility checklist, signed and dated by a member of the study team
9. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center OnCore Database

Registrations may be submitted Monday through Friday between 8am and 5pm CT. Urgent late afternoon or early morning enrollments should be planned in advance and coordinated with the Washington University research coordinator. Registration will be confirmed by the research coordinator or his/her delegate by email within one business day. Verification of eligibility and registration should be kept in the patient chart.

All patients at all sites must be registered through the Siteman Cancer Center OnCore database at Washington University.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. Patients

will also be identified by first, middle, and last initials. If the patient has no middle initial, a dash will be used on the case report forms (CRFs). All data will be recorded with this identification number on the appropriate CRFs.

5.0 INVESTIGATIONAL PLAN

5.1 Summary of Study Design

This is an open-label, single-arm, Phase II, biomarker study of patients with AML or MDS who are treated with 10 consecutive days of decitabine. FAB-M3 AML is excluded from this trial, because other well-tolerated options exist for the treatment of FAB-M3 acute promyelocytic leukemia. AML patients who are < 60 years old and have not received cytotoxic induction chemotherapy are also excluded, because cytotoxic therapy offers a curative possibility for these patients. MDS patients without cytopenias are also excluded because these patients can be followed with watchful-waiting.

All patients will undergo bone marrow biopsies pre-study, on Cycle 1 Day 10 ± 1, and on Day 28 ± 4 of Cycles 1, 2, 4, and 6. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression. Patients will provide a skin biopsy sample pre-study. See Section 8.0 for details on correlative studies.

Patients will receive decitabine 20 mg/m^2/day as a 1-hour infusion on consecutive Days 1-10 in 28-day cycles. The dose will be calculated based on actual patient weight at the beginning of each cycle.

Once AML patients achieve morphologic complete remission with incomplete blood count recovery (CRi) or MDS patients achieve a marrow complete response (Marrow CR) as defined in Section 9.0, decitabine administration may be decreased to 20 mg/m2/day for ten consecutive days every 6 weeks, to limit myelosuppressive toxicity and improve count recovery. After 2 cycles at 6 week intervals, the dose may be reduce to 20 mg/m2/day for five consecutive days in 6 week cycles, at the discretion of the treating physician. After two cycles of five consecutive days of treatment, if the patient meets the definition of CR with complete cytogenetic response as defined in Section 9.0, administration may further be decreased to three consecutive days per cycle at the discretion of the treating physician.

It will be a logistical goal of this trial consistently to return preliminary exome results to the treating physician by the end of Cycle 1 and validated results by the end of Cycle 2. However, this logistical goal is not a primary or secondary aim of this study. Given the limited data regarding mutation presence and drug response, we do not anticipate that the results will directly influence therapeutic decisions made by the treating physician. However, the development of comprehensive genomic pipelines and integration of this data into a clinical trial setting will be required before personalized medicine can be realized.

5.2 Study Procedures

5.2.1 Pre-study Procedures

Pre-study procedures must take place no more than 14 days prior to the first dose of decitabine except where noted below.

1. Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics, and FISH.
2. Bone marrow or peripheral blood for flow cytometry
3. Skin biopsy sample (collected under HRPO# 201011766). There is no required time frame for this sample; it may have been collected months or even years prior to the first dose of decitabine.

5.2.2 Baseline Evaluation

Baseline evaluations must take place no more than 7 days prior to the first dose of decitabine.

1. Medical history
2. Physical exam including height, weight, and ECOG performance score.
3. Record transfusion requirements for 8 weeks before first dose of study drug
4. CBC with differential and platelets
5. Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH
6. Urine βHCG pregnancy test if appropriate
7. Clinical pathology should be carefully reviewed to rule-out acute promyelocytic leukemia (M3-AML), and discussion between the pathologist and treating physician should take place if warranted by morphology or immunophenotype.

5.2.3 Day 1 of Each Cycle

1. Physical exam, including weight
2. CBC with differential and platelets
3. Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH

5.2.4 Cycle 1 Days 1-10

Decitabine administration, 20 mg/m^2 per day given as a 1-hour continuous intravenous infusion. The dose will be calculated based on actual patient weight at

the beginning of each cycle. Patients may be premedicated with lorazepam, prochlorperazine, palonosetron, or ondansetron at the treating physician's discretion.

The clinical research coordinator should monitor for final FISH results to confirm that PML/RARA screening is negative.

5.2.5 Cycle 1 Day 10 ± 1

Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics, and FISH.

5.2.6 Weekly

CBC with differential and platelets. After the first cycle, this may be changed to every other week at the discretion of the treating physician.

5.2.7 Every Other Week

Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH. After the first cycle, this may be changed to every month at the discretion of the treating physician.

5.2.8 Cycles 1, 2, 4 and 6 Day 28 ± 4 days

Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include: correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics, and FISH.

Patients who achieve with clinical response (CRi or better) and are receiving cycles every 6 weeks, will undergo bone marrow biopsies on day 42 ± 4.

5.2.9 End-of-Study Procedures

1. Physical exam
2. CBC with differential and platelets.
3. Serum chemistries including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, and LDH.
4. Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include morphologic assessment, cytogenetics, and FISH.

5.2.10 Post-study Follow-up

Patients are to be followed according to standard patient care procedures for 30 days following the date of the last decitabine dose to monitor for toxicity, survival, and response. Thereafter, the investigator or designee will record information on subsequent therapy, disease progression, and/or death every 6 months for 2 years after last dose of study drug or until the patient is lost to follow-up or dies.

5.3 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented in the case report forms.

In the absence of treatment delays due to adverse events, treatment may continue indefinitely or until one of the following criteria applies:

- Documented and confirmed disease progression
- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious non-compliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study

Patients who prematurely discontinue treatment for any reason will be followed as indicated in the study calendar.

5.4 Concomitant Therapy and Supportive Care Guidelines

5.4.1 Chemotherapy

Patients cannot receive any chemotherapy within 21 days of study enrollment and must have recovered from any treatment-related toxicities. However, patients may be receiving hydrea at enrollment and may continue on hydrea through Cycle 2 of decitabine. An indication for hydrea following 2 cycles of decitabine is evidence that the patient is not responding and decitabine should be discontinued. Other than decitabine, patients cannot receive chemotherapy during this trial.

5.4.2 Growth Factors

Erythroid growth factors should not be routinely administered to patients during the study.

Thrombopoietin receptor agonists should not be routinely administered to patients during the study.

Filgrastim (G-CSF) or sargramostim (GM-CSF) may be administered to patients with septic shock or febrile neutropenia at the discretion of the treating physician.

5.4.3 Transfusions

- *Packed red blood cells (RBCs) transfusion*:
  RBC transfusions will be administered at the discretion of the treating physician.

- *Platelet transfusion*:
  Platelet transfusions will be administered at the discretion of the treating physician

5.4.4 Prophylactic Antimicrobial Agents

Antimicrobial agents will be administered at the discretion of the treating physician. Common antimicrobial prophylaxis during cycles 1 and 2 include acyclovir, ciprofloxacin, and fluconazole.

5.4.5 Radiotherapy

Patients cannot receive radiotherapy within 14 days prior to study enrollment or while on study.

6.0 DOSE MODIFICATIONS

A criterion for discontinuation of the study is a delay of > 8 weeks between any two cycles.

6.1 Delay for Cytopenia or Infection

As cytopenias are a defining feature of AML and MDS, their presence alone does not generally warrant dose modifications or delays. Patients will receive four cycles of therapy without adjustment for any cytopenia. Following Cycle 4, dose modifications or delays for cytopenias will be made at the discretion of the investigator.

Patients with febrile neutropenia (temperature ≥ 38.5° with ANC < 1,000/μl) or systemic infections should have treatment held until clinical resolution of the infection.

6.2 Delay for Organ Dysfunction

If renal dysfunction (creatinine ≥ 3 X institutional upper limit of normal) or hepatic dysfunction (bilirubin ≥ 2.5 mg/dl or ALT/AST ≥ 3 X institutional upper limit of normal) occurs, therapy may not resume until the abnormalities have resolved to below the limits stated above. If renal or hepatic dysfunction occurs during a treatment course, the next dose of decitabine should be held, and serum chemistries should be checked twice per week. Decitabine can be resumed at full dose when creatinine, bilirubin, ALT and AST are below the limits listed above.

For grade 3-4 non-hematologic toxicities related to decitabine, decitabine treatment should be delayed until recovery to baseline or to grade ≤ 1. Decitabine may be reintroduced with a 50% dose reduction, or at full dose at the discretion of the investigator.

6.3 Modification in Dose or Schedule for Treatment Response

Once AML patients achieve morphologic complete remission with incomplete blood count recovery (CRi) or MDS patients achieve a marrow complete response (Marrow CR) as defined in Section 9.0, decitabine administration may be decreased to 20 mg/m2/day for ten consecutive days, and the cycles may be administered every 6 weeks to limit myelosuppressive toxicity, at the discretion of the treating physician. After two cycles at six week intervals, the dose may be reduced to 20 mg/m2/day for five consecutive days of treatment in six week intervals. If the patient meets the definition of CR with complete cytogenetic response as defined in Section 9.0, administration may further be decreased to three consecutive days per cycle at the discretion of the treating physician.

7.0 PHARMACEUTICAL INFORMATION

[redacted]

8.0 CORRELATIVE STUDIES SAMPLE COLLECTIONS

Bone marrow samples will be collected pre-study, on Cycle 1 Day 10 ± 1, and on Day 28 ± 4 of Cycle 1, 2, 4, and 6 for correlative studies. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression. The bone marrow samples will be collected under HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Diseases") that all patients will be concurrently enrolled in.

Skin biopsy sample will be collected as part of the patient's participation in HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Malignancies"). There is no required time frame for this sample, and it may have been collected months or even years prior to the first dose of decitabine.

9.0 EFFICACY, SAFETY AND CORRELATIVE STUDY MEASURES

A secondary endpoint of this study is to estimate morphologic complete remission rate.

9.1 Response Criteria - AML

AML Patients will be assessed for response according to the IWG criteria:[1]

Morphologic complete remission (CR) – Defined as morphologic leukemia-free state, defined as less than 5% blasts in aspirate with marrow spicules and a count of ≥200 nucleated cells and no blasts with Auer rods or any persistence of extramedullary disease. When erythroid precursors constitute less than 50% of bone marrow nucleated cells, the percentage of blasts is based on all nucleated cells; when there are 50% or more erythroid cells, the percentage blasts should be based on the nonerythroid cells. The presence of mild megaloblastoid changes may be permitted if they are thought to be consistent with treatment effect. However, persistence of pretreatment abnormalities (eg, pseudo-Pelger-Hüet cells, ringed sideroblasts, dysplastic megakaryocytes) are not consistent with CR. In addition, ANC >1000/µl and platelet count of ≥100,000/µl. Patient must be independent of transfusions. No duration requirement for this designation.

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

Morphologic complete remission with incomplete blood count recovery (CRi) – Defined as morphologic complete recovery with the exception of neutropenia <1000/µl or thrombocytopenia <100,000/µl.

Partial remission (PR) – Defined as meeting the above criteria for complete remission, with the following exceptions: decrease of ≥50% in the percentage of blast to 5-25% in the bone marrow aspirate. A value of ≤5% blasts in bone marrow with Auer rods present may also be considered a partial remission.

Stable Disease (SD) – Defined as not meeting the definitions of CR, CRi, PR, PD, or recurrence/morphologic relapse.

Progressive disease (PD) – Defined as a clear increase in bone marrow blast count, typically a greater than 50% increase compared with baseline, or new extramedullary disease. Patients with a > 50% increase in peripheral blast count with an increase of the total peripheral white blood cell count to > 10,000/µl should undergo evaluation by bone marrow biopsy to access for progressive disease.

Recurrence/morphologic relapse **-** Defined as relapse following 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. New dysplastic changes is considered relapse.

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.

9.2 Response Criteria - MDS

MDS Patients will be assessed for response according to the IWG criteria:[69]

Complete remission (CR) – Defined as ≤ 5% myeloblasts with normal maturation of all cell lines in the bone marrow and peripheral blood values of Hgb ≥ 11g/dL, Platelets ≥ 100 x 10^9/L, Neutrophils ≥ 1.0 x 10^9/L, and 0% blasts. Persistent dysplasia does not exclude CR but will be noted.

Marrow Complete Response (Marrow CR) – Defined as ≤ 5% myeloblasts in the bone marrow and a decrease by ≥ 50% from pretreatment values, but not meeting the definition of CR above.

Partial Remission (PR) – Defined as meeting the definition of CR above with a decrease of myeloeblasts in the bone marrow by ≥ 50% from pretreatment values, but absolute myeloblasts still >5%.

Stable Disease (SD) – Defined as not meeting the definitions of CR, Marrow CR, PR, SD, PD, or recurrence/morphologic relapse.

Progressive Disease//Relapse (PD) – Defined as ≥ 50% increase in blasts to > 5% blasts (for patients with less than 5% blasts at baseline only), ≥ 50% increase to > 10% blasts (for patients with 5-10% blasts at baseline only), ≥ 50% increase to > 20% blasts (for patients with l0-20% blasts at baseline only), ≥ 50% increase to > 30% blasts (for patients with 20-30% blasts at baseline only) or any of the following: At least 50% decrement from maximum remission/response in granulocytes or platelets, reduction in Hgb by ≥ 2 g/dL, or New or worsened transfusion dependence not related to study drug toxicity. Or for patients with a CR, Marrow CR, or PR as defined above and subsequently development of one of the following: Return to pretreatment bone marrow blast percentage, decrement of ≥ 50% from maximum remission/response levels in granulocytes or platelets, or reduction in Hgb concentration by ≥ 1.5 g/dL or transfusion dependence.

9.2.1 Hematologic Improvement (HI)

Progressive disease as defined above nullifies hematologic improvement.

Erythroid response requires all of the following (only required if pretreatment HgB <11 g/dL):

- Hgb increase by ≥ 1.5 g/dL
- Relevant reduction of units of RBC transfusions by an absolute number of at least 4 RBC transfusions/8 wk compared with the pretreatment transfusion number in the previous 8 wk. Only RBC transfusions given for a Hgb of ≤ 9.0 g/dL pretreatment will count in the RBC transfusion response evaluation

Platelet response requires one of the following (only required if pretreatment platelets < 100 x 10^9/L):

- Absolute increase of ≥ 30 x 10^9/L (for patients starting with > 20 x 10^9/L platelets)
- Increase from < 20 x 10^9/L to > 20 x 10^9/L and absolute increase ≥ 100% (for patients starting with < 20 x 10^9/L)

Neutrophil response requires the following (only required if pretreatment ANC <1.0 x 10^9/L):

- At least 100% increase and an absolute increase > 0.5 x 10^9/L

9.3.1 Cytogenetic Response

Complete Cytogenetic Response – Defined as reversion to a normal karyotype. For this study, reversion of a normal karyotype is defined as no clonal abnormalities detected in a minimum of 20 mitotic cells. Progressive disease as defined above nullifies cytogenetic response.

9.3 Guidelines for Evaluation of Disease

Bone marrow biopsy should be performed on Day 28 ± 4 of cycles 1, 2, 4 and 6 for clinical disease assessment. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression.

9.4 Other Secondary Efficacy Measures

9.4.1 Treatment Failure, Overall Survival and Event-free Survival

Treatment failure is defined as failure to achieve a complete or partial remission following 4 cycles of therapy.

Overall survival is defined as the date of first dose of study drug to the date of death from any cause. Patients still alive at the time of the final analysis will be right-censored.

Event-free survival is defined as the interval from the date of first dose of study drug to date of treatment failure, recurrence, death due to any cause, or loss to follow up.

9.4.2 Duration of Remission and Relapse-free Survival for Patients Having Complete Remission

Duration of complete remission is defined as the interval from the date that complete remission is documented to the date of recurrence, death due to any cause, or loss 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.

9.4.3 Safety

Safety evaluation will include assessments of adverse experiences, medical history, physical examinations, vital signs, and laboratory assessments at baseline and throughout the study period. Local laboratories may be used for this study.

10.0 REGULATORY AND REPORTING REQUIREMENTS

The entities providing oversight of safety and compliance with the protocol require reporting as outlined below.

The Washington University Human Research Protection Office (HRPO) requires that all events meeting the definition of unanticipated problem or serious noncompliance be reported as outlined in Section 10.2.

The FDA requires that all serious and unexpected adverse events be reported as outlined in Section 10.6. In addition, any fatal or life-threatening adverse experiences where there is a reasonable possibility of relationship to study intervention must be reported.

10.1 Definitions

10.1.1 Adverse Events (AEs)

Definition: any unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site: http://ctep.cancer.gov/reporting/ctc.html. Grade 1-2 events will be recorded for the first fifty enrolled patients. Subsequently, only events graded as 3 or greater will be recorded. Fifty patients should be sufficient to estimate the true incidence of any adverse event to within ± 14% with 95% confidence intervals.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website: (http://www.hhs.gov/ohrp/policy/AdvEvntGuid.htm).

10.1.2 Serious Adverse Event (SAE)

Definition: any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions)
- A congenital anomaly/birth defect

- Any other experience which, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

All unexpected SAEs must be reported to the FDA.

10.1.3 Unexpected Adverse Experience

Definition: any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure (or risk information, if an IB is not required or available).

Events that are both serious AND unexpected must be reported to the FDA.

10.1.4 Life-Threatening Adverse Experience

Definition: any adverse drug experience that places the subject (in the view of the investigator) at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.

Life-threatening adverse experiences must be reported to the FDA.

10.1.5 Unanticipated Problems

Definition:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

10.1.6 Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

10.1.7 Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

10.1.8 Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

Local IRB Pre-approval of all protocol exceptions must be obtained prior to the event. For secondary sites, the Washington University PI will issue approval of the exception, but it must also be submitted to the local IRB with documentation of approval forwarded to Washington University. Washington University IRB approval is not required for protocol exceptions occurring at secondary sites.

10.2 Reporting to the Human Research Protection Office (HRPO) at Washington University:

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH or SLCH institution, or that impacts participants or the conduct of the study.
- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within **10 working days** of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification to the PI of the event.

10.3 Reporting to the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The PI is required to notify the QASMC of any unanticipated problem occurring at WU or any BJH or SLCH institution that has been reported to and acknowledged by HRPO as reportable. (Unanticipated problems reported to HRPO and withdrawn during the review process need not be reported to QASMC.)

QASMC must be notified within **10 days** of receipt of IRB acknowledgment via email to a QASMC auditor.

10.4 Reporting Requirements for Secondary Sites

The research team at each secondary site is required to promptly notify the Washington University PI and research coordinator of all reportable events (as described in Section 7.6) within **1 working day** of the occurrence of the event or notification of the secondary site's PI of the event. This notification may take place via email if there is not yet enough information for a formal written report (using either an FDA MedWatch form if required or an institutional SAE reporting form if not). A formal written report must be sent to the Washington University PI and research coordinator within **10 working days** of the occurrence of the event or notification of the secondary site's PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification of the secondary site's PI of the event.

The research team at a secondary site is responsible for following its site's guidelines for reporting applicable events to its site's IRB according to its own institutional guidelines. The research team at Washington University is responsible for reporting all applicable events to the FDA.

10.5 Reporting to Secondary Sites

The Washington University PI (or designee) will notify the research team at each secondary site of all reportable events that have occurred at other sites within **10 working days** of the occurrence of the event or notification of the PI of the event. This includes events that take place both at Washington University and at other secondary sites, if applicable.

10.6 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. **PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC.** It is the responsibility of the Washington University principal investigator to report any unanticipated problem to the FDA as follows:

- Report any unexpected fatal or life-threatening adverse experiences (Section 10.1.4) associated with use of the drug by telephone or fax no later than **7 calendar days** after initial receipt of the information.
- Report any serious, unexpected adverse experiences (Section 10.1.2), as well as results from animal studies that suggest significant clinical risk within **15 calendar days** after initial receipt of this information.

All MedWatch forms will be sent by the investigator or investigator's team to the FDA at the following address or by fax:

Food and Drug Administration

Center for Drug Evaluation and Research
Division of Oncology Drug Products
5901-B Ammendale Rd.
Beltsville, MD 20705-1266
FAX: 1-800-FDA-0178

Secondary sites must submit a completed MedWatch form to the Washington University PI and research coordinator within **4 calendar days** (for fatal or life-threatening adverse experiences) or **11 calendar days** (for serious, unexpected adverse experiences). The Washington University PI will be responsible for submitting all MedWatch forms from secondary sites to the FDA within the timeframes specified above.

10.7 Timeframe for Tracking Reportable Events:

Adverse events will be tracked for 30 days following the last day of study treatment, or until the start of a new chemotherapy/treatment, whichever comes first.

11.0 DATA SAFETY MONITORING PLAN

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Data and Safety Monitoring Committee (DSMC) will meet to review toxicity data at least every 6 months following the activation of the first secondary site. The report will be prepared by the statistician and will be submitted to the Quality Assurance and Safety Monitoring Committee (QASMC). This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual including numbers from participating sites
- Protocol activation date at each participating site
- Average rate of accrual observed in year 1, year 2, and subsequent years at each participating site
- Expected accrual end date and accrual by site
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities at all participating sites
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

Until such a time as the first secondary site activates this protocol, a semi-annual DSM report to be prepared by the study team will be submitted to the QASM Committee beginning 6 months after study activation at Washington University.

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

A DSMC will consist of no fewer than 3 members including 2 clinical investigators and a biostatistician. Like investigators, DSMC members are subject to the Washington University School of Medicine policies regarding standards of conduct. Individuals invited to serve on the DSMC will disclose any potential conflicts of interest to the trial principal investigator and/or appropriate university officials, in accordance with institution policies. Potential conflicts that develop during a trial or a member's tenure on a DSMC must also be disclosed.

Refer to the Washington University Quality Assurance and Data Safety Monitoring Committee Policies and Procedures for full details on the responsibilities of the DSMC at http://www.siteman.wustl.edu/uploadedFiles/Research_Programs/Clinical_Research_Resources/Protocol_Review_and_Monitoring_Committee/QASMCQualityAssurcance.pdf

12.0 AUDITING

Since Washington University is the coordinating center, each site will be audited annually by Siteman Cancer Center personnel (QASMC) unless the outside institution has an auditing mechanism in place and can provide a report. The outside sites will be asked to send copies of all audit materials, including source documentation. The audit notification will be sent to the Washington University Research Patient Coordinator, who will obtain the audit materials from the participating institution.

Notification of an upcoming audit will be sent to the research team one month ahead of the audit. Once accrual numbers are confirmed, and approximately 30 days prior to the audit, a list of the cases selected for review (up to 10 for each site) will be sent to the research team. However, if during the audit the need arises to review cases not initially selected, the research team will be asked to provide the additional charts within two working days.

Additional details regarding the Auditing Policies and Procedures can be found at https://siteman.wustl.edu/wp-content/uploads/2015/QASMC-Policies-and-Procedures-03.31.2015.pdf

13.0 MULTICENTER REGULATORY REQUIREMENTS

Washington University requires that each participating site sends its informed consent document to be reviewed and approved by the Washington University Regulatory Coordinator (or designee) prior to IRB/IEC submission.

Site activation is defined as when the secondary site has received official written documentation from the coordinating center that the site has been approved to begin enrollment. At a minimum, each participating institution must have the following documents on file at Washington University prior to study activation:

- Documentation of IRB approval of the study in the form of a letter or other official document from the participating institution's IRB. This documentation must show which version of the protocol was approved by the IRB.
- Documentation of IRB approval of an informed consent form. The consent must include a statement that data will be shared with Washington University, including the Quality Assurance and Safety Monitoring Committee (QASMC), the DSMC (if applicable), and the Washington University study team.
- Documentation of FWA, signed FDA Form 1572 (if applicable), and the CVs of all participating investigators.
- Protocol signature page signed and dated by the investigator at each participating site.

The coordinating center Principal Investigator (or designee) is responsible for disseminating to the participating sites all study updates, amendments, reportable adverse events, etc. Protocol/consent modifications and IB updates will be forwarded electronically to the secondary sites within 4 weeks of obtaining Washington University IRB approval. Activated secondary sites are expected to submit protocol/consent/IB modifications to their local IRBs within 4 weeks of receipt unless otherwise noted. Upon the secondary sites obtaining local IRB approval, documentation of such shall be sent to the Washington University study team within 2 weeks of receipt of approval.

Documentation of participating sites' IRB approval of annual continuing reviews, protocol amendments or revisions, all SAE reports, and all protocol violations/deviations/exceptions must be kept on file at Washington University.

The investigator or a designee from each institution must participate in a regular conference call to update and inform regarding the progress of the trial.

14.0 STATISTICAL ANALYSIS METHODS AND SAMPLE SIZE

14.1 Analysis Populations

Intent-to-Treat - The intent-to-treat (ITT) population will be defined as all patients who receive at least one dose of decitabine.

Efficacy-Evaluable - The efficacy-evaluable (EE) population will be defined as all patients who receive at least one cycle of treatment.

Safety - The safety population will be defined as all patients with at least one dose of decitabine.

14.2 Statistical Analysis

14.2.1 Descriptive Analyses

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

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.

14.2.2 Primary Endpoint Analysis

The primary end-point of this phase II study is the correlation biomarkers with overall response in AML/MDS patients to decitabine.

We will correlate the presence of patient specific mutations (especially mutations in *DNMT3A, IDH1, IDH2,* and *TET2*) with overall response rates. Mutation rates in these genes and in other patient-specific genes will be correlated with overall response and their respective 95% confidence intervals will be provided.

Statistical considerations. The relationship between AML/MDS-associated mutations and the responsiveness to decitabine will be described by contingency tables and assessed using Chi-square test or Fisher's exact test as appropriate. The differentially expressed genes between responders and non-responders will be determined using the significance analysis of microarray (SAM). Multivariate logistic regression will also be fit to adjust the potential confounding effects of other demographic and clinical characteristics. The resultant critical *P* values will be corrected for multiple testing. Since the Bonferroni correction is known to be too conservative, a permutation analysis (e.g., 10,000 random assignments for

responsive status) will be used to derive an empirical critical *P* value. The new critical p-value is the number of permutation tests which yield a lower p-value than the original test divided by the total number of permutation tests.[61]

A power analysis was performed using Chi-square test for the association between mutation and response to decitabine. The frequency of *DNMT3A, IDH1/2,* and *TET2* mutations in AML and MDS patients are each between 15% and 30% in multiple cohorts.[29-37] **Table 3** shows the minimal detectable differences of response rates between mutant and wild-type cases, assuming an expected overall 45% CR/CRi rate and 80% power. For an average of 20% frequency of a mutated gene, for example, 100 patients will provide 80% power to detect 30% difference at a 2-sided 0.1 alpha error. The purpose of this study is to determine whether any of these mutations result in a clinically significant association that would warrant pre-treatment testing. We believe that a reasonable cut off would be if at least 30% of clinical response could be attributed to the presence of a specific mutation. However, we anticipate that more power could be achieved as we assess patient groups with combined characteristics (e.g. *DNMT3A* mutation or loss of *DNMT3A* expression). In addition, we expect that the majority of the enrolled 125 patients can be used in the analysis (since all of our molecular information will be acquired within 30 days of enrollment, we anticipate a drop-out rate of 20% or less).

Table 3. Detectable mutation associated response rates (80% power, $\alpha = 0.05$)

Total N	% of patients with a given mutation	Detectable response difference	Sensitivity of mutation to predict response at minimum detectable response difference*
100	15%	0.40	80%
	20%	0.35	75%
	30%	0.30	67%
	40%	0.27	61%
	50%	0.26	58%

* Assuming the overall response rate (CR/Cri) of 45%

14.2.3 Secondary Endpoint Analyses

The overall response rate (CR/CRi/PR) and complete response rate (CR/CRi) will be compared with historical controls[2], and the 95% confidence intervals will be provided.

The rate of patient specific mutation clearance after 10, 28, and 56 days of therapy will be determined using an Illumina based platform. We will compare the rate of mutation clearance between the patients who achieve a CR/CRi after 4 cycles and those who do not. We anticipate that we will identify between 6 and 25 exonic SNVs per case (the range obtained with >200 AML samples sequenced to date with whole genome or exome sequencing), and that the expected 95% confidence interval of the mean variant allele frequency will be ±2.56% and ±1.25%,

respectively, if we assume an anticipated standard deviation of 3.2% within the same clone. The change of tumor burden over time and its association with clinical response will be assessed using 2-way ANOVA for repeated measurement data, followed by the ad-hoc multiple comparisons for between-group differences (e.g., responders vs. non-responders) at given time points as well as the between-time differences within each group. The resultant critical *P* values will also be corrected using permutation analysis for multiple testing.

The percent decrease in bone marrow methylcytosine will be calculated on day 10±1. We will compare this reduction with previously published data and between the patients who achieve CR/CRi and those who do not. The correlation of peak plasma levels of decitabine with response will be assessed using 2-sample t-test or Mann-Whitney non-parametric rank-sum test as appropriate. The change of total bone marrow DNA methylcytosine from baseline and its association with both steady-state plasma drug levels and response will be assessed using 2-way ANOVA for repeated measurement data, followed by the ad-hoc multiple comparisons for between-group differences (e.g., responders vs. non-responders) and between-time differences. Similar analyses will be performed for the correlation of expression profiles and SNP analysis with pharmacokinetic data.

Statistical power justification. A power analysis was performed using two-sample t-test for between-group difference and paired t-test for the change over time. For tumor clearance, this will provide > 99% power to detect a 10% difference in the means at two separate time points for clones with 6 or more variants, and > 98% power to detect a 15% difference if there are 3 or more variants per clone with $\alpha = 0.05$ (power will decrease to 91% and 82%, respectively, in cases where the standard deviation is 4.7%, the maximum we have observed thus far). For pharmacologic correlations, we anticipate 85% power to determine a 25 ng/ml difference between the average C_{max} of 45 evaluable responders and 55 evaluable non-responders with an anticipated average C_{max} of 70 ng/ml and a standard deviation of 40 ng/ml within the entire cohort, and with $\alpha = 0.05$.[22] We believe a less significant difference would not be sufficient to warrant application in clinical decision making (i.e. whether to continue therapy for more than 1 cycle). We anticipate 99% power to determine a difference of 20% in the reduction of bone marrow methylcytosine between 45 evaluable responders and 55 evaluable non-responders with an expected standard deviation of 15% and $\alpha = 0.05$.[28, 66-68] The difference in methylcytosine reduction previously observed was 30% between patients achieving CR/PR and those with only hematologic improvement.[28]

Time to relapse and time to death will also be listed for each patient.

14.2.4 Safety Analysis

Extent of Exposure – Duration (number of cycles/infusions) of decitabine doses (cumulative and intensity) will be summarized.

Adverse Events - All adverse events will be coded using NCI CTCAE v. 4.0. 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. In addition, adverse events will be summarized by investigator attribution of relationship to study medication and by grade. Similar summaries will be provided by treatment cycle.

Clinical Laboratory Evaluation – Applicable laboratory parameters will be graded according to NCI CTCAE version 4.0. The incidence of maximum grade (number and percent of patients experiencing the maximum grade during the study) will be summarized for each laboratory parameter. Similar summaries will be provided by treatment cycle. Graphical displays of select hematological parameters will also be provided.

Vital Signs, Physical Findings, and Other Safety Observations – Vital signs, findings from physical examinations, ECOG scores and weights will be presented in listing format.

14.2.5 Description of Planned Subgroup Analyses

The following subgroups (if sufficient numbers of patients) will be explored with respect to the primary and overall survival endpoints.

- Age (60-69 years old vs ≥ 70 years old)
- Cytogenetics
- Patients with AML-associated mutations in *DNMT3A, TET2, IDH1,* or *IDH2* vs those who do not have somatic variants in these genes.

14.3 Sample Size

We anticipate initially enrolling 25 patients at Washington University. Once we establish a routine exome analysis with validation and calculation of the rate of patient-specific mutation clearance, we will enroll an additional 100 patients from at least 2 additional centers.

15.0 DATA SUBMISSION SCHEDULE

Case Report Form	Submission Schedule
Original Consent Form	Prior to registration
Patient Registration Form Disease Diagnosis Form Inclusion Criteria Worksheet Exclusion Criteria Worksheet	Prior to starting treatment
Treatment Record Form	To be maintained throughout a patient's treatment cycle. The complete record is due at completion of treatment.
Bone Marrow Sample Collection Form	To be maintained throughout the course of the study at designated bone marrow sample collections time points (See Appendix 2).
Pathology: Bone Marrow Biopsy	To be completed upon the return of pathology report for each bone marrow biopsy.
Study Calendar Form	To be completed with the date of each time point upon occurrence.
Labs Form	To be completed upon the return of laboratory reports for each study time point (See Appendix 2)
Dose Modifications	To be maintained throughout the course of the study if dose modifications are performed.
Therapy Response Form	To be filled out upon remission (prior to, and/or on therapy)
End of Study Form	Due at the completion of the EOS visit, at the time of death, and/or at the time that the patient is lost to follow-up
Follow Up Form	• 30 days post date of last decitabine dose. • Every 6 months for 2 years after the last dose of study drug.
Record of Adverse Events	At the time of any AE

Any queries generated by Washington University must be responded to within 28 days of receipt by the participating site. The Washington University research team will conduct a regular review of data status at all secondary sites, with appropriate corrective action to be requested as needed.

Appendix 1 ECOG Performance Scale

Activity Status	Description
0	Asymptomatic, fully active, and able to carry on all predisease performance without restrictions.
1	Symptomatic, fully ambulatory but restricted in physically strenuous activity and able to carry out performance of a light or sedentary nature, eg, light housework, office work.
2	Symptomatic, ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours: in bed less than 50% of day.
3	Symptomatic, capable of only limited self-care, confined to bed or chair more than 50% of waking hours, but not bedridden.
4	Completely disabled. Cannot carry on any self-care. Totally bedridden.
5	Dead

Appendix 2 Study Flow Chart

Study Flow Chart

Test	Pre-study[1]	Base-line[2]	All Cycles Day 1	All Cycles Days 1-10[3]	Cycle 1 Day 4 ± 1	Cycle 1 Day 10 ± 1 and 28 ± 4	Every week	Every 2 weeks	Even cycles Day 28±4	End of Study
Informed Consent	X									
Medical History		X								
Physical Exam		X[a]	X[b]							X
Transfusion Requirements[c]		X[c]	Ongoing							
Bone Marrow Aspirate and Biopsy	X[d]					X[d]			X[d]	X[d]
Flow Cytometry[e]	X									
Pregnancy test		X								
CBC with differential and platelets		X	X				X[f]			X
Serum Chemistries[g]		X	X					X[f]		X
Decitabine administration[h]				X						
Adverse Events			Ongoing							
Post-study follow-up										X[i]

[1] To be performed within 14 days of the first dose of decitabine.
[2] To be performed within 7 days of the first dose of decitabine.
[3] Decitabine may be reduced to Days 1-5 or Days 1-3. See Section 6.3 for details.
[a] To include height, weight, and ECOG.
[b] To include weight.
[c] Care should be given to record the frequency of transfusions from 8 weeks prior to first dose of study drug through the duration of the study.
[d] Bone marrow biopsy and aspirate should be performed at baseline, at Cycle 1 Day 10 ± 1, and at Day 28 ± 4 of Cycles 1, 2, 4 and 6 for clinical disease assessment. Patients who achieve with clinical response (CRi or better) and are receiving cycles every 6 weeks, will undergo bone marrow biopsies on day 42 ± 4. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression. Standard bone marrow procurement procedures will be followed for collection of the tissue. To include: correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics and FISH at all time points. A skin biopsy sample (collected under HRPO# 201011766) is required at pre-study, but may have been collected months or even years prior to first dose of decitabine.
[e] On bone marrow or peripheral blood.
[f] After Cycle 1, these labs may be obtained every other week (CBC) or once a month (Chemistries) at the discretion of the treating physician.
[g] Serum chemistries include bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, and LDH.
[h] Decitabine treatment will be 20 mg/m^2 over a 1-hour IV infusion on consecutive Days 1-10 every 28-day cycle. The dose will be calculated based on actual patient weight at the beginning of each cycle. Patients may be premedicated with lorazepam, prochlorperazine, palonosetron, or ondansetron at the treating physician's discretion.
[i] Patients will be followed for 30 days following the date of the last decitabine dose to monitor for toxicity, survival and response. Thereafter, the investigator or designee will record information on subsequent therapy, disease progression, and death, every 6 months for 2 years after first dose of study drug or until the patient is lost to follow-up.

16.0 REFERENCES

1. Cheson, B.D., et al., *Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia.* J Clin Oncol, 2003. **21**(24): p. 4642-9.
2. Cashen, A.F., et al., *Multicenter, phase II study of decitabine for the first-line treatment of older patients with acute myeloid leukemia.* J Clin Oncol, 2010. **28**(4): p. 556-61.
3. Blum, W., et al., *Clinical response and miR-29b predictive significance in older AML patients treated with a 10-day schedule of decitabine.* Proc Natl Acad Sci U S A, 2010. **107**(16): p. 7473-8.
4. Schlenk, R.F., et al., *Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia.* N Engl J Med, 2008. **358**(18): p. 1909-18.
5. Erba, H.P., *Has there been progress in the treatment of older patients with acute myeloid leukemia?* Best Pract Res Clin Haematol, 2010. **23**(4): p. 495-501.
6. Heaney, M.L. and D.W. Golde, *Myelodysplasia.* N Engl J Med, 1999. **340**(21): p. 1649-60.
7. Bennett, J.M., et al., *Proposals for the classification of the myelodysplastic syndromes.* Br J Haematol, 1982. **51**(2): p. 189-99.
8. Kantarjian, H., et al., *Results of intensive chemotherapy in 998 patients age 65 years or older with acute myeloid leukemia or high-risk myelodysplastic syndrome: predictive prognostic models for outcome.* Cancer, 2006. **106**(5): p. 1090-8.
9. Martin, M.G. and C.N. Abboud, *Induction therapy for elderly patients with acute myeloid leukemia.* Blood Rev, 2008. **22**(6): p. 311-20.
10. Bow, E.J., et al., *Therapy of untreated acute myeloid leukemia in the elderly: remission-induction using a non-cytarabine-containing regimen of mitoxantrone plus etoposide.* J Clin Oncol, 1996. **14**(4): p. 1345-52.
11. Goldstone, A.H., et al., *Attempts to improve treatment outcomes in acute myeloid leukemia (AML) in older patients: the results of the United Kingdom Medical Research Council AML11 trial.* Blood, 2001. **98**(5): p. 1302-11.
12. Stone, R.M., *The difficult problem of acute myeloid leukemia in the older adult.* CA Cancer J Clin, 2002. **52**(6): p. 363-71.
13. Liu Yin, J.A., et al., *Mitozantrone and cytosine arabinoside as first-line therapy in elderly patients with acute myeloid leukaemia.* Br J Haematol, 1991. **79**(3): p. 415-20.
14. Stone, R.M., et al., *Granulocyte-macrophage colony-stimulating factor after initial chemotherapy for elderly patients with primary acute myelogenous leukemia. Cancer and Leukemia Group B.* N Engl J Med, 1995. **332**(25): p. 1671-7.
15. Kantarjian, H., et al., *Decitabine improves patient outcomes in myelodysplastic syndromes.* Cancer, 2006. **106**(8): p. 1794-1803.

16. Lubbert, M., et al., *A multicenter phase II trial of Decitabine as first-line treatment of older AML patients judged unfit for induction chemotherapy.* Haematologica, 2011. **97**(3): p. 393-401.
17. Issa, J.P., et al., *Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies.* Blood, 2004. **103**(5): p. 1635-40.
18. Petti, M.C., et al., *Pilot study of 5-aza-2'-deoxycytidine (Decitabine) in the treatment of poor prognosis acute myelogenous leukemia patients: preliminary results.* Leukemia, 1993. **7 Suppl 1**: p. 36-41.
19. Wijermans, P.W., et al., *Continuous infusion of low-dose 5-Aza-2'-deoxycytidine in elderly patients with high-risk myelodysplastic syndrome.* Leukemia, 1997. **11**(1): p. 1-5.
20. Kantarjian, H., et al., *Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study.* Cancer, 2006. **106**(8): p. 1794-803.
21. Wijermans, P.W., et al., *An epigenetic approach to the treatment of advanced MDS; the experience with the DNA demethylating agent 5-aza-2'-deoxycytidine (decitabine) in 177 patients.* Ann Hematol, 2005. **84 Suppl 1**: p. 9-17.
22. Cashen, A.F., et al., *Pharmacokinetics of decitabine administered as a 3-h infusion to patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS).* Cancer Chemother Pharmacol, 2008. **61**(5): p. 759-66.
23. Chabot, G.G., J. Bouchard, and R.L. Momparler, *Kinetics of deamination of 5-aza-2'-deoxycytidine and cytosine arabinoside by human liver cytidine deaminase and its inhibition by 3-deazauridine, thymidine or uracil arabinoside.* Biochem Pharmacol, 1983. **32**(7): p. 1327-8.
24. Momparler, R.L., et al., *Cell cycle effects and cellular pharmacology of 5-aza-2'-deoxycytidine.* Cancer Chemother Pharmacol, 1984. **13**(3): p. 191-4.
25. Raza, A., et al., *Biological significance of cell cycle kinetics in 128 standard risk newly diagnosed patients with acute myelocytic leukemia.* Br J Haematol, 1991. **79**(1): p. 33-9.
26. Brons, P.P., et al., *Proliferation patterns in acute myeloid leukemia: leukemic clonogenic growth and in vivo cell cycle kinetics.* Ann Hematol, 1993. **66**(5): p. 225-33.
27. Raza, A., et al., *Direct relationship between remission duration in acute myeloid leukemia and cell cycle kinetics: a leukemia intergroup study.* Blood, 1990. **76**(11): p. 2191-7.
28. Shen, L., et al., *DNA methylation predicts survival and response to therapy in patients with myelodysplastic syndromes.* J Clin Oncol, 2010. **28**(4): p. 605-13.
29. Tefferi, A., et al., *IDH1 and IDH2 mutation studies in 1473 patients with chronic-, fibrotic- or blast-phase essential thrombocythemia, polycythemia vera or myelofibrosis.* Leukemia, 2010. **24**(7): p. 1302-9.
30. Ley, T.J., et al., *DNMT3A mutations in acute myeloid leukemia.* N Engl J Med, 2010. **363**(25): p. 2424-33.
31. Mardis, E.R., et al., *Recurring mutations found by sequencing an acute myeloid leukemia genome.* N Engl J Med, 2009. **361**(11): p. 1058-66.

32. Abdel-Wahab, O., J. Patel, and R.L. Levine, *Clinical Implications of Novel Mutations in Epigenetic Modifiers in AML.* Hematol Oncol Clin North Am, 2011. **25**(6): p. 1119-33.
33. Reuther, G.W., *Recurring mutations in myeloproliferative neoplasms alter epigenetic regulation of gene expression.* Am J Cancer Res, 2011. **1**(6): p. 752-62.
34. Paschka, P., et al., *IDH1 and IDH2 mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3 internal tandem duplication.* J Clin Oncol, 2010. **28**(22): p. 3636-43.
35. Shen, Y., et al., *Gene mutation patterns and their prognostic impact in a cohort of 1185 patients with acute myeloid leukemia.* Blood, 2011. **118**(20): p. 5593-603.
36. Tefferi, A., et al., *Detection of mutant TET2 in myeloid malignancies other than myeloproliferative neoplasms: CMML, MDS, MDS/MPN and AML.* Leukemia, 2009. **23**(7): p. 1343-5.
37. Walter, M.J., et al., *Recurrent DNMT3A mutations in patients with myelodysplastic syndromes.* Leukemia, 2011. **25**(7): p. 1153-8.
38. Lin, J., et al., *Recurrent DNMT3A R882 Mutations in Chinese Patients with Acute Myeloid Leukemia and Myelodysplastic Syndrome.* PLoS One, 2011. **6**(10): p. e26906.
39. Bejar, R., et al., *Clinical effect of point mutations in myelodysplastic syndromes.* N Engl J Med, 2011. **364**(26): p. 2496-506.
40. Walter, M.J., et al., *Clonal architecture of secondary acute myeloid leukemia.* N Engl J Med, 2012. **366**(12): p. 1090-8.
41. Kantarjian, H.M., et al., *Survival advantage with decitabine versus intensive chemotherapy in patients with higher risk myelodysplastic syndrome: comparison with historical experience.* Cancer, 2007. **109**(6): p. 1133-7.
42. Lubbert, M., et al., *Continued Low-Dose Decitabine (DAC) Is an Active First-Line Treatment in All Cytogenetic Subgroups of Older AML Patients: Results of the FR00331 Multicenter Phase II Study.* ASH Annual Meeting Abstracts, 2007. **110**(11): p. 300-.
43. Blum, W., et al., *Phase I Study of Decitabine Alone or in Combination With Valproic Acid in Acute Myeloid Leukemia.* J Clin Oncol, 2007. **25**(25): p. 3884-3891.
44. Issa, J.-P.J., et al., *Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies.* Blood, 2004. **103**(5): p. 1635-1640.
45. Garcia-Manero, G., et al., *Phase 1/2 study of the combination of 5-aza-2'-deoxycytidine with valproic acid in patients with leukemia.* Blood, 2006. **108**(10): p. 3271-3279.
46. Issa, J.P., S.B. Baylin, and J.G. Herman, *DNA methylation changes in hematologic malignancies: biologic and clinical implications.* Leukemia, 1997. **11 Suppl 1**: p. S7-11.
47. Ganetzky, R.D., et al., *Differences Between Normal and Leukemic Stem Cell-Specific Methylome Indicates Aberrantly Silenced Genes Involved in the*

Pathogenesis of Malignant Evolution. ASH Annual Meeting Abstracts, 2008. **112**(11): p. 599-.

48. Christman, J.K., *5-Azacytidine and 5-aza-2'-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy.* Oncogene, 2002. **21**(35): p. 5483-95.
49. Lubbert, M., *DNA methylation inhibitors in the treatment of leukemias, myelodysplastic syndromes and hemoglobinopathies: clinical results and possible mechanisms of action.* Curr Top Microbiol Immunol, 2000. **249**: p. 135-64.
50. Kantarjian, H., et al., *Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia.* Blood, 2007. **109**(1): p. 52-57.
51. Steensma, D.P., et al., *Multicenter Study of Decitabine Administered Daily for 5 Days Every 4 Weeks to Adults With Myelodysplastic Syndromes: The Alternative Dosing for Outpatient Treatment (ADOPT) Trial.* J Clin Oncol, 2009: p. JCO.2008.19.6550.
52. Cashen, A.F., et al., *Preliminary Results of a Multicenter Phase II Trial of 5-Day Decitabine as Front-Line Therapy for Elderly Patients with Acute Myeloid Leukemia (AML).* ASH Annual Meeting Abstracts, 2008. **112**(11): p. 560-.
53. Jabbour, E., et al., *Evolution of decitabine development: accomplishments, ongoing investigations, and future strategies.* Cancer, 2008. **112**(11): p. 2341-51.
54. Kantarjian, H., et al., *Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia.* Blood, 2007. **109**(1): p. 52-7.
55. Blum, W.G., Klisovic, R., Liu, S., Kefauver, C., Grever, M. R., Schaaf, L., Chan, K., Byrd, J. C., Villalona-Calero, M., Marcucci, G. *Efficacy of a novel schedule of decitabine in previously untreated AML, age 60 or older*. in *ASCO Annual Meeting*. 2009.
56. van Groeningen, C.J., et al., *Phase I and pharmacokinetic study of 5-aza-2'-deoxycytidine (NSC 127716) in cancer patients.* Cancer Res, 1986. **46**(9): p. 4831-6.
57. Rivard, G.E., et al., *Phase I study on 5-aza-2'-deoxycytidine in children with acute leukemia.* Leuk Res, 1981. **5**(6): p. 453-62.
58. Lin, K.T., R.L. Momparler, and G.E. Rivard, *Sample preparation for the determination of 5-aza-2'-deoxycytidine in plasma by high-performance liquid chromatography.* J Chromatogr, 1985. **345**(1): p. 162-7.
59. Momparler, R.L., G.E. Rivard, and M. Gyger, *Clinical trial on 5-aza-2'-deoxycytidine in patients with acute leukemia.* Pharmacol Ther, 1985. **30**(3): p. 277-86.
60. Raska, K., Jurovcik, M., Sormova., Z., *On the metabolism of 5-azacytidine and 5-aza-2'-deoxycytidine in mice.* Coll Czech Chem Commun, 1965. **30**: p. 3001-3005.
61. Momparler, R.L., et al., *Pilot phase I-II study on 5-aza-2'-deoxycytidine (Decitabine) in patients with metastatic lung cancer.* Anticancer Drugs, 1997. **8**(4): p. 358-68.

62. Aparicio, A., et al., *Phase I trial of continuous infusion 5-aza-2'-deoxycytidine.* Cancer Chemother Pharmacol, 2003. **51**(3): p. 231-9.
63. Bryan, J., et al., *Pharmacokinetic evaluation of decitabine for the treatment of leukemia.* Expert Opin Drug Metab Toxicol, 2011. **7**(5): p. 661-72.
64. Scandura, J.M., et al., *Phase 1 study of epigenetic priming with decitabine prior to standard induction chemotherapy for patients with AML.* Blood, 2011. **118**(6): p. 1472-80.
65. Westfall, P.H. and S.A. Young, *Resampling-based multiple testing: examples and methods for p-value adjustment*. 1993, New York: John Wiley & Sons, Inc.
66. Samlowski, W.E., et al., *Evaluation of a 7-day continuous intravenous infusion of decitabine: inhibition of promoter-specific and global genomic DNA methylation.* J Clin Oncol, 2005. **23**(17): p. 3897-905.
67. Blum, W., et al., *Phase I study of decitabine alone or in combination with valproic acid in acute myeloid leukemia.* J Clin Oncol, 2007. **25**(25): p. 3884-91.
68. Yang, A.S., et al., *DNA methylation changes after 5-aza-2'-deoxycytidine therapy in patients with leukemia.* Cancer Res, 2006. **66**(10): p. 5495-503.
69. Cheson, B.D., et al., *Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia.* Blood, 2006. **108**(2): p. 419-25.

Genomic Predictors of Decitabine Response in AML/MDS

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Genomic Predictors of Decitabine Response in AML/MDS

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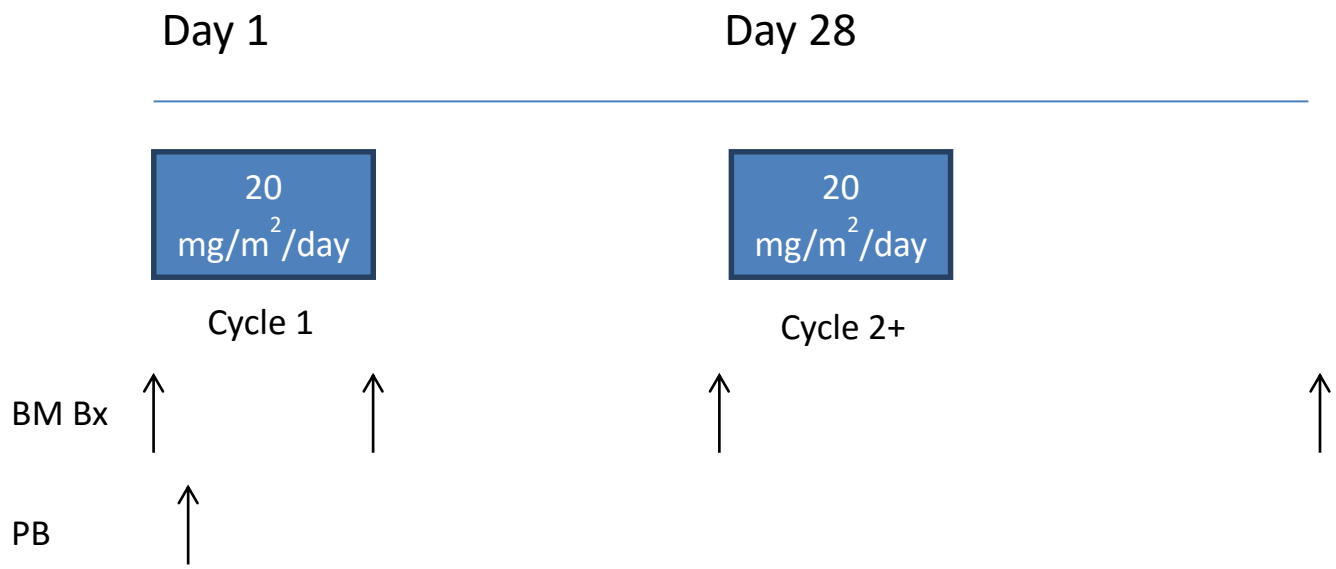
Genomic Predictors of Decitabine Response in AML/MDS
Principal Investigator Signature Page

Principal Investigator:	John Welch, M.D., Ph.D.	
	Signature of Investigator	Date
	Printed Name of Investigator	
	By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IRB/HRPO procedures, the Declaration of Helsinki, ICH Good Clinical Practices guidelines, and the applicable parts of the United States Code of Federal Regulations or local regulations governing the conduct of clinical studies.	

Study Schema:



Patients with progressive disease after two cycles (defined as a clear increase in bone marrow blast count, typically a > 50% increase in bone marrow blasts over baseline) should be removed from study.

Patients with stable disease may continue on study and should undergo a diagnostic bone marrow biopsy at the end of even numbered cycles.

Once AML patients achieve morphologic complete remission with incomplete blood count recovery (CRi) or MDS patients achieve a marrow complete response (Marrow CR) as defined in Section 9.0, decitabine administration may be decreased to 20 mg/m2/day for ten consecutive days every 6 weeks, to limit myelosuppressive toxicity and improve count recovery. After 2 cycles at 6 week intervals, the dose may be reduce to 20 mg/m2/day for five consecutive days in 6 week cycles, at the discretion of the treating physcian. After two cycles of five consecutive days of treatment, if the patient meets the definition of CR with complete cytogenetic response as defined in Section 9.0, administration may further be decreased to three consecutive days per cycle at the discretion of the treating physician.

Sample collection for correlative studies:

Bone marrow biopsies should be performed at baseline, at Cycle 1 Day 10 ± 1, and at Day 28 ± 4 of Cycles 1, 2, 4, and 6. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression.

Genomic Predictors of Decitabine Response in AML/MDS

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1.0 INTRODUCTION

1.1 Rationale

Personalized therapy for cancer requires that 1) patient-specific factors (e.g. patient-specific mutations, expression profiles, or pharmacogenomics) influence treatment algorithms, and 2) once therapy is initiated, *in vivo* responsiveness is used to guide further therapy. The modern era of genomics has provided significant insights into cancer pathogenesis by describing patterns of germline and somatic variants, as well as defining disease-specific patterns of gene expression. However, the application and integration of this data with therapeutics remains a difficult task.

[redacted]

AML and MDS are two closely related hematological disorders characterized by ineffective hematopoiesis and malignant expansion of clonal myeloid cells. Both AML and MDS frequently occur in elderly patients and some cases are secondary and/or related to long-term bone marrow damage after radiation or chemotherapy. Current therapy for AML consists of cytotoxic chemotherapy followed by consolidation chemotherapy or stem cell transplantation. Using these approaches, current cure rates may be as high as 60% for patients younger than 60 with good risk cytogenetics (e.g. t(15;17), t(8;21), inv(16)).[4] Outcomes in patients ≥ 60 years old and with relapsed disease remain poor with 2 year survival of only ~25%.[5] Historically, the available treatment for MDS has been symptomatic and has not been shown to be effective in producing sustained improvement in hematopoiesis or in delaying leukemic evolution[6, 7].

Current AML therapy is poorly tolerated in elderly and pre-treated patients due to hematologic and non-hematologic toxicities; following such therapy, patients typically require 3-4 weeks of hospitalization for treatment of neutropenic fevers and for regular transfusions. Even among the relatively healthy patients enrolled in clinical trials, induction mortality in the elderly can be as high as 20-40% following standard induction therapy,[8-11] and moderate to severe non-hematologic toxicity occurs in at least an additional 30% of patients.[9, 12-14] Consequently, many older AML patients are not offered traditional intensive chemotherapy, but receive supportive care alone.[15] More effective and less toxic therapies are needed to provide durable remissions in a significant proportion of elderly AML in populations.

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We and others have recently used whole genome sequencing to identify recurrent, somatic mutations in AML and MDS patients in four genes that are involved in methylcytosine regulation: *DNMT3A, IDH1, IDH2,* and *TET2.*[29-37] Collectively, nearly half of all AML/MDS patients have a mutation in one of these four genes. *DNMT3A* mutations are associated with an adverse outcome in AML patients treated with cytotoxic chemotherapy.[35, 38] However, it is not yet clear whether the presence of mutations in one or more of these genes is associated with response or resistance to decitabine.

In this study, we will treat patients with the current state-of-the-art decitabine schedule.[3] We will perform exome sequencing on all patients and correlate response with mutation status, rate of mutation clearance, expression profile, plasma decitabine levels, and DNA

methylcytosine content reduction. This approach will comprehensively assess genomic and pharmacologic parameters that could clinically predict patient response to decitabine.

In this study, we will combine analysis of patients with AML and MDS. Recent genomic analysis has shown that AML and MDS have shared mutations in many of the same genes, shared patterns of mutation acquisition, and shared patterns of mutation evolution.[39, 40] Furthermore, MDS, like AML, presents with clonal hematopoiesis, even when the morphologic blast count is zero.[36]Because this study will identify and track the rate of clearance of patient specific mutations, and because decitabine has been shown to have efficacy in both AML and MDS patients, [2, 3, 16-19, 41-45] combining analysis of AML and MDS patients is reasonable. Asymptomatic MDS patients are excluded because these patients are typically followed with watchful-waiting rather than with chemotherapy.

1.2 Decitabine and Hypomethylating agents in AML/MDS

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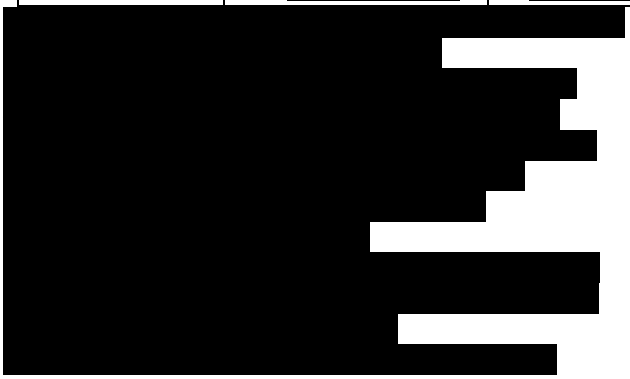

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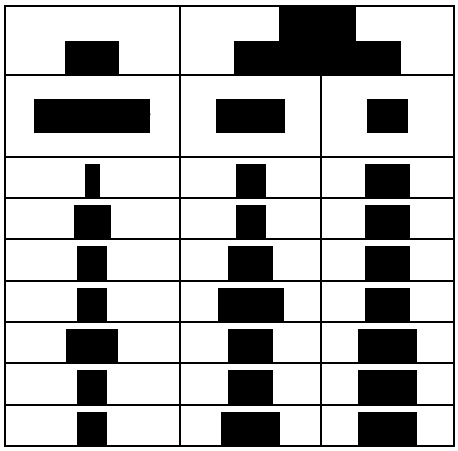

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1.3 Correlative Studies

All correlative studies will be collected under HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Diseases") that all patients will be concurrently enrolled in. The goals of the correlative studies include:

1. To correlate patient-specific mutations with response to decitabine.
   We will perform exome sequencing of each patient's pre-study bone marrow sample and skin sample. We will determine AML/MDS-associated variants found in the bone marrow, but not skin sample. We will correlate the presence of mutations with response (especially mutations in *DNMT3A, IDH1, IDH2,* and *TET2*). This study is powered to identify mutations with >80% sensitivity to predict decitabine response if used as a clinical assay.

2. To correlate the velocity and depth of response with response to decitabine.
   We will assess tumor burden by allele frequency of patient-specific mutations in bone marrow samples collected pre study, Cycle 1 Day 10±1, Cycle 1 Day 28±4, and Cycle 2 Day 28±4. We will assess mutations in at least 24 recurrently mutated genes (including, but not limited to: *FLT3, DNMT3A, NPM1, TET2, WT1, IDH1, IDH2, RUNX1, NRAS, TP53, U2AF1, KRAS, PHF6, MSTP9, PTPN11, KIT, SMC3, PRUNE2, ETV6, CEBPA, SMC1A, ASXL1, STAG2, RAD21*) as well as all patient-specific mutations discovered during exome sequencing. The leukemic allele frequency will be measured using the Illumnia MySeq or HiSeq platform using DNA capture by gene specific biotinylated-oligonucleotides. Patient bone marrow samples will be assessed pre-study, Cycle 1 Day 10 ± 1, Cycle 1 Day 28 ± 4, and Cycle 2 Day 28 ± 4 and compared with patient specific skin samples (normal control). The velocity of response will be calculated as the natural log rate of patient-specific mutation allele frequency clearance. Depth of response will be defined as the lowest patient-specific mutation

allele frequency in a bone marrow sample. The velocity and depth of tumor response will be correlated to:

- Achievement of clinical CR,
- Time to best response,
- Mutational status,
- Time to disease progression.

Because all patient-specific exome-discovered mutations will be assessed a second time in the primary bone marrow sample as well as the Cycle 1 Day 10 ± 1, Cycle 1 Day 28 ± 4, and Cycle 2 Day 28 ± 4 bone marrow samples, this study will also serve to validate all exome-discovered mutations. This is important, because exome sequencing is associated with a 20-50% false-discovery rate depending on type I error tolerated (mutation-calling algorithms are typically set for greater sensitivity than specificity). Thus, many mutations discovered during exome sequencing are incorrect. This second analysis of these mutations will server to exclude these false positive calls.

3. To correlate the bone marrow expression profile from pre-study and Cycle 1 Day 10 ± 1 with response to decitabine.
   We will correlate expression signatures, and changes in expression signatures, with:
   - Achievement of clinical CR,
   - Mutation status.

4. To correlate the extent of bone marrow DNA hypomethylation on Cycle 1 Day 10 ± 1 with response to decitabine.
   We will assess the percent reduction in bone marrow methylcytosine content using LC-MS. Patients will be evaluated pre-study and Cycle 1 Day 10 ± 1. The change in DNA methylcytosine content will be correlated to:
   - Achievement of clinical CR,
   - Change in expression signature.

   This will determine whether inadequate blast exposure to decitabine correlates with drug resistance.

2.0 OBJECTIVES

2.1 Primary Objective

To determine whether patient-specific mutations correlate with clinical overall response to decitabine in patients with AML or MDS.

2.2 Secondary Objectives

- To determine whether 10 consecutive days per cycle of decitabine improves outcomes compared with historical controls treated with 5 consecutive days per cycle of decitabine.

- To determine whether other biomarkers correlate with clinical overall response to decitabine in patients with AML or MDS. These will include:

    1. The velocity and depth of patient-specific mutation clearance;
    2. The bone marrow expression profile and change in expression profile during decitabine treatment;
    3. The decrease in bone marrow methylcytosine content in response to decitabine.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

One of the following:

- Patient must have non-M3 AML and be ≥ 60 years of age OR
- non-M3 AML with relapsed disease OR
- Symptomatic MDS with one of the following:
  - □ Symptomatic anemia with either hemoglobin < 10.0 g/dL or requiring RBC transfusion
  - □ Thrombocytopenia with a history of two or more platelet counts < 50,000/mcL or a significant hemorrhage requiring platelet transfusions
  - □ Neutropenia with two or more ANC < 1,000/mcL

All of the following:

1. Patient must have an ECOG performance status ≤ 2.
2. Patient must have >10% disease burden measured by cytomorphology, flow cytometry, or cytogenetics.
3. Patient must have peripheral white blood cell count ≤ 50,000/mcl.
4. Patient must have adequate organ function, defined as:
   a. Total bilirubin ≤ 1.5 x ULN
   b. AST/ALT ≤ 2.5 x ULN
   c. Serum creatinine ≤ 2.0 x ULN
5. Patient must have undergone ≤ 2 cycles of prior hypomethylating agent (decitabine or azacitidine).
6. Patient must be enrolled in HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Diseases").
7. Patient must be ≥ 18 years of age.
8. Patient must be able to understand and willing to sign an IRB-approved written informed consent document.

3.2 Exclusion Criteria

1. Patient must not be pregnant or nursing.
2. Patient must not have known CNS leukemia.
3. Patient must not have a history of positive HIV serology.
4. Patient must not have a history of positive Hepatitis C serology.
5. Patient must not have undergone a prior allogeneic stem cell transplant.
6. Patient must not have any uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, ongoing or active GVHD, congestive heart failure of NYHA class 3 or 4, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situation that would limit compliance with study requirements.
7. Patient must not have had radiation therapy within 14 days of enrollment.
8. Patient must not have received any chemotherapy within 21 days of enrollment, and any acute treatment-related toxicities must have returned to baseline. Patients

may be receiving hydrea at time of enrollment.

3.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4.0 REGISTRATION PROCEDURES

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility by Washington University
2. Registration of patient in the Siteman Cancer Center database
3. Assignment of unique patient number (UPN)

Once the patient has been entered in the Siteman Cancer Center database, the WUSM coordinator will forward verification of enrollment and the UPN via email.

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility collecting the information listed below and scanning and emailing it to the research coordinator listed in the *Siteman Cancer Center Clinical Trials Core Protocol Procedures for Secondary Sites* packet at least one business day prior to registering patient:

1. Your name and contact information (telephone number, fax number, and email address)
2. Your site PI's name, the registering MD's name, and your institution name
3. Patient's race, sex, and DOB
4. Three letters (or two letters and a dash) for the patient's initials
5. Currently approved protocol version date
6. Copy of signed consent form (patient name may be blacked out)
7. Planned date of enrollment
8. Completed eligibility checklist, signed and dated by a member of the study team
9. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center Database

Registrations may be submitted Monday through Friday between 8am and 5pm CT. Urgent late afternoon or early morning enrollments should be planned in advance and coordinated with the Washington University research coordinator. Registration will be confirmed by the research coordinator or his/her delegate by email within one business day. Verification of eligibility and registration should be kept in the patient chart.

All patients at all sites must be registered through the Siteman Cancer Center database at Washington University.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. Patients will also be identified by first, middle, and last initials. If the patient has no middle initial, a dash will be used on the case report forms (CRFs). All data will be recorded with this identification number on the appropriate CRFs.

5.0 INVESTIGATIONAL PLAN

5.1 Summary of Study Design

This is an open-label, single-arm, Phase II, biomarker study of patients with AML or MDS who are treated with 10 consecutive days of decitabine. FAB-M3 AML is excluded from this trial, because other well-tolerated options exist for the treatment of FAB-M3 acute promyelocytic leukemia. AML patients who are < 60 years old and have not received cytotoxic induction chemotherapy are also excluded, because cytotoxic therapy offers a curative possibility for these patients. MDS patients without cytopenias are also excluded because these patients can be followed with watchful-waiting.

All patients will undergo bone marrow biopsies pre-study, on Cycle 1 Day 10 ± 1, and on Day 28 ± 4 of Cycles 1, 2, 4, and 6. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression. Patients will provide a skin biopsy sample pre-study. See Section 8.0 for details on correlative studies.

Patients will receive decitabine 20 mg/m^2/day as a 1-hour infusion on consecutive Days 1-10 in 28-day cycles. The dose will be calculated based on actual patient weight at the beginning of each cycle.

Once AML patients achieve morphologic complete remission with incomplete blood count recovery (CRi) or MDS patients achieve a marrow complete response (Marrow CR) as defined in Section 9.0, decitabine administration may be decreased to 20 mg/m2/day for ten consecutive days every 6 weeks, to limit myelosuppressive toxicity and improve count recovery. After 2 cycles at 6 week intervals, the dose may be reduce to 20 mg/m2/day for five consecutive days in 6 week cycles, at the discretion of the treating physician. After two cycles of five consecutive days of treatment, if the patient meets the definition of CR with complete cytogenetic response as defined in Section 9.0, administration may further be decreased to three consecutive days per cycle at the discretion of the treating physician.

It will be a logistical goal of this trial consistently to return preliminary exome results to the treating physician by the end of Cycle 1 and validated results by the end of Cycle 2. However, this logistical goal is not a primary or secondary aim of this study. Given the limited data regarding mutation presence and drug response, we do not anticipate that the results will directly influence therapeutic decisions made by the treating physician. However, the development of comprehensive genomic pipelines and integration of this data into a clinical trial setting will be required before personalized medicine can be realized.

5.2 Study Procedures

5.2.1 Pre-study Procedures

Pre-study procedures must take place no more than 14 days prior to the first dose of decitabine except where noted below.

1. Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics, and FISH.
2. Bone marrow or peripheral blood for flow cytometry
3. Skin biopsy sample (collected under HRPO# 201011766). There is no required time frame for this sample; it may have been collected months or even years prior to the first dose of decitabine.

5.2.2 Baseline Evaluation

Baseline evaluations must take place no more than 7 days prior to the first dose of decitabine.

1. Medical history
2. Physical exam including height, weight, and ECOG performance score.
3. Record transfusion requirements for 8 weeks before first dose of study drug
4. CBC with differential and platelets
5. Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH
6. Urine βHCG pregnancy test if appropriate

5.2.3 Day 1 of Each Cycle

1. Physical exam, including weight
2. CBC with differential and platelets
3. Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH

5.2.4 Cycle 1 Days 1-10

Decitabine administration, 20 mg/m^2 per day given as a 1-hour continuous intravenous infusion. The dose will be calculated based on actual patient weight at the beginning of each cycle. Patients may be premedicated with lorazepam, prochlorperazine, palonosetron, or ondansetron at the treating physician's discretion.

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5.2.5 Cycle 1 Day 10 ± 1

Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics, and FISH.

5.2.6 Weekly

CBC with differential and platelets. After the first cycle, this may be changed to every other week at the discretion of the treating physician.

5.2.7 Every Other Week

Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH. After the first cycle, this may be changed to every month at the discretion of the treating physician.

5.2.8 Cycles 1, 2, 4 and 6 Day 28 ± 4 days

Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include: correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics, and FISH.

Patients who achieve with clinical response (CRi or better) and are receiving cycles every 6 weeks, will undergo bone marrow biopsies on day 42 ± 4.

5.2.9 End-of-Study Procedures

1. Physical exam
2. CBC with differential and platelets.
3. Serum chemistries including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, and LDH.
4. Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include morphologic assessment, cytogenetics, and FISH.

5.2.10 Post-study Follow-up

Patients are to be followed according to standard patient care procedures for 30 days following the date of the last decitabine dose to monitor for toxicity, survival,

and response. Thereafter, the investigator or designee will record information on subsequent therapy, disease progression, and/or death every 6 months for 2 years after last dose of study drug or until the patient is lost to follow-up or dies.

5.3 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented in the case report forms.

In the absence of treatment delays due to adverse events, treatment may continue indefinitely or until one of the following criteria applies:

- Documented and confirmed disease progression
- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious non-compliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study

Patients who prematurely discontinue treatment for any reason will be followed as indicated in the study calendar.

5.4 Concomitant Therapy and Supportive Care Guidelines

5.4.1 Chemotherapy

Patients cannot receive any chemotherapy within 21 days of study enrollment and must have recovered from any treatment-related toxicities. However, patients may be receiving hydrea at enrollment and may continue on hydrea through Cycle 2 of decitabine. An indication for hydrea following 2 cycles of decitabine is evidence that the patient is not responding and decitabine should be discontinued. Other than decitabine, patients cannot receive chemotherapy during this trial.

5.4.2 Growth Factors

Erythroid growth factors should not be routinely administered to patients during the study.

Thrombopoietin receptor agonists should not be routinely administered to patients during the study.

Filgrastim (G-CSF) or sargramostim (GM-CSF) may be administered to patients with septic shock or febrile neutropenia at the discretion of the treating physician.

5.4.3 Transfusions

- *Packed red blood cells (RBCs) transfusion*:
  RBC transfusions will be administered at the discretion of the treating physician.

- *Platelet transfusion*:
  Platelet transfusions will be administered at the discretion of the treating physician

5.4.4 Prophylactic Antimicrobial Agents

Antimicrobial agents will be administered at the discretion of the treating physician. Common antimicrobial prophylaxis during cycles 1 and 2 include acyclovir, ciprofloxacin, and fluconazole.

5.4.5 Radiotherapy

Patients cannot receive radiotherapy within 14 days prior to study enrollment or while on study.

6.0 DOSE MODIFICATIONS

A criterion for discontinuation of the study is a delay of > 8 weeks between any two cycles.

6.1 Delay for Cytopenia or Infection

As cytopenias are a defining feature of AML and MDS, their presence alone does not generally warrant dose modifications or delays. Patients will receive four cycles of therapy without adjustment for any cytopenia. Following Cycle 4, dose modifications or delays for cytopenias will be made at the discretion of the investigator.

Patients with febrile neutropenia (temperature ≥ 38.5° with ANC < 1,000/μl) or systemic infections should have treatment held until clinical resolution of the infection.

6.2 Delay for Organ Dysfunction

If renal dysfunction (creatinine ≥ 3 X institutional upper limit of normal) or hepatic dysfunction (bilirubin ≥ 2.5 mg/dl or ALT/AST ≥ 3 X institutional upper limit of normal) occurs, therapy may not resume until the abnormalities have resolved to below the limits stated above. If renal or hepatic dysfunction occurs during a treatment course, the next dose of decitabine should be held, and serum chemistries should be checked twice per week. Decitabine can be resumed at full dose when creatinine, bilirubin, ALT and AST are below the limits listed above.

For grade 3-4 non-hematologic toxicities related to decitabine, decitabine treatment should be delayed until recovery to baseline or to grade ≤ 1. Decitabine may be reintroduced with a 50% dose reduction, or at full dose at the discretion of the investigator.

6.3 Modification in Dose or Schedule for Treatment Response

Once AML patients achieve morphologic complete remission with incomplete blood count recovery (CRi) or MDS patients achieve a marrow complete response (Marrow CR) as defined in Section 9.0, decitabine administration may be decreased to 20 mg/m2/day for ten consecutive days, and the cycles may be administered every 6 weeks to limit myelosuppressive toxicity, at the discretion of the treating physician. After two cycles at six week intervals, the dose may be reduced to 20 mg/m2/day for five consecutive days of treatment in six week intervals. If the patient meets the definition of CR with complete cytogenetic response as defined in Section 9.0, administration may further be decreased to three consecutive days per cycle at the discretion of the treating physician.

7.0 PHARMACEUTICAL INFORMATION

[redacted]

8.0 CORRELATIVE STUDIES SAMPLE COLLECTIONS

Bone marrow samples will be collected pre-study, on Cycle 1 Day 10 ± 1, and on Day 28 ± 4 of Cycle 1, 2, 4, and 6 for correlative studies. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression. The bone marrow samples will be collected under HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Diseases") that all patients will be concurrently enrolled in.

Skin biopsy sample will be collected as part of the patient's participation in HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Malignancies"). There is no required time frame for this sample, and it may have been collected months or even years prior to the first dose of decitabine.

9.0 EFFICACY, SAFETY AND CORRELATIVE STUDY MEASURES

A secondary endpoint of this study is to estimate morphologic complete remission rate.

9.1 Response Criteria - AML

AML Patients will be assessed for response according to the IWG criteria:[1]

Morphologic complete remission (CR) – Defined as morphologic leukemia-free state, defined as less than 5% blasts in aspirate with marrow spicules and a count of ≥200 nucleated cells and no blasts with Auer rods or any persistence of extramedullary disease. When erythroid precursors constitute less than 50% of bone marrow nucleated cells, the percentage of blasts is based on all nucleated cells; when there are 50% or more erythroid cells, the percentage blasts should be based on the nonerythroid cells. The presence of mild megaloblastoid changes may be permitted if they are thought to be consistent with treatment effect. However, persistence of pretreatment abnormalities (eg, pseudo-Pelger-Hüet cells, ringed sideroblasts, dysplastic megakaryocytes) are not consistent with CR. In addition, ANC >1000/µl and platelet count of ≥100,000/µl. Patient must be independent of transfusions. No duration requirement for this designation.

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

Morphologic complete remission with incomplete blood count recovery (CRi) – Defined as morphologic complete recovery with the exception of neutropenia <1000/µl or thrombocytopenia <100,000/µl.

Partial remission (PR) – Defined as meeting the above criteria for complete remission, with the following exceptions: decrease of ≥50% in the percentage of blast to 5-25% in the bone marrow aspirate. A value of ≤5% blasts in bone marrow with Auer rods present may also be considered a partial remission.

Stable Disease (SD) – Defined as not meeting the definitions of CR, CRi, PR, PD, or recurrence/morphologic relapse.

Progressive disease (PD) – Defined as a clear increase in bone marrow blast count, typically a greater than 50% increase compared with baseline, or new extramedullary disease. Patients with a > 50% increase in peripheral blast count with an increase of the total peripheral white blood cell count to > 10,000/µl should undergo evaluation by bone marrow biopsy to access for progressive disease.

Recurrence/morphologic relapse **-** Defined as relapse following 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. New dysplastic changes is considered relapse.

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.

9.2 Response Criteria - MDS

MDS Patients will be assessed for response according to the IWG criteria:[69]

Complete remission (CR) – Defined as ≤ 5% myeloblasts with normal maturation of all cell lines in the bone marrow and peripheral blood values of Hgb ≥ 11g/dL, Platelets ≥ 100 x 10^9/L, Neutrophils ≥ 1.0 x 10^9/L, and 0% blasts. Persistent dysplasia does not exclude CR but will be noted.

Marrow Complete Response (Marrow CR) – Defined as ≤ 5% myeloblasts in the bone marrow and a decrease by ≥ 50% from pretreatment values, but not meeting the definition of CR above.

Partial Remission (PR) – Defined as meeting the definition of CR above with a decrease of myeloeblasts in the bone marrow by ≥ 50% from pretreatment values, but absolute myeloblasts still >5%.

Stable Disease (SD) – Defined as not meeting the definitions of CR, Marrow CR, PR, SD, PD, or recurrence/morphologic relapse.

Progressive Disease//Relapse (PD) – Defined as ≥ 50% increase in blasts to > 5% blasts (for patients with less than 5% blasts at baseline only), ≥ 50% increase to > 10% blasts (for patients with 5-10% blasts at baseline only), ≥ 50% increase to > 20% blasts (for patients with l0-20% blasts at baseline only), ≥ 50% increase to > 30% blasts (for patients with 20-30% blasts at baseline only) or any of the following: At least 50% decrement from maximum remission/response in granulocytes or platelets, reduction in Hgb by ≥ 2 g/dL, or New or worsened transfusion dependence not related to study drug toxicity. Or for patients with a CR, Marrow CR, or PR as defined above and subsequently development of one of the following: Return to pretreatment bone marrow blast percentage, decrement of ≥ 50% from maximum remission/response levels in granulocytes or platelets, or reduction in Hgb concentration by ≥ 1.5 g/dL or transfusion dependence.

9.2.1 Hematologic Improvement (HI)

Progressive disease as defined above nullifies hematologic improvement.

Erythroid response requires all of the following (only required if pretreatment HgB <11 g/dL):

- Hgb increase by ≥ 1.5 g/dL
- Relevant reduction of units of RBC transfusions by an absolute number of at least 4 RBC transfusions/8 wk compared with the pretreatment transfusion number in the previous 8 wk. Only RBC transfusions given for a Hgb of ≤ 9.0 g/dL pretreatment will count in the RBC transfusion response evaluation

Platelet response requires one of the following (only required if pretreatment platelets < 100 x 10^9/L):

- Absolute increase of ≥ 30 x 10^9/L (for patients starting with > 20 x 10^9/L platelets)
- Increase from < 20 x 10^9/L to > 20 x 10^9/L and absolute increase ≥ 100% (for patients starting with < 20 x 10^9/L)

Neutrophil response requires the following (only required if pretreatment ANC <1.0 x 10^9/L):

- At least 100% increase and an absolute increase > 0.5 x 10^9/L

9.3.1 Cytogenetic Response

Complete Cytogenetic Response – Defined as reversion to a normal karyotype. For this study, reversion of a normal karyotype is defined as no clonal abnormalities detected in a minimum of 20 mitotic cells. Progressive disease as defined above nullifies cytogenetic response.

9.3 Guidelines for Evaluation of Disease

Bone marrow biopsy should be performed on Day 28 ± 4 of cycles 1, 2, 4 and 6 for clinical disease assessment. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression.

9.4 Other Secondary Efficacy Measures

9.4.1 Treatment Failure, Overall Survival and Event-free Survival

Treatment failure is defined as failure to achieve a complete or partial remission following 4 cycles of therapy.

Overall survival is defined as the date of first dose of study drug to the date of death from any cause. Patients still alive at the time of the final analysis will be right-censored.

Event-free survival is defined as the interval from the date of first dose of study drug to date of treatment failure, recurrence, death due to any cause, or loss to follow up.

9.4.2 Duration of Remission and Relapse-free Survival for Patients Having Complete Remission

Duration of complete remission is defined as the interval from the date that complete remission is documented to the date of recurrence, death due to any cause,

or loss 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.

9.4.3 Safety

Safety evaluation will include assessments of adverse experiences, medical history, physical examinations, vital signs, and laboratory assessments at baseline and throughout the study period. Local laboratories may be used for this study.

10.0 ADVERSE EVENT REPORTING

10.1 Adverse Events (AEs)

Definition: any unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site: http://ctep.cancer.gov/reporting/ctc.html. Grade 1-2 events will be recorded for the first fifty enrolled patients. Subsequently, only events graded as 3 or greater will be recorded. Fifty patients should be sufficient to estimate the true incidence of any adverse event to within ± 14% with 95% confidence intervals.
Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website: (http://www.hhs.gov/ohrp/policy/AdvEvntGuid.htm).

10.2 Unanticipated Problems

Definition:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

10.3 Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

10.4 Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

10.5 Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

Local IRB Pre-approval of all protocol exceptions must be obtained prior to the event. For secondary sites, the Washington University PI will issue approval of the exception, but it must also be submitted to the local IRB with documentation of approval forwarded to Washington University. HRPO approval is not required for protocol exceptions occurring at secondary sites.

10.6 Reporting to the Human Research Protection Office (HRPO) and the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University:

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH or SLCH institution, or that impacts participants or the conduct of the study.
- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within **10 working days** of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification to the PI of the event.

10.7 Reporting Requirements for Secondary Sites

The research team at each secondary site is required to promptly notify the Washington University PI and research coordinator of all reportable events (as described in Section 7.6) within **1 working day** of the occurrence of the event or notification of the secondary site's PI of the event. This notification may take place via email if there is not yet enough information for a formal written report (using either an FDA MedWatch form if required or an institutional SAE reporting form if not). A formal written report must be sent to the Washington University PI and research coordinator within **10 working days** of the occurrence of the event or notification of the secondary site's PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification of the secondary site's PI of the event.

The research team at a secondary site is responsible for following its site's guidelines for reporting applicable events to its site's IRB according to its own institutional guidelines.

10.8 Reporting to Secondary Sites

The Washington University PI (or designee) will notify the research team at each secondary site of all reportable events that have occurred at other sites (as described in Section 7.6) within **10 working days** of the occurrence of the event or notification of the PI of the event. This includes events that take place both at Washington University and at other secondary sites, if applicable.

10.9 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. **PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC.** It is the responsibility of the investigator to report any unanticipated problem to the FDA as follows:

- Report any unexpected fatal or life-threatening adverse experiences associated with use of the drug (i.e., there is a reasonable possibility that the experience may have been caused by the drug) by telephone or fax no later than **7 calendar days** after initial receipt of the information. A life-threatening adverse experience is defined as any adverse drug experience that places the subject (in the view of the investigator) at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Report any serious, unexpected adverse experiences, as well as results from animal studies that suggest significant clinical risk within **15 calendar days** after initial receipt of this information. A serious adverse drug experience is defined as any adverse drug experience occurring at any dose that results in any of the following outcomes:
    - Death
    - A life-threatening adverse drug experience
    - Inpatient hospitalization or prolongation of existing hospitalization
    - A persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions)
    - A congenital anomaly/birth defect
    - Any other experience which, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

  An unexpected adverse drug experience is defined as any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure (or risk information, if an IB is not required or available).

All MedWatch forms will be sent by the investigator or investigator's team to the FDA at the following address or by fax:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Oncology Drug Products
5901-B Ammendale Rd.
Beltsville, MD 20705-1266
FAX: 1-800-FDA-0178

Secondary sites must submit a completed MedWatch form to the Washington University PI and research coordinator within **4 calendar days** (for fatal or life-threatening adverse experiences) or **11 calendar days** (for serious, unexpected adverse experiences). The Washington University PI will be responsible for submitting all MedWatch forms from secondary sites to the FDA within the timeframes specified above.

10.10 Timeframe for Tracking Reportable Events:

Reportable adverse events will be tracked for 30 days following the last day of study treatment, or until the start of a new chemotherapy/treatment, whichever comes first.

11.0 DATA SAFETY MONITORING PLAN

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months following activation of the first secondary site.

The Principal Investigator will review all patient data at least every six months, and provide a semi-annual report to the QASMC. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual including numbers from participating sites
- Protocol activation date at each participating site
- Average rate of accrual observed in year 1, year 2, and subsequent years at each participating site
- Expected accrual end date and accrual by site
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities at all participating sites
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

Until such a time as the first secondary site activates this protocol, a semi-annual DSM report to be prepared by the study team will be submitted to the QASM Committee beginning 6 months after study activation at Washington University.

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

12.0 AUDITING

Since Washington University is the coordinating center, each site will be audited annually by Siteman Cancer Center personnel (QASMC) unless the outside institution has an auditing mechanism in place and can provide a report. The outside sites will be asked to send copies of all audit materials, including source documentation. The audit notification will be sent to the Washington University Research Patient Coordinator, who will obtain the audit materials from the participating institution.

Notification of an upcoming audit will be sent to the research team one month ahead of the audit. Once accrual numbers are confirmed, and approximately 30 days prior to the audit, a list of the cases selected for review (up to 10 for each site) will be sent to the research team. However, if during the audit the need arises to review cases not initially selected, the research team will be asked to provide the additional charts within two working days.

Additional details regarding the Auditing Policies and procedures can be found at http://www.siteman.wustl.edu/uploadedFiles/Research_Programs/Clinical_Research_Resources/Protocol_Review_and_Monitoring_Committee/QASMCQualityAssurcance.pdf

13.0 MULTICENTER REGULATORY REQUIREMENTS

Washington University requires that each participating site sends its informed consent document to be reviewed and approved by the Washington University Regulatory Coordinator (or designee) prior to IRB/IEC submission.

Each participating institution must have the following documents on file at Washington University prior to first subject enrollment:

- Documentation of IRB approval of the study in the form of a letter or other official document from the participating institution's IRB. This documentation must show which version of the protocol was approved by the IRB.
- Documentation of IRB approval of an informed consent form. The consent must include a statement that data will be shared with Washington University, including the Quality Assurance and Safety Monitoring Committee (QASMC), the DSMC (if applicable), and the Washington University study team.
- Documentation of FWA, signed FDA Form 1572, and signed and dated CVs of all participating investigators.
- Documentation of training in protection of human subjects by all participating investigators.
- Protocol signature page signed and dated by the investigator at each participating site.

The Principal Investigator is responsible for disseminating to the participating sites all study updates, amendments, reportable adverse events, etc. There will be one current version of the protocol document at any given time and each participating institution will utilize that document. Protocol/consent modifications and IB updates will be forwarded electronically to the secondary sites within 2 weeks of obtaining Washington University IRB approval with acknowledgement of receipt requested. Secondary sites are to submit protocol/consent/IB modifications to their local IRBs within 4 weeks of receipt, and confirmation of submission must be forwarded to the appropriate contact person on the Washington University study team at the time of submission. Upon the secondary sites obtaining local IRB approval, documentation of such shall be sent to the Washington University study team within 2 weeks of receipt of approval.

Documentation of participating sites' IRB approval of annual continuing reviews, protocol amendments or revisions, all SAE reports, and all protocol violations/deviations/exceptions must be kept on file at Washington University.

The investigator or a designee from each institution must participate in a regular conference call to update and inform regarding the progress of the trial.

14.0 STATISTICAL ANALYSIS METHODS AND SAMPLE SIZE

14.1 Analysis Populations

Intent-to-Treat - The intent-to-treat (ITT) population will be defined as all patients who receive at least one dose of decitabine.

Efficacy-Evaluable - The efficacy-evaluable (EE) population will be defined as all patients who receive at least one cycle of treatment.

Safety - The safety population will be defined as all patients with at least one dose of decitabine.

14.2 Statistical Analysis

14.2.1 Descriptive Analyses

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

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.

14.2.2 Primary Endpoint Analysis

The primary end-point of this phase II study is the correlation biomarkers with overall response in AML/MDS patients to decitabine.

We will correlate the presence of patient specific mutations (especially mutations in *DNMT3A, IDH1, IDH2,* and *TET2*) with overall response rates. Mutation rates in these genes and in other patient-specific genes will be correlated with overall response and their respective 95% confidence intervals will be provided.

Statistical considerations. The relationship between AML/MDS-associated mutations and the responsiveness to decitabine will be described by contingency tables and assessed using Chi-square test or Fisher's exact test as appropriate. The differentially expressed genes between responders and non-responders will be determined using the significance analysis of microarray (SAM). Multivariate logistic regression will also be fit to adjust the potential confounding effects of other demographic and clinical characteristics. The resultant critical *P* values will be corrected for multiple testing. Since the Bonferroni correction is known to be too conservative, a permutation analysis (e.g., 10,000 random assignments for

responsive status) will be used to derive an empirical critical *P* value. The new critical p-value is the number of permutation tests which yield a lower p-value than the original test divided by the total number of permutation tests.[61]

A power analysis was performed using Chi-square test for the association between mutation and response to decitabine. The frequency of *DNMT3A, IDH1/2,* and *TET2* mutations in AML and MDS patients are each between 15% and 30% in multiple cohorts.[29-37] **Table 3** shows the minimal detectable differences of response rates between mutant and wild-type cases, assuming an expected overall 45% CR/CRi rate and 80% power. For an average of 20% frequency of a mutated gene, for example, 100 patients will provide 80% power to detect 30% difference at a 2-sided 0.1 alpha error. The purpose of this study is to determine whether any of these mutations result in a clinically significant association that would warrant pre-treatment testing. We believe that a reasonable cut off would be if at least 30% of clinical response could be attributed to the presence of a specific mutation. However, we anticipate that more power could be achieved as we assess patient groups with combined characteristics (e.g. *DNMT3A* mutation or loss of *DNMT3A* expression). In addition, we expect that the majority of the enrolled 125 patients can be used in the analysis (since all of our molecular information will be acquired within 30 days of enrollment, we anticipate a drop-out rate of 20% or less).

Table 3. Detectable mutation associated response rates (80% power, $\alpha = 0.05$)

Total N	% of patients with a given mutation	Detectable response difference	Sensitivity of mutation to predict response at minimum detectable response difference*
100	15%	0.40	80%
	20%	0.35	75%
	30%	0.30	67%
	40%	0.27	61%
	50%	0.26	58%

* Assuming the overall response rate (CR/Cri) of 45%

14.2.3 Secondary Endpoint Analyses

The overall response rate (CR/CRi/PR) and complete response rate (CR/CRi) will be compared with historical controls[2], and the 95% confidence intervals will be provided.

The rate of patient specific mutation clearance after 10, 28, and 56 days of therapy will be determined using an Illumina based platform. We will compare the rate of mutation clearance between the patients who achieve a CR/CRi after 4 cycles and those who do not. We anticipate that we will identify between 6 and 25 exonic SNVs per case (the range obtained with >200 AML samples sequenced to date with whole genome or exome sequencing), and that the expected 95% confidence interval of the mean variant allele frequency will be ±2.56% and ±1.25%,

respectively, if we assume an anticipated standard deviation of 3.2% within the same clone. The change of tumor burden over time and its association with clinical response will be assessed using 2-way ANOVA for repeated measurement data, followed by the ad-hoc multiple comparisons for between-group differences (e.g., responders vs. non-responders) at given time points as well as the between-time differences within each group. The resultant critical *P* values will also be corrected using permutation analysis for multiple testing.

The percent decrease in bone marrow methylcytosine will be calculated on day 10±1. We will compare this reduction with previously published data and between the patients who achieve CR/CRi and those who do not. The correlation of peak plasma levels of decitabine with response will be assessed using 2-sample t-test or Mann-Whitney non-parametric rank-sum test as appropriate. The change of total bone marrow DNA methylcytosine from baseline and its association with both steady-state plasma drug levels and response will be assessed using 2-way ANOVA for repeated measurement data, followed by the ad-hoc multiple comparisons for between-group differences (e.g., responders vs. non-responders) and between-time differences. Similar analyses will be performed for the correlation of expression profiles and SNP analysis with pharmacokinetic data.

Statistical power justification. A power analysis was performed using two-sample t-test for between-group difference and paired t-test for the change over time. For tumor clearance, this will provide > 99% power to detect a 10% difference in the means at two separate time points for clones with 6 or more variants, and > 98% power to detect a 15% difference if there are 3 or more variants per clone with $\alpha = 0.05$ (power will decrease to 91% and 82%, respectively, in cases where the standard deviation is 4.7%, the maximum we have observed thus far). For pharmacologic correlations, we anticipate 85% power to determine a 25 ng/ml difference between the average C_{max} of 45 evaluable responders and 55 evaluable non-responders with an anticipated average C_{max} of 70 ng/ml and a standard deviation of 40 ng/ml within the entire cohort, and with $\alpha = 0.05$.[22] We believe a less significant difference would not be sufficient to warrant application in clinical decision making (i.e. whether to continue therapy for more than 1 cycle). We anticipate 99% power to determine a difference of 20% in the reduction of bone marrow methylcytosine between 45 evaluable responders and 55 evaluable non-responders with an expected standard deviation of 15% and $\alpha = 0.05$.[28, 66-68] The difference in methylcytosine reduction previously observed was 30% between patients achieving CR/PR and those with only hematologic improvement.[28]

Time to relapse and time to death will also be listed for each patient.

14.2.4 Safety Analysis

Extent of Exposure – Duration (number of cycles/infusions) of decitabine doses (cumulative and intensity) will be summarized.

Adverse Events - All adverse events will be coded using NCI CTCAE v. 4.0. 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. In addition, adverse events will be summarized by investigator attribution of relationship to study medication and by grade. Similar summaries will be provided by treatment cycle.

Clinical Laboratory Evaluation – Applicable laboratory parameters will be graded according to NCI CTCAE version 4.0. The incidence of maximum grade (number and percent of patients experiencing the maximum grade during the study) will be summarized for each laboratory parameter. Similar summaries will be provided by treatment cycle. Graphical displays of select hematological parameters will also be provided.

Vital Signs, Physical Findings, and Other Safety Observations – Vital signs, findings from physical examinations, ECOG scores and weights will be presented in listing format.

14.2.5 Description of Planned Subgroup Analyses

The following subgroups (if sufficient numbers of patients) will be explored with respect to the primary and overall survival endpoints.

- Age (60-69 years old vs ≥ 70 years old)
- Cytogenetics
- Patients with AML-associated mutations in *DNMT3A, TET2, IDH1,* or *IDH2* vs those who do not have somatic variants in these genes.

14.3 Sample Size

We anticipate initially enrolling 25 patients at Washington University. Once we establish a routine exome analysis with validation and calculation of the rate of patient-specific mutation clearance, we will enroll an additional 100 patients from at least 2 additional centers.

15.0 DATA SUBMISSION SCHEDULE

Case Report Form	Submission Schedule
Original Consent Form	*Prior to registration*
Patient Registration Form *Disease Diagnosis Form* *Inclusion Criteria Worksheet* *Exclusion Criteria Worksheet*	*Prior to starting treatment*
Treatment Record Form	*To be maintained throughout a patient's treatment cycle. The complete record is due at completion of treatment.*
Bone Marrow Sample Collection Form	*To be maintained throughout the course of the study at designated bone marrow sample collections time points (See Appendix 2).*
Pathology: Bone Marrow Biopsy	*To be completed upon the return of pathology report for each bone marrow biopsy.*
Study Calendar Form	*To be completed with the date of each time point upon occurrence.*
Labs Form	*To be completed upon the return of laboratory reports for each study time point (See Appendix 2)*
Dose Modifications	*To be maintained throughout the course of the study if dose modifications are performed.*
Therapy Response Form	*To be filled out upon remission (prior to, and/or on therapy)*
End of Study Form	*Due at the completion of the EOS visit, at the time of death, and/or at the time that the patient is lost to follow-up*
Follow Up Form	• *30 days post date of last decitabine dose.* • *Every 6 months for 2 years after the last dose of study drug.*
Record of Adverse Events	*At the time of any AE*

Any queries generated by Washington University must be responded to within 28 days of receipt by the participating site. The Washington University research team will conduct a regular review of data status at all secondary sites, with appropriate corrective action to be requested as needed.

Appendix 1 ECOG Performance Scale

Activity Status	Description
0	Asymptomatic, fully active, and able to carry on all predisease performance without restrictions.
1	Symptomatic, fully ambulatory but restricted in physically strenuous activity and able to carry out performance of a light or sedentary nature, eg, light housework, office work.
2	Symptomatic, ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours: in bed less than 50% of day.
3	Symptomatic, capable of only limited self-care, confined to bed or chair more than 50% of waking hours, but not bedridden.
4	Completely disabled. Cannot carry on any self-care. Totally bedridden.
5	Dead

Appendix 2 Study Flow Chart

Study Flow Chart

Test	Pre-study[1]	Base-line[2]	All Cycles Day 1	All Cycles Days 1-10[3]	Cycle 1 Day 4 ± 1	Cycle 1 Day 10 ± 1 and 28 ± 4	Every week	Every 2 weeks	Even cycles Day 28±4	End of Study
Informed Consent	X									
Medical History		X								
Physical Exam		X[a]	X[b]							X
Transfusion Requirements[c]		X[c]	Ongoing							
Bone Marrow Aspirate and Biopsy	X[d]					X[d]			X[d]	X[d]
Flow Cytometry[e]	X									
Pregnancy test		X								
CBC with differential and platelets		X	X				X[f]			X
Serum Chemistries[g]		X	X					X[f]		X
Decitabine administration[h]				X						
Adverse Events			Ongoing							
Post-study follow-up										X[i]

[1] To be performed within 14 days of the first dose of decitabine.
[2] To be performed within 7 days of the first dose of decitabine.
[3] Decitabine may be reduced to Days 1-5 or Days 1-3. See Section 6.3 for details.
[a] To include height, weight, and ECOG.
[b] To include weight.
[c] Care should be given to record the frequency of transfusions from 8 weeks prior to first dose of study drug through the duration of the study.
[d] Bone marrow biopsy and aspirate should be performed at baseline, at Cycle 1 Day 10 ± 1, and at Day 28 ± 4 of Cycles 1, 2, 4 and 6 for clinical disease assessment. Patients who achieve with clinical response (CRi or better) and are receiving cycles every 6 weeks, will undergo bone marrow biopsies on day 42 ± 4. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression. Standard bone marrow procurement procedures will be followed for collection of the tissue. To include: correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics and FISH at all time points. A skin biopsy sample (collected under HRPO# 201011766) is required at pre-study, but may have been collected months or even years prior to first dose of decitabine.
[e] On bone marrow or peripheral blood.
[f] After Cycle 1, these labs may be obtained every other week (CBC) or once a month (Chemistries) at the discretion of the treating physician.
[g] Serum chemistries include bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, and LDH.
[h] Decitabine treatment will be 20 mg/m^2 over a 1-hour IV infusion on consecutive Days 1-10 every 28-day cycle. The dose will be calculated based on actual patient weight at the beginning of each cycle. Patients may be premedicated with lorazepam, prochlorperazine, palonosetron, or ondansetron at the treating physician's discretion.
[i] Patients will be followed for 30 days following the date of the last decitabine dose to monitor for toxicity, survival and response. Thereafter, the investigator or designee will record information on subsequent therapy, disease progression, and death, every 6 months for 2 years after first dose of study drug or until the patient is lost to follow-up.

16.0 REFERENCES

1. Cheson, B.D., et al., *Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia.* J Clin Oncol, 2003. **21**(24): p. 4642-9.
2. Cashen, A.F., et al., *Multicenter, phase II study of decitabine for the first-line treatment of older patients with acute myeloid leukemia.* J Clin Oncol, 2010. **28**(4): p. 556-61.
3. Blum, W., et al., *Clinical response and miR-29b predictive significance in older AML patients treated with a 10-day schedule of decitabine.* Proc Natl Acad Sci U S A, 2010. **107**(16): p. 7473-8.
4. Schlenk, R.F., et al., *Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia.* N Engl J Med, 2008. **358**(18): p. 1909-18.
5. Erba, H.P., *Has there been progress in the treatment of older patients with acute myeloid leukemia?* Best Pract Res Clin Haematol, 2010. **23**(4): p. 495-501.
6. Heaney, M.L. and D.W. Golde, *Myelodysplasia.* N Engl J Med, 1999. **340**(21): p. 1649-60.
7. Bennett, J.M., et al., *Proposals for the classification of the myelodysplastic syndromes.* Br J Haematol, 1982. **51**(2): p. 189-99.
8. Kantarjian, H., et al., *Results of intensive chemotherapy in 998 patients age 65 years or older with acute myeloid leukemia or high-risk myelodysplastic syndrome: predictive prognostic models for outcome.* Cancer, 2006. **106**(5): p. 1090-8.
9. Martin, M.G. and C.N. Abboud, *Induction therapy for elderly patients with acute myeloid leukemia.* Blood Rev, 2008. **22**(6): p. 311-20.
10. Bow, E.J., et al., *Therapy of untreated acute myeloid leukemia in the elderly: remission-induction using a non-cytarabine-containing regimen of mitoxantrone plus etoposide.* J Clin Oncol, 1996. **14**(4): p. 1345-52.
11. Goldstone, A.H., et al., *Attempts to improve treatment outcomes in acute myeloid leukemia (AML) in older patients: the results of the United Kingdom Medical Research Council AML11 trial.* Blood, 2001. **98**(5): p. 1302-11.
12. Stone, R.M., *The difficult problem of acute myeloid leukemia in the older adult.* CA Cancer J Clin, 2002. **52**(6): p. 363-71.
13. Liu Yin, J.A., et al., *Mitozantrone and cytosine arabinoside as first-line therapy in elderly patients with acute myeloid leukaemia.* Br J Haematol, 1991. **79**(3): p. 415-20.
14. Stone, R.M., et al., *Granulocyte-macrophage colony-stimulating factor after initial chemotherapy for elderly patients with primary acute myelogenous leukemia. Cancer and Leukemia Group B.* N Engl J Med, 1995. **332**(25): p. 1671-7.
15. Kantarjian, H., et al., *Decitabine improves patient outcomes in myelodysplastic syndromes.* Cancer, 2006. **106**(8): p. 1794-1803.

16. Lubbert, M., et al., *A multicenter phase II trial of Decitabine as first-line treatment of older AML patients judged unfit for induction chemotherapy.* Haematologica, 2011. **97**(3): p. 393-401.
17. Issa, J.P., et al., *Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies.* Blood, 2004. **103**(5): p. 1635-40.
18. Petti, M.C., et al., *Pilot study of 5-aza-2'-deoxycytidine (Decitabine) in the treatment of poor prognosis acute myelogenous leukemia patients: preliminary results.* Leukemia, 1993. **7 Suppl 1**: p. 36-41.
19. Wijermans, P.W., et al., *Continuous infusion of low-dose 5-Aza-2'-deoxycytidine in elderly patients with high-risk myelodysplastic syndrome.* Leukemia, 1997. **11**(1): p. 1-5.
20. Kantarjian, H., et al., *Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study.* Cancer, 2006. **106**(8): p. 1794-803.
21. Wijermans, P.W., et al., *An epigenetic approach to the treatment of advanced MDS; the experience with the DNA demethylating agent 5-aza-2'-deoxycytidine (decitabine) in 177 patients.* Ann Hematol, 2005. **84 Suppl 1**: p. 9-17.
22. Cashen, A.F., et al., *Pharmacokinetics of decitabine administered as a 3-h infusion to patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS).* Cancer Chemother Pharmacol, 2008. **61**(5): p. 759-66.
23. Chabot, G.G., J. Bouchard, and R.L. Momparler, *Kinetics of deamination of 5-aza-2'-deoxycytidine and cytosine arabinoside by human liver cytidine deaminase and its inhibition by 3-deazauridine, thymidine or uracil arabinoside.* Biochem Pharmacol, 1983. **32**(7): p. 1327-8.
24. Momparler, R.L., et al., *Cell cycle effects and cellular pharmacology of 5-aza-2'-deoxycytidine.* Cancer Chemother Pharmacol, 1984. **13**(3): p. 191-4.
25. Raza, A., et al., *Biological significance of cell cycle kinetics in 128 standard risk newly diagnosed patients with acute myelocytic leukemia.* Br J Haematol, 1991. **79**(1): p. 33-9.
26. Brons, P.P., et al., *Proliferation patterns in acute myeloid leukemia: leukemic clonogenic growth and in vivo cell cycle kinetics.* Ann Hematol, 1993. **66**(5): p. 225-33.
27. Raza, A., et al., *Direct relationship between remission duration in acute myeloid leukemia and cell cycle kinetics: a leukemia intergroup study.* Blood, 1990. **76**(11): p. 2191-7.
28. Shen, L., et al., *DNA methylation predicts survival and response to therapy in patients with myelodysplastic syndromes.* J Clin Oncol, 2010. **28**(4): p. 605-13.
29. Tefferi, A., et al., *IDH1 and IDH2 mutation studies in 1473 patients with chronic-, fibrotic- or blast-phase essential thrombocythemia, polycythemia vera or myelofibrosis.* Leukemia, 2010. **24**(7): p. 1302-9.
30. Ley, T.J., et al., *DNMT3A mutations in acute myeloid leukemia.* N Engl J Med, 2010. **363**(25): p. 2424-33.
31. Mardis, E.R., et al., *Recurring mutations found by sequencing an acute myeloid leukemia genome.* N Engl J Med, 2009. **361**(11): p. 1058-66.

32. Abdel-Wahab, O., J. Patel, and R.L. Levine, *Clinical Implications of Novel Mutations in Epigenetic Modifiers in AML.* Hematol Oncol Clin North Am, 2011. **25**(6): p. 1119-33.
33. Reuther, G.W., *Recurring mutations in myeloproliferative neoplasms alter epigenetic regulation of gene expression.* Am J Cancer Res, 2011. **1**(6): p. 752-62.
34. Paschka, P., et al., *IDH1 and IDH2 mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3 internal tandem duplication.* J Clin Oncol, 2010. **28**(22): p. 3636-43.
35. Shen, Y., et al., *Gene mutation patterns and their prognostic impact in a cohort of 1185 patients with acute myeloid leukemia.* Blood, 2011. **118**(20): p. 5593-603.
36. Tefferi, A., et al., *Detection of mutant TET2 in myeloid malignancies other than myeloproliferative neoplasms: CMML, MDS, MDS/MPN and AML.* Leukemia, 2009. **23**(7): p. 1343-5.
37. Walter, M.J., et al., *Recurrent DNMT3A mutations in patients with myelodysplastic syndromes.* Leukemia, 2011. **25**(7): p. 1153-8.
38. Lin, J., et al., *Recurrent DNMT3A R882 Mutations in Chinese Patients with Acute Myeloid Leukemia and Myelodysplastic Syndrome.* PLoS One, 2011. **6**(10): p. e26906.
39. Bejar, R., et al., *Clinical effect of point mutations in myelodysplastic syndromes.* N Engl J Med, 2011. **364**(26): p. 2496-506.
40. Walter, M.J., et al., *Clonal architecture of secondary acute myeloid leukemia.* N Engl J Med, 2012. **366**(12): p. 1090-8.
41. Kantarjian, H.M., et al., *Survival advantage with decitabine versus intensive chemotherapy in patients with higher risk myelodysplastic syndrome: comparison with historical experience.* Cancer, 2007. **109**(6): p. 1133-7.
42. Lubbert, M., et al., *Continued Low-Dose Decitabine (DAC) Is an Active First-Line Treatment in All Cytogenetic Subgroups of Older AML Patients: Results of the FR00331 Multicenter Phase II Study.* ASH Annual Meeting Abstracts, 2007. **110**(11): p. 300-.
43. Blum, W., et al., *Phase I Study of Decitabine Alone or in Combination With Valproic Acid in Acute Myeloid Leukemia.* J Clin Oncol, 2007. **25**(25): p. 3884-3891.
44. Issa, J.-P.J., et al., *Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies.* Blood, 2004. **103**(5): p. 1635-1640.
45. Garcia-Manero, G., et al., *Phase 1/2 study of the combination of 5-aza-2'-deoxycytidine with valproic acid in patients with leukemia.* Blood, 2006. **108**(10): p. 3271-3279.
46. Issa, J.P., S.B. Baylin, and J.G. Herman, *DNA methylation changes in hematologic malignancies: biologic and clinical implications.* Leukemia, 1997. **11 Suppl 1**: p. S7-11.
47. Ganetzky, R.D., et al., *Differences Between Normal and Leukemic Stem Cell-Specific Methylome Indicates Aberrantly Silenced Genes Involved in the*

Pathogenesis of Malignant Evolution. ASH Annual Meeting Abstracts, 2008. **112**(11): p. 599-.

48. Christman, J.K., *5-Azacytidine and 5-aza-2'-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy.* Oncogene, 2002. **21**(35): p. 5483-95.
49. Lubbert, M., *DNA methylation inhibitors in the treatment of leukemias, myelodysplastic syndromes and hemoglobinopathies: clinical results and possible mechanisms of action.* Curr Top Microbiol Immunol, 2000. **249**: p. 135-64.
50. Kantarjian, H., et al., *Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia.* Blood, 2007. **109**(1): p. 52-57.
51. Steensma, D.P., et al., *Multicenter Study of Decitabine Administered Daily for 5 Days Every 4 Weeks to Adults With Myelodysplastic Syndromes: The Alternative Dosing for Outpatient Treatment (ADOPT) Trial.* J Clin Oncol, 2009: p. JCO.2008.19.6550.
52. Cashen, A.F., et al., *Preliminary Results of a Multicenter Phase II Trial of 5-Day Decitabine as Front-Line Therapy for Elderly Patients with Acute Myeloid Leukemia (AML).* ASH Annual Meeting Abstracts, 2008. **112**(11): p. 560-.
53. Jabbour, E., et al., *Evolution of decitabine development: accomplishments, ongoing investigations, and future strategies.* Cancer, 2008. **112**(11): p. 2341-51.
54. Kantarjian, H., et al., *Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia.* Blood, 2007. **109**(1): p. 52-7.
55. Blum, W.G., Klisovic, R., Liu, S., Kefauver, C., Grever, M. R., Schaaf, L., Chan, K., Byrd, J. C., Villalona-Calero, M., Marcucci, G. *Efficacy of a novel schedule of decitabine in previously untreated AML, age 60 or older*. in *ASCO Annual Meeting*. 2009.
56. van Groeningen, C.J., et al., *Phase I and pharmacokinetic study of 5-aza-2'-deoxycytidine (NSC 127716) in cancer patients.* Cancer Res, 1986. **46**(9): p. 4831-6.
57. Rivard, G.E., et al., *Phase I study on 5-aza-2'-deoxycytidine in children with acute leukemia.* Leuk Res, 1981. **5**(6): p. 453-62.
58. Lin, K.T., R.L. Momparler, and G.E. Rivard, *Sample preparation for the determination of 5-aza-2'-deoxycytidine in plasma by high-performance liquid chromatography.* J Chromatogr, 1985. **345**(1): p. 162-7.
59. Momparler, R.L., G.E. Rivard, and M. Gyger, *Clinical trial on 5-aza-2'-deoxycytidine in patients with acute leukemia.* Pharmacol Ther, 1985. **30**(3): p. 277-86.
60. Raska, K., Jurovcik, M., Sormova., Z., *On the metabolism of 5-azacytidine and 5-aza-2'-deoxycytidine in mice.* Coll Czech Chem Commun, 1965. **30**: p. 3001-3005.
61. Momparler, R.L., et al., *Pilot phase I-II study on 5-aza-2'-deoxycytidine (Decitabine) in patients with metastatic lung cancer.* Anticancer Drugs, 1997. **8**(4): p. 358-68.

62. Aparicio, A., et al., *Phase I trial of continuous infusion 5-aza-2'-deoxycytidine.* Cancer Chemother Pharmacol, 2003. **51**(3): p. 231-9.
63. Bryan, J., et al., *Pharmacokinetic evaluation of decitabine for the treatment of leukemia.* Expert Opin Drug Metab Toxicol, 2011. **7**(5): p. 661-72.
64. Scandura, J.M., et al., *Phase 1 study of epigenetic priming with decitabine prior to standard induction chemotherapy for patients with AML.* Blood, 2011. **118**(6): p. 1472-80.
65. Westfall, P.H. and S.A. Young, *Resampling-based multiple testing: examples and methods for p-value adjustment*. 1993, New York: John Wiley & Sons, Inc.
66. Samlowski, W.E., et al., *Evaluation of a 7-day continuous intravenous infusion of decitabine: inhibition of promoter-specific and global genomic DNA methylation.* J Clin Oncol, 2005. **23**(17): p. 3897-905.
67. Blum, W., et al., *Phase I study of decitabine alone or in combination with valproic acid in acute myeloid leukemia.* J Clin Oncol, 2007. **25**(25): p. 3884-91.
68. Yang, A.S., et al., *DNA methylation changes after 5-aza-2'-deoxycytidine therapy in patients with leukemia.* Cancer Res, 2006. **66**(10): p. 5495-503.
69. Cheson, B.D., et al., *Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia.* Blood, 2006. **108**(2): p. 419-25.

Genomic Predictors of Decitabine Response in AML/MDS

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Genomic Predictors of Decitabine Response in AML/MDS

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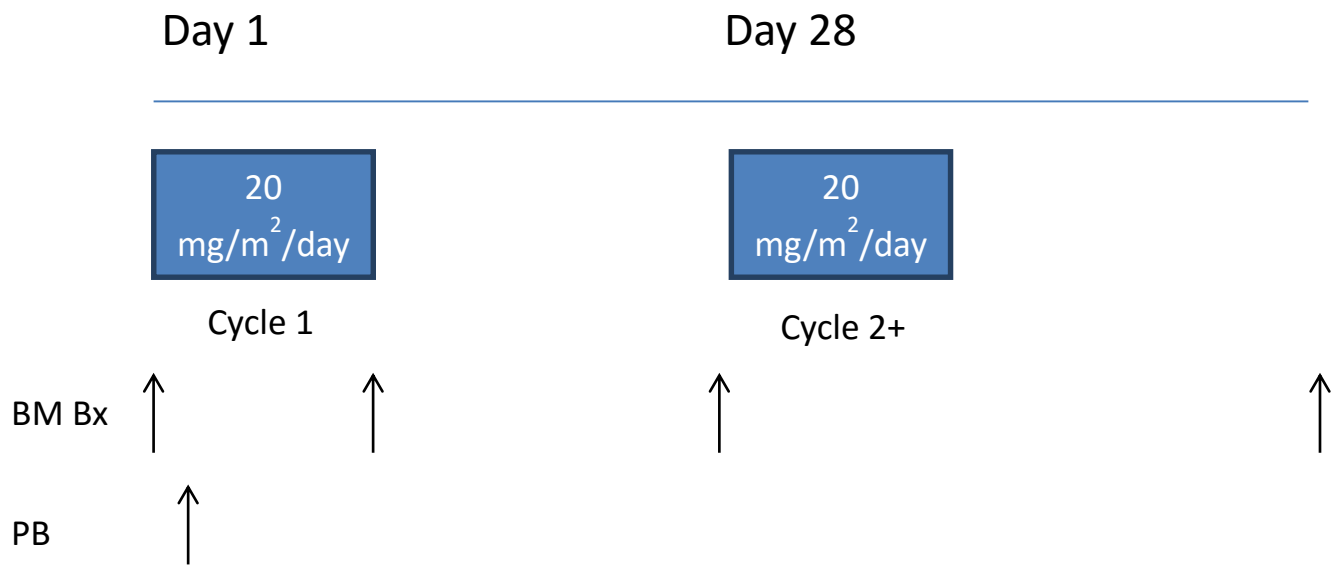
CONFIDENTIAL

Genomic Predictors of Decitabine Response in AML/MDS
Principal Investigator Signature Page

Principal Investigator:	John Welch, M.D., Ph.D.	
	Signature of Investigator	Date
	Printed Name of Investigator	
	By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IRB/HRPO procedures, the Declaration of Helsinki, ICH Good Clinical Practices guidelines, and the applicable parts of the United States Code of Federal Regulations or local regulations governing the conduct of clinical studies.	

Study Schema:



Patients with progressive disease after two cycles (defined as a clear increase in bone marrow blast count, typically a > 50% increase in bone marrow blasts over baseline) should be removed from study.

Patients with stable disease may continue on study and should undergo a diagnostic bone marrow biopsy at the end of even numbered cycles.

Once AML patients achieve morphologic complete remission with incomplete blood count recovery (CRi) or MDS patients achieve a marrow complete response (Marrow CR) as defined in Section 9.0, decitabine administration may be decreased to 20 mg/m2/day for ten consecutive days every 6 weeks, to limit myelosuppressive toxicity and improve count recovery. After 2 cycles at 6 week intervals, the dose may be reduce to 20 mg/m2/day for five consecutive days in 6 week cycles, at the discretion of the treating physcian. After two cycles of five consecutive days of treatment, if the patient meets the definition of CR with complete cytogenetic response as defined in Section 9.0, administration may further be decreased to three consecutive days per cycle at the discretion of the treating physician.

Sample collection for correlative studies:

Bone marrow biopsies should be performed at baseline, at Cycle 1 Day 10 ± 1, and at Day 28 ± 4 of Cycles 1, 2, 4, and 6. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression.

Peripheral blood samples should be collected on Day 4± 1 of Cycle 1. Collection should be obtained 45 ± 15 minutes after decitabine infusion is initiated.

Genomic Predictors of Decitabine Response in AML/MDS

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1.0 INTRODUCTION

1.1 Rationale

Personalized therapy for cancer requires that 1) patient-specific factors (e.g. patient-specific mutations, expression profiles, or pharmacogenomics) influence treatment algorithms, and 2) once therapy is initiated, *in vivo* responsiveness is used to guide further therapy. The modern era of genomics has provided significant insights into cancer pathogenesis by describing patterns of germline and somatic variants, as well as defining disease-specific patterns of gene expression. However, the application and integration of this data with therapeutics remains a difficult task.

[redacted]

AML and MDS are two closely related hematological disorders characterized by ineffective hematopoiesis and malignant expansion of clonal myeloid cells. Both AML and MDS frequently occur in elderly patients and some cases are secondary and/or related to long-term bone marrow damage after radiation or chemotherapy. Current therapy for AML consists of cytotoxic chemotherapy followed by consolidation chemotherapy or stem cell transplantation. Using these approaches, current cure rates may be as high as 60% for patients younger than 60 with good risk cytogenetics (e.g. t(15;17), t(8;21), inv(16)).[4] Outcomes in patients ≥ 60 years old and with relapsed disease remain poor with 2 year survival of only ~25%.[5] Historically, the available treatment for MDS has been symptomatic and has not been shown to be effective in producing sustained improvement in hematopoiesis or in delaying leukemic evolution[6, 7].

Current AML therapy is poorly tolerated in elderly and pre-treated patients due to hematologic and non-hematologic toxicities; following such therapy, patients typically require 3-4 weeks of hospitalization for treatment of neutropenic fevers and for regular transfusions. Even among the relatively healthy patients enrolled in clinical trials, induction mortality in the elderly can be as high as 20-40% following standard induction therapy,[8-11] and moderate to severe non-hematologic toxicity occurs in at least an additional 30% of patients.[9, 12-14] Consequently, many older AML patients are not offered traditional intensive chemotherapy, but receive supportive care alone.[15] More effective and less toxic therapies are needed to provide durable remissions in a significant proportion of elderly AML in populations.

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We and others have recently used whole genome sequencing to identify recurrent, somatic mutations in AML and MDS patients in four genes that are involved in methylcytosine regulation: *DNMT3A, IDH1, IDH2,* and *TET2.*[29-37] Collectively, nearly half of all AML/MDS patients have a mutation in one of these four genes. *DNMT3A* mutations are associated with an adverse outcome in AML patients treated with cytotoxic chemotherapy.[35, 38] However, it is not yet clear whether the presence of mutations in one or more of these genes is associated with response or resistance to decitabine.

In this study, we will treat patients with the current state-of-the-art decitabine schedule.[3] We will perform exome sequencing on all patients and correlate response with mutation status, rate of mutation clearance, expression profile, plasma decitabine levels, and DNA

methylcytosine content reduction. This approach will comprehensively assess genomic and pharmacologic parameters that could clinically predict patient response to decitabine.

In this study, we will combine analysis of patients with AML and MDS. Recent genomic analysis has shown that AML and MDS have shared mutations in many of the same genes, shared patterns of mutation acquisition, and shared patterns of mutation evolution.[39, 40] Furthermore, MDS, like AML, presents with clonal hematopoiesis, even when the morphologic blast count is zero.[36]Because this study will identify and track the rate of clearance of patient specific mutations, and because decitabine has been shown to have efficacy in both AML and MDS patients, [2, 3, 16-19, 41-45] combining analysis of AML and MDS patients is reasonable. Asymptomatic MDS patients are excluded because these patients are typically followed with watchful-waiting rather than with chemotherapy.

1.2 Decitabine and Hypomethylating agents in AML/MDS

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1.3 Correlative Studies

With exception of the peripheral blood sample collected on Cycle 1 Day 4, all correlative studies will be collected under HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Diseases") that all patients will be concurrently enrolled in. The goals of the correlative studies include:

1. To correlate patient-specific mutations with response to decitabine.
   We will perform exome sequencing of each patient's pre-study bone marrow sample and skin sample. We will determine AML/MDS-associated variants found in the bone marrow, but not skin sample. We will correlate the presence of mutations with response (especially mutations in *DNMT3A, IDH1, IDH2,* and *TET2*). This study is powered to identify mutations with >80% sensitivity to predict decitabine response if used as a clinical assay.

2. To correlate the velocity and depth of response with response to decitabine.
   We will assess tumor burden by allele frequency of patient-specific mutations in bone marrow samples collected pre study, Cycle 1 Day 10±1, Cycle 1 Day 28±4, and Cycle 2 Day 28±4. We will assess mutations in at least 24 recurrently mutated genes (including, but not limited to: *FLT3, DNMT3A, NPM1, TET2, WT1, IDH1, IDH2, RUNX1, NRAS, TP53, U2AF1, KRAS, PHF6, MSTP9, PTPN11, KIT, SMC3, PRUNE2, ETV6, CEBPA, SMC1A, ASXL1, STAG2, RAD21*) as well as all patient-specific mutations discovered during exome sequencing. The leukemic allele frequency will be measured using the Illumnia MySeq or HiSeq platform using DNA capture by gene specific biotinylated-oligonucleotides. Patient bone marrow samples will be assessed pre-study, Cycle 1 Day 10 ± 1, Cycle 1 Day 28 ± 4, and Cycle 2 Day 28 ± 4 and compared with patient specific skin samples (normal control). The velocity of response will be calculated as the natural log rate of patient-specific mutation allele frequency clearance. Depth of response will be defined as the lowest patient-specific mutation

allele frequency in a bone marrow sample. The velocity and depth of tumor response will be correlated to:

- Achievement of clinical CR,
- Time to best response,
- Mutational status,
- Time to disease progression.

Because all patient-specific exome-discovered mutations will be assessed a second time in the primary bone marrow sample as well as the Cycle 1 Day 10 ± 1, Cycle 1 Day 28 ± 4, and Cycle 2 Day 28 ± 4 bone marrow samples, this study will also serve to validate all exome-discovered mutations. This is important, because exome sequencing is associated with a 20-50% false-discovery rate depending on type I error tolerated (mutation-calling algorithms are typically set for greater sensitivity than specificity). Thus, many mutations discovered during exome sequencing are incorrect. This second analysis of these mutations will server to exclude these false positive calls.

3. To correlate the bone marrow expression profile from pre-study and Cycle 1 Day 10 ± 1 with response to decitabine.
   We will correlate expression signatures, and changes in expression signatures, with:
   - Achievement of clinical CR,
   - Mutation status.

4. To correlate steady-state plasma decitabine concentration on Cycle 1 Day 4 ± 1 with:
   - Achievement of CR.

5. To correlate the extent of bone marrow DNA hypomethylation on Cycle 1 Day 10 ± 1 with response to decitabine.
   We will assess the percent reduction in bone marrow methylcytosine content using LC-MS. Patients will be evaluated pre-study and Cycle 1 Day 10 ± 1. The change in DNA methylcytosine content will be correlated to:
   - Achievement of clinical CR,
   - Change in expression signature.

   This will determine whether inadequate blast exposure to decitabine correlates with drug resistance.

2.0 OBJECTIVES

2.1 Primary Objective

To determine whether patient-specific mutations correlate with clinical overall response to decitabine in patients with AML or MDS.

2.2 Secondary Objectives

- To determine whether 10 consecutive days per cycle of decitabine improves outcomes compared with historical controls treated with 5 consecutive days per cycle of decitabine.

- To determine whether other biomarkers correlate with clinical overall response to decitabine in patients with AML or MDS. These will include:

    1. The velocity and depth of patient-specific mutation clearance;
    2. The bone marrow expression profile and change in expression profile during decitabine treatment;
    3. The steady-state plasma decitabine concentrations;
    4. The decrease in bone marrow methylcytosine content in response to decitabine.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

One of the following:

- Patient must have non-M3 AML and be ≥ 60 years of age OR
- non-M3 AML with relapsed disease OR
- Symptomatic MDS with one of the following:
  - □ Symptomatic anemia with either hemoglobin < 10.0 g/dL or requiring RBC transfusion
  - □ Thrombocytopenia with a history of two or more platelet counts < 50,000/mcL or a significant hemorrhage requiring platelet transfusions
  - □ Neutropenia with two or more ANC < 1,000/mcL

All of the following:

1. Patient must have an ECOG performance status ≤ 2.
2. Patient must have >10% disease burden measured by cytomorphology, flow cytometry, or cytogenetics.
3. Patient must have peripheral white blood cell count ≤ 50,000/mcl.
4. Patient must have adequate organ function, defined as:
   a. Total bilirubin ≤ 1.5 x ULN
   b. AST/ALT ≤ 2.5 x ULN
   c. Serum creatinine ≤ 2.0 x ULN
5. Patient must have undergone ≤ 2 cycles of prior hypomethylating agent (decitabine or azacitidine).
6. Patient must be enrolled in HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Diseases").
7. Patient must be ≥ 18 years of age.
8. Patient must be able to understand and willing to sign an IRB-approved written informed consent document.

3.2 Exclusion Criteria

1. Patient must not be pregnant or nursing.
2. Patient must not have known CNS leukemia.
3. Patient must not have a history of positive HIV serology.
4. Patient must not have a history of positive Hepatitis C serology.
5. Patient must not have undergone a prior allogeneic stem cell transplant.
6. Patient must not have any uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, ongoing or active GVHD, congestive heart failure of NYHA class 3 or 4, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situation that would limit compliance with study requirements.
7. Patient must not have had radiation therapy within 14 days of enrollment.
8. Patient must not have received any chemotherapy within 21 days of enrollment, and any acute treatment-related toxicities must have returned to baseline. Patients

may be receiving hydrea at time of enrollment.

3.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4.0 REGISTRATION PROCEDURES

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility by Washington University
2. Registration of patient in the Siteman Cancer Center database
3. Assignment of unique patient number (UPN)

Once the patient has been entered in the Siteman Cancer Center database, the WUSM coordinator will forward verification of enrollment and the UPN via email.

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility collecting the information listed below and scanning and emailing it to the research coordinator listed in the *Siteman Cancer Center Clinical Trials Core Protocol Procedures for Secondary Sites* packet at least one business day prior to registering patient:

1. Your name and contact information (telephone number, fax number, and email address)
2. Your site PI's name, the registering MD's name, and your institution name
3. Patient's race, sex, and DOB
4. Three letters (or two letters and a dash) for the patient's initials
5. Currently approved protocol version date
6. Copy of signed consent form (patient name may be blacked out)
7. Planned date of enrollment
8. Completed eligibility checklist, signed and dated by a member of the study team
9. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center Database

Registrations may be submitted Monday through Friday between 8am and 5pm CT. Urgent late afternoon or early morning enrollments should be planned in advance and coordinated with the Washington University research coordinator. Registration will be confirmed by the research coordinator or his/her delegate by email within one business day. Verification of eligibility and registration should be kept in the patient chart.

All patients at all sites must be registered through the Siteman Cancer Center database at Washington University.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. Patients will also be identified by first, middle, and last initials. If the patient has no middle initial, a dash will be used on the case report forms (CRFs). All data will be recorded with this identification number on the appropriate CRFs.

5.0 INVESTIGATIONAL PLAN

5.1 Summary of Study Design

This is an open-label, single-arm, Phase II, biomarker study of patients with AML or MDS who are treated with 10 consecutive days of decitabine. FAB-M3 AML is excluded from this trial, because other well-tolerated options exist for the treatment of FAB-M3 acute promyelocytic leukemia. AML patients who are < 60 years old and have not received cytotoxic induction chemotherapy are also excluded, because cytotoxic therapy offers a curative possibility for these patients. MDS patients without cytopenias are also excluded because these patients can be followed with watchful-waiting.

All patients will undergo bone marrow biopsies pre-study, on Cycle 1 Day 10 ± 1, and on Day 28 ± 4 of Cycles 1, 2, 4, and 6. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression. Patients will provide a skin biopsy sample pre-study. Patients will also provide peripheral blood samples on Cycle 1 Day 4 ± 1 for pharmacokinetic studies. See Section 8.0 for details on correlative studies.

Patients will receive decitabine 20 mg/m^2/day as a 1-hour infusion on consecutive Days 1-10 in 28-day cycles. The dose will be calculated based on actual patient weight at the beginning of each cycle.

Once AML patients achieve morphologic complete remission with incomplete blood count recovery (CRi) or MDS patients achieve a marrow complete response (Marrow CR) as defined in Section 9.0, decitabine administration may be decreased to 20 mg/m2/day for ten consecutive days every 6 weeks, to limit myelosuppressive toxicity and improve count recovery. After 2 cycles at 6 week intervals, the dose may be reduce to 20 mg/m2/day for five consecutive days in 6 week cycles, at the discretion of the treating physician. After two cycles of five consecutive days of treatment, if the patient meets the definition of CR with complete cytogenetic response as defined in Section 9.0, administration may further be decreased to three consecutive days per cycle at the discretion of the treating physician.

It will be a logistical goal of this trial consistently to return preliminary exome results to the treating physician by the end of Cycle 1 and validated results by the end of Cycle 2. However, this logistical goal is not a primary or secondary aim of this study. Given the limited data regarding mutation presence and drug response, we do not anticipate that the results will directly influence therapeutic decisions made by the treating physician. However, the development of comprehensive genomic pipelines and integration of this data into a clinical trial setting will be required before personalized medicine can be realized.

5.2 Study Procedures

5.2.1 Pre-study Procedures

Pre-study procedures must take place no more than 14 days prior to the first dose of decitabine except where noted below.

1. Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics, and FISH.
2. Bone marrow or peripheral blood for flow cytometry
3. Skin biopsy sample (collected under HRPO# 201011766). There is no required time frame for this sample; it may have been collected months or even years prior to the first dose of decitabine.

5.2.2 Baseline Evaluation

Baseline evaluations must take place no more than 7 days prior to the first dose of decitabine.

1. Medical history
2. Physical exam including height, weight, and ECOG performance score.
3. Record transfusion requirements for 8 weeks before first dose of study drug
4. CBC with differential and platelets
5. Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH
6. Urine βHCG pregnancy test if appropriate

5.2.3 Day 1 of Each Cycle

1. Physical exam, including weight
2. CBC with differential and platelets
3. Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH

5.2.4 Cycle 1 Days 1-10

Decitabine administration, 20 mg/m^2 per day given as a 1-hour continuous intravenous infusion. The dose will be calculated based on actual patient weight at the beginning of each cycle. Patients may be premedicated with lorazepam, prochlorperazine, palonosetron, or ondansetron at the treating physician's discretion.

5.2.5 Cycle 1 Day 4 ± 1

Obtain 3 mL peripheral blood for correlative studies. Sample must be obtained 45 minutes ± 15 minutes after beginning the infusion, before the decitabine infusion is complete, and should be obtained from the opposite arm as the infusion. The sample should be collected in a 3 mL K3EDTA tube pre-loaded with 8 mcL of tetrahydrouridine (THU 500 mcg/mL), an inhibitor of cytidine deaminase. The sample should be processed by the Tissue Procurement Core Facility at the Siteman Cancer Center. Plasma should be separated and stored per protocols.

5.2.6 Cycle 1 Day 10 ± 1

Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics, and FISH.

5.2.7 Weekly

CBC with differential and platelets. After the first cycle, this may be changed to every other week at the discretion of the treating physician.

5.2.8 Every Other Week

Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH. After the first cycle, this may be changed to every month at the discretion of the treating physician.

5.2.9 Cycles 1, 2, 4 and 6 Day 28 ± 4 days

Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include: correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics, and FISH.

Patients who achieve with clinical response (CRi or better) and are receiving cycles every 6 weeks, will undergo bone marrow biopsies on day 42 ± 4.

5.2.10 End-of-Study Procedures

1. Physical exam
2. CBC with differential and platelets.
3. Serum chemistries including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, and LDH.

4. Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include morphologic assessment, cytogenetics, and FISH.

5.2.11 Post-study Follow-up

Patients are to be followed according to standard patient care procedures for 30 days following the date of the last decitabine dose to monitor for toxicity, survival, and response. Thereafter, the investigator or designee will record information on subsequent therapy, disease progression, and/or death every 6 months for 2 years after last dose of study drug or until the patient is lost to follow-up or dies.

5.3 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented in the case report forms.

In the absence of treatment delays due to adverse events, treatment may continue indefinitely or until one of the following criteria applies:

- Documented and confirmed disease progression
- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious non-compliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study

Patients who prematurely discontinue treatment for any reason will be followed as indicated in the study calendar.

5.4 Concomitant Therapy and Supportive Care Guidelines

5.4.1 Chemotherapy

Patients cannot receive any chemotherapy within 21 days of study enrollment and must have recovered from any treatment-related toxicities. However, patients may be receiving hydrea at enrollment and may continue on hydrea through Cycle 2 of decitabine. An indication for hydrea following 2 cycles of decitabine is evidence

that the patient is not responding and decitabine should be discontinued. Other than decitabine, patients cannot receive chemotherapy during this trial.

5.4.2 Growth Factors

Erythroid growth factors should not be routinely administered to patients during the study.

Thrombopoietin receptor agonists should not be routinely administered to patients during the study.

Filgrastim (G-CSF) or sargramostim (GM-CSF) may be administered to patients with septic shock or febrile neutropenia at the discretion of the treating physician.

5.4.3 Transfusions

- *Packed red blood cells (RBCs) transfusion*:
  RBC transfusions will be administered at the discretion of the treating physician.

- *Platelet transfusion*:
  Platelet transfusions will be administered at the discretion of the treating physician

5.4.4 Prophylactic Antimicrobial Agents

Antimicrobial agents will be administered at the discretion of the treating physician. Common antimicrobial prophylaxis during cycles 1 and 2 include acyclovir, ciprofloxacin, and fluconazole.

5.4.5 Radiotherapy

Patients cannot receive radiotherapy within 14 days prior to study enrollment or while on study.

6.0 DOSE MODIFICATIONS

A criterion for discontinuation of the study is a delay of > 8 weeks between any two cycles.

6.1 Delay for Cytopenia or Infection

As cytopenias are a defining feature of AML and MDS, their presence alone does not generally warrant dose modifications or delays. Patients will receive four cycles of therapy without adjustment for any cytopenia. Following Cycle 4, dose modifications or delays for cytopenias will be made at the discretion of the investigator.

Patients with febrile neutropenia (temperature ≥ 38.5° with ANC < 1,000/μl) or systemic infections should have treatment held until clinical resolution of the infection.

6.2 Delay for Organ Dysfunction

If renal dysfunction (creatinine ≥ 3 X institutional upper limit of normal) or hepatic dysfunction (bilirubin ≥ 2.5 mg/dl or ALT/AST ≥ 3 X institutional upper limit of normal) occurs, therapy may not resume until the abnormalities have resolved to below the limits stated above. If renal or hepatic dysfunction occurs during a treatment course, the next dose of decitabine should be held, and serum chemistries should be checked twice per week. Decitabine can be resumed at full dose when creatinine, bilirubin, ALT and AST are below the limits listed above.

For grade 3-4 non-hematologic toxicities related to decitabine, decitabine treatment should be delayed until recovery to baseline or to grade ≤ 1. Decitabine may be reintroduced with a 50% dose reduction, or at full dose at the discretion of the investigator.

6.3 Modification in Dose or Schedule for Treatment Response

Once AML patients achieve morphologic complete remission with incomplete blood count recovery (CRi) or MDS patients achieve a marrow complete response (Marrow CR) as defined in Section 9.0, decitabine administration may be decreased to 20 mg/m2/day for ten consecutive days, and the cycles may be administered every 6 weeks to limit myelosuppressive toxicity, at the discretion of the treating physician. After two cycles at six week intervals, the dose may be reduced to 20 mg/m2/day for five consecutive days of treatment in six week intervals. If the patient meets the definition of CR with complete cytogenetic response as defined in Section 9.0, administration may further be decreased to three consecutive days per cycle at the discretion of the treating physician.

7.0 PHARMACEUTICAL INFORMATION

[redacted]

8.0 CORRELATIVE STUDIES SAMPLE COLLECTIONS

Bone marrow samples will be collected pre-study, on Cycle 1 Day 10 ± 1, and on Day 28 ± 4 of Cycle 1, 2, 4, and 6 for correlative studies. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression. The bone marrow samples will be collected under HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Diseases") that all patients will be concurrently enrolled in.

Skin biopsy sample will be collected as part of the patient's participation in HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Malignancies"). There is no required time frame for this sample, and it may have been collected months or even years prior to the first dose of decitabine.

Peripheral blood sample (3 mL) will be collected on Cycle 1 Day 4 ± 1. Sample must be obtained 45 minutes ± 15 minutes after beginning the infusion, before the decitabine infusion is complete, and should be obtained from the opposite arm as the infusion. The sample should be collected in a 3 mL K3EDTA tube pre-loaded with 8 mcL of tetrahydrouridine (THU 500 mcg/mL), an inhibitor of cytidine deaminase. The sample should be processed by the Tissue Procurement Core Facility at the Siteman Cancer Center. Plasma should be separated and stored per protocols.

9.0 EFFICACY, SAFETY AND CORRELATIVE STUDY MEASURES

A secondary endpoint of this study is to estimate morphologic complete remission rate.

9.1 Response Criteria - AML

AML Patients will be assessed for response according to the IWG criteria:[1]

Morphologic complete remission (CR) – Defined as morphologic leukemia-free state, defined as less than 5% blasts in aspirate with marrow spicules and a count of ≥200 nucleated cells and no blasts with Auer rods or any persistence of extramedullary disease. When erythroid precursors constitute less than 50% of bone marrow nucleated cells, the percentage of blasts is based on all nucleated cells; when there are 50% or more erythroid cells, the percentage blasts should be based on the nonerythroid cells. The presence of mild megaloblastoid changes may be permitted if they are thought to be consistent with treatment effect. However, persistence of pretreatment abnormalities (eg, pseudo-Pelger-Hüet cells, ringed sideroblasts, dysplastic megakaryocytes) are not consistent with CR. In addition, ANC >1000/µl and platelet count of ≥100,000/µl. Patient must be independent of transfusions. No duration requirement for this designation.

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

Morphologic complete remission with incomplete blood count recovery (CRi) – Defined as morphologic complete recovery with the exception of neutropenia <1000/µl or thrombocytopenia <100,000/µl.

Partial remission (PR) – Defined as meeting the above criteria for complete remission, with the following exceptions: decrease of ≥50% in the percentage of blast to 5-25% in the bone marrow aspirate. A value of ≤5% blasts in bone marrow with Auer rods present may also be considered a partial remission.

Stable Disease (SD) – Defined as not meeting the definitions of CR, CRi, PR, PD, or recurrence/morphologic relapse.

Progressive disease (PD) – Defined as a clear increase in bone marrow blast count, typically a greater than 50% increase compared with baseline, or new extramedullary disease. Patients with a > 50% increase in peripheral blast count with an increase of the total peripheral white blood cell count to > 10,000/µl should undergo evaluation by bone marrow biopsy to access for progressive disease.

Recurrence/morphologic relapse **-** Defined as relapse following 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. New dysplastic changes is considered relapse.

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.

9.2 Response Criteria - MDS

MDS Patients will be assessed for response according to the IWG criteria:[69]

Complete remission (CR) – Defined as ≤ 5% myeloblasts with normal maturation of all cell lines in the bone marrow and peripheral blood values of Hgb ≥ 11g/dL, Platelets ≥ 100 x 10^9/L, Neutrophils ≥ 1.0 x 10^9/L, and 0% blasts. Persistent dysplasia does not exclude CR but will be noted.

Marrow Complete Response (Marrow CR) – Defined as ≤ 5% myeloblasts in the bone marrow and a decrease by ≥ 50% from pretreatment values, but not meeting the definition of CR above.

Partial Remission (PR) – Defined as meeting the definition of CR above with a decrease of myeloeblasts in the bone marrow by ≥ 50% from pretreatment values, but absolute myeloblasts still >5%.

Stable Disease (SD) – Defined as not meeting the definitions of CR, Marrow CR, PR, SD, PD, or recurrence/morphologic relapse.

Progressive Disease//Relapse (PD) – Defined as ≥ 50% increase in blasts to > 5% blasts (for patients with less than 5% blasts at baseline only), ≥ 50% increase to > 10% blasts (for patients with 5-10% blasts at baseline only), ≥ 50% increase to > 20% blasts (for patients with l0-20% blasts at baseline only), ≥ 50% increase to > 30% blasts (for patients with 20-30% blasts at baseline only) or any of the following: At least 50% decrement from maximum remission/response in granulocytes or platelets, reduction in Hgb by ≥ 2 g/dL, or New or worsened transfusion dependence not related to study drug toxicity. Or for patients with a CR, Marrow CR, or PR as defined above and subsequently development of one of the following: Return to pretreatment bone marrow blast percentage, decrement of ≥ 50% from maximum remission/response levels in granulocytes or platelets, or reduction in Hgb concentration by ≥ 1.5 g/dL or transfusion dependence.

9.2.1 Hematologic Improvement (HI)

Progressive disease as defined above nullifies hematologic improvement.

Erythroid response requires all of the following (only required if pretreatment HgB <11 g/dL):

- Hgb increase by ≥ 1.5 g/dL
- Relevant reduction of units of RBC transfusions by an absolute number of at least 4 RBC transfusions/8 wk compared with the pretreatment transfusion number in the previous 8 wk. Only RBC transfusions given for a Hgb of ≤ 9.0 g/dL pretreatment will count in the RBC transfusion response evaluation

Platelet response requires one of the following (only required if pretreatment platelets < 100 x 10^9/L):

- Absolute increase of ≥ 30 x 10^9/L (for patients starting with > 20 x 10^9/L platelets)
- Increase from < 20 x 10^9/L to > 20 x 10^9/L and absolute increase ≥ 100% (for patients starting with < 20 x 10^9/L)

Neutrophil response requires the following (only required if pretreatment ANC <1.0 x 10^9/L):

- At least 100% increase and an absolute increase > 0.5 x 10^9/L

9.3.1 Cytogenetic Response

Complete Cytogenetic Response – Defined as reversion to a normal karyotype. For this study, reversion of a normal karyotype is defined as no clonal abnormalities detected in a minimum of 20 mitotic cells. Progressive disease as defined above nullifies cytogenetic response.

9.3 Guidelines for Evaluation of Disease

Bone marrow biopsy should be performed on Day 28 ± 4 of cycles 1, 2, 4 and 6 for clinical disease assessment. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression.

9.4 Other Secondary Efficacy Measures

9.4.1 Treatment Failure, Overall Survival and Event-free Survival

Treatment failure is defined as failure to achieve a complete or partial remission following 4 cycles of therapy.

Overall survival is defined as the date of first dose of study drug to the date of death from any cause. Patients still alive at the time of the final analysis will be right-censored.

Event-free survival is defined as the interval from the date of first dose of study drug to date of treatment failure, recurrence, death due to any cause, or loss to follow up.

9.4.2 Duration of Remission and Relapse-free Survival for Patients Having Complete Remission

Duration of complete remission is defined as the interval from the date that complete remission is documented to the date of recurrence, death due to any cause,

or loss 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.

9.4.3 Safety

Safety evaluation will include assessments of adverse experiences, medical history, physical examinations, vital signs, and laboratory assessments at baseline and throughout the study period. Local laboratories may be used for this study.

10.0 ADVERSE EVENT REPORTING

10.1 Adverse Events (AEs)

Definition: any unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site: http://ctep.cancer.gov/reporting/ctc.html. Grade 1-2 events will be recorded for the first fifty enrolled patients. Subsequently, only events graded as 3 or greater will be recorded. Fifty patients should be sufficient to estimate the true incidence of any adverse event to within ± 14% with 95% confidence intervals.
Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website: (http://www.hhs.gov/ohrp/policy/AdvEvntGuid.htm).

10.2 Unanticipated Problems

Definition:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

10.3 Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

10.4 Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

10.5 Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

Local IRB Pre-approval of all protocol exceptions must be obtained prior to the event. For secondary sites, the Washington University PI will issue approval of the exception, but it must also be submitted to the local IRB with documentation of approval forwarded to Washington University. HRPO approval is not required for protocol exceptions occurring at secondary sites.

10.6 Reporting to the Human Research Protection Office (HRPO) and the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University:

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH or SLCH institution, or that impacts participants or the conduct of the study.
- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within **10 working days** of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification to the PI of the event.

10.7 Reporting Requirements for Secondary Sites

The research team at each secondary site is required to promptly notify the Washington University PI and research coordinator of all reportable events (as described in Section 7.6) within **1 working day** of the occurrence of the event or notification of the secondary site's PI of the event. This notification may take place via email if there is not yet enough information for a formal written report (using either an FDA MedWatch form if required or an institutional SAE reporting form if not). A formal written report must be sent to the Washington University PI and research coordinator within **10 working days** of the occurrence of the event or notification of the secondary site's PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification of the secondary site's PI of the event.

The research team at a secondary site is responsible for following its site's guidelines for reporting applicable events to its site's IRB according to its own institutional guidelines.

10.8 Reporting to Secondary Sites

The Washington University PI (or designee) will notify the research team at each secondary site of all reportable events that have occurred at other sites (as described in Section 7.6) within **10 working days** of the occurrence of the event or notification of the PI of the event. This includes events that take place both at Washington University and at other secondary sites, if applicable.

10.9 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. **PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC.** It is the responsibility of the investigator to report any unanticipated problem to the FDA as follows:

- Report any unexpected fatal or life-threatening adverse experiences associated with use of the drug (i.e., there is a reasonable possibility that the experience may have been caused by the drug) by telephone or fax no later than **7 calendar days** after initial receipt of the information. A life-threatening adverse experience is defined as any adverse drug experience that places the subject (in the view of the investigator) at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Report any serious, unexpected adverse experiences, as well as results from animal studies that suggest significant clinical risk within **15 calendar days** after initial receipt of this information. A serious adverse drug experience is defined as any adverse drug experience occurring at any dose that results in any of the following outcomes:
    - Death
    - A life-threatening adverse drug experience
    - Inpatient hospitalization or prolongation of existing hospitalization
    - A persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions)
    - A congenital anomaly/birth defect
    - Any other experience which, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

  An unexpected adverse drug experience is defined as any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure (or risk information, if an IB is not required or available).

All MedWatch forms will be sent by the investigator or investigator's team to the FDA at the following address or by fax:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Oncology Drug Products
5901-B Ammendale Rd.
Beltsville, MD 20705-1266
FAX: 1-800-FDA-0178

Secondary sites must submit a completed MedWatch form to the Washington University PI and research coordinator within **4 calendar days** (for fatal or life-threatening adverse experiences) or **11 calendar days** (for serious, unexpected adverse experiences). The Washington University PI will be responsible for submitting all MedWatch forms from secondary sites to the FDA within the timeframes specified above.

10.10 Timeframe for Reporting Required Events:

Reportable adverse events will be tracked for 30 days following the last day of study treatment, or until the start of a new chemotherapy/treatment, whichever comes first.

Deaths	
Any reportable death while on study or within 30 days of study	Immediately, within 24 hours, to PI and the IRB
Any reportable death while off study	Immediately, within 24 hours, to PI and the IRB
Adverse Events/Unanticipated Problems	
Any reportable adverse events as described in Sections 10.1 and 10.2 (other than death) and 10.7	Immediately, within 24 hours to PI and within 10 working days to the IRB, and within 7 or 15 days to the FDA
All adverse events are to be reported per protocol regardless of attribution should be submitted cumulatively	Include in DSM report
Noncompliance and Serious Noncompliance	
All noncompliance and serious noncompliance as described in Sections 10.3 and 10.4	Immediately, within 24 hours, to PI and within 10 working days to the IRB

11.0 DATA SAFETY MONITORING PLAN

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months following activation of the first secondary site.

The Principal Investigator will review all patient data at least every six months, and provide a semi-annual report to the QASMC. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual including numbers from participating sites
- Protocol activation date at each participating site
- Average rate of accrual observed in year 1, year 2, and subsequent years at each participating site
- Expected accrual end date and accrual by site
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities at all participating sites
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

Until such a time as the first secondary site activates this protocol, a semi-annual DSM report to be prepared by the study team will be submitted to the QASM Committee beginning 6 months after study activation at Washington University.

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

12.0 AUDITING

Since Washington University is the coordinating center, each site will be audited annually by Siteman Cancer Center personnel (QASMC) unless the outside institution has an auditing mechanism in place and can provide a report. The outside sites will be asked to send copies of all audit materials, including source documentation. The audit notification will be sent to the Washington University Research Patient Coordinator, who will obtain the audit materials from the participating institution.

Notification of an upcoming audit will be sent to the research team one month ahead of the audit. Once accrual numbers are confirmed, and approximately 30 days prior to the audit, a list of the cases selected for review (up to 10 for each site) will be sent to the research team. However, if during the audit the need arises to review cases not initially selected, the research team will be asked to provide the additional charts within two working days.

Additional details regarding the Auditing Policies and procedures can be found at http://www.siteman.wustl.edu/uploadedFiles/Research_Programs/Clinical_Research_Resources/Protocol_Review_and_Monitoring_Committee/QASMCQualityAssurcance.pdf

13.0 MULTICENTER REGULATORY REQUIREMENTS

Washington University requires that each participating site sends its informed consent document to be reviewed and approved by the Washington University Regulatory Coordinator (or designee) prior to IRB/IEC submission.

Each participating institution must have the following documents on file at Washington University prior to first subject enrollment:

- Documentation of IRB approval of the study in the form of a letter or other official document from the participating institution's IRB. This documentation must show which version of the protocol was approved by the IRB.
- Documentation of IRB approval of an informed consent form. The consent must include a statement that data will be shared with Washington University, including the Quality Assurance and Safety Monitoring Committee (QASMC), the DSMC (if applicable), and the Washington University study team.
- Documentation of FWA, signed FDA Form 1572, and signed and dated CVs of all participating investigators.
- Documentation of training in protection of human subjects by all participating investigators.
- Protocol signature page signed and dated by the investigator at each participating site.

The Principal Investigator is responsible for disseminating to the participating sites all study updates, amendments, reportable adverse events, etc. There will be one current version of the protocol document at any given time and each participating institution will utilize that document. Protocol/consent modifications and IB updates will be forwarded electronically to the secondary sites within 2 weeks of obtaining Washington University IRB approval with acknowledgement of receipt requested. Secondary sites are to submit protocol/consent/IB modifications to their local IRBs within 4 weeks of receipt, and confirmation of submission must be forwarded to the appropriate contact person on the Washington University study team at the time of submission. Upon the secondary sites obtaining local IRB approval, documentation of such shall be sent to the Washington University study team within 2 weeks of receipt of approval.

Documentation of participating sites' IRB approval of annual continuing reviews, protocol amendments or revisions, all SAE reports, and all protocol violations/deviations/exceptions must be kept on file at Washington University.

The investigator or a designee from each institution must participate in a regular conference call to update and inform regarding the progress of the trial.

14.0 STATISTICAL ANALYSIS METHODS AND SAMPLE SIZE

14.1 Analysis Populations

Intent-to-Treat - The intent-to-treat (ITT) population will be defined as all patients who receive at least one dose of decitabine.

Efficacy-Evaluable - The efficacy-evaluable (EE) population will be defined as all patients who receive at least one cycle of treatment.

Safety - The safety population will be defined as all patients with at least one dose of decitabine.

14.2 Statistical Analysis

14.2.1 Descriptive Analyses

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

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.

14.2.2 Primary Endpoint Analysis

The primary end-point of this phase II study is the correlation biomarkers with overall response in AML/MDS patients to decitabine.

We will correlate the presence of patient specific mutations (especially mutations in *DNMT3A, IDH1, IDH2,* and *TET2*) with overall response rates. Mutation rates in these genes and in other patient-specific genes will be correlated with overall response and their respective 95% confidence intervals will be provided.

Statistical considerations. The relationship between AML/MDS-associated mutations and the responsiveness to decitabine will be described by contingency tables and assessed using Chi-square test or Fisher's exact test as appropriate. The differentially expressed genes between responders and non-responders will be determined using the significance analysis of microarray (SAM). Multivariate logistic regression will also be fit to adjust the potential confounding effects of other demographic and clinical characteristics. The resultant critical *P* values will be corrected for multiple testing. Since the Bonferroni correction is known to be too conservative, a permutation analysis (e.g., 10,000 random assignments for

responsive status) will be used to derive an empirical critical *P* value. The new critical p-value is the number of permutation tests which yield a lower p-value than the original test divided by the total number of permutation tests.[61]

A power analysis was performed using Chi-square test for the association between mutation and response to decitabine. The frequency of *DNMT3A, IDH1/2,* and *TET2* mutations in AML and MDS patients are each between 15% and 30% in multiple cohorts.[29-37] **Table 3** shows the minimal detectable differences of response rates between mutant and wild-type cases, assuming an expected overall 45% CR/CRi rate and 80% power. For an average of 20% frequency of a mutated gene, for example, 100 patients will provide 80% power to detect 30% difference at a 2-sided 0.1 alpha error. The purpose of this study is to determine whether any of these mutations result in a clinically significant association that would warrant pre-treatment testing. We believe that a reasonable cut off would be if at least 30% of clinical response could be attributed to the presence of a specific mutation. However, we anticipate that more power could be achieved as we assess patient groups with combined characteristics (e.g. *DNMT3A* mutation or loss of *DNMT3A* expression). In addition, we expect that the majority of the enrolled 125 patients can be used in the analysis (since all of our molecular information will be acquired within 30 days of enrollment, we anticipate a drop-out rate of 20% or less).

Table 3. Detectable mutation associated response rates (80% power, $\alpha = 0.05$)

Total N	% of patients with a given mutation	Detectable response difference	Sensitivity of mutation to predict response at minimum detectable response difference*
100	15%	0.40	80%
	20%	0.35	75%
	30%	0.30	67%
	40%	0.27	61%
	50%	0.26	58%

* Assuming the overall response rate (CR/Cri) of 45%

14.2.3 Secondary Endpoint Analyses

The overall response rate (CR/CRi/PR) and complete response rate (CR/CRi) will be compared with historical controls[2], and the 95% confidence intervals will be provided.

The rate of patient specific mutation clearance after 10, 28, and 56 days of therapy will be determined using an Illumina based platform. We will compare the rate of mutation clearance between the patients who achieve a CR/CRi after 4 cycles and those who do not. We anticipate that we will identify between 6 and 25 exonic SNVs per case (the range obtained with >200 AML samples sequenced to date with whole genome or exome sequencing), and that the expected 95% confidence interval of the mean variant allele frequency will be ±2.56% and ±1.25%,

respectively, if we assume an anticipated standard deviation of 3.2% within the same clone. The change of tumor burden over time and its association with clinical response will be assessed using 2-way ANOVA for repeated measurement data, followed by the ad-hoc multiple comparisons for between-group differences (e.g., responders vs. non-responders) at given time points as well as the between-time differences within each group. The resultant critical *P* values will also be corrected using permutation analysis for multiple testing.

The steady-state plasma decitabine concentration on day 4 ± 1 will be measured and correlated with clinical overall response.

The percent decrease in bone marrow methylcytosine will be calculated on day 10±1. We will compare this reduction with previously published data and between the patients who achieve CR/CRi and those who do not. The correlation of peak plasma levels of decitabine with response will be assessed using 2-sample t-test or Mann-Whitney non-parametric rank-sum test as appropriate. The change of total bone marrow DNA methylcytosine from baseline and its association with both steady-state plasma drug levels and response will be assessed using 2-way ANOVA for repeated measurement data, followed by the ad-hoc multiple comparisons for between-group differences (e.g., responders vs. non-responders) and between-time differences. Similar analyses will be performed for the correlation of expression profiles and SNP analysis with pharmacokinetic data.

Statistical power justification. A power analysis was performed using two-sample t-test for between-group difference and paired t-test for the change over time. For tumor clearance, this will provide > 99% power to detect a 10% difference in the means at two separate time points for clones with 6 or more variants, and > 98% power to detect a 15% difference if there are 3 or more variants per clone with $\alpha = 0.05$ (power will decrease to 91% and 82%, respectively, in cases where the standard deviation is 4.7%, the maximum we have observed thus far). For pharmacologic correlations, we anticipate 85% power to determine a 25 ng/ml difference between the average C_{max} of 45 evaluable responders and 55 evaluable non-responders with an anticipated average C_{max} of 70 ng/ml and a standard deviation of 40 ng/ml within the entire cohort, and with $\alpha = 0.05$.[22] We believe a less significant difference would not be sufficient to warrant application in clinical decision making (i.e. whether to continue therapy for more than 1 cycle). We anticipate 99% power to determine a difference of 20% in the reduction of bone marrow methylcytosine between 45 evaluable responders and 55 evaluable non-responders with an expected standard deviation of 15% and $\alpha = 0.05$.[28, 66-68] The difference in methylcytosine reduction previously observed was 30% between patients achieving CR/PR and those with only hematologic improvement.[28]

Time to relapse and time to death will also be listed for each patient.

14.2.4 Safety Analysis

Extent of Exposure – Duration (number of cycles/infusions) of decitabine doses (cumulative and intensity) will be summarized.

Adverse Events - All adverse events will be coded using NCI CTCAE v. 4.0. 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. In addition, adverse events will be summarized by investigator attribution of relationship to study medication and by grade. Similar summaries will be provided by treatment cycle.

Clinical Laboratory Evaluation – Applicable laboratory parameters will be graded according to NCI CTCAE version 4.0. The incidence of maximum grade (number and percent of patients experiencing the maximum grade during the study) will be summarized for each laboratory parameter. Similar summaries will be provided by treatment cycle. Graphical displays of select hematological parameters will also be provided.

Vital Signs, Physical Findings, and Other Safety Observations – Vital signs, findings from physical examinations, ECOG scores and weights will be presented in listing format.

14.2.5 Description of Planned Subgroup Analyses

The following subgroups (if sufficient numbers of patients) will be explored with respect to the primary and overall survival endpoints.

- Age (60-69 years old vs ≥ 70 years old)
- Cytogenetics
- Patients with AML-associated mutations in *DNMT3A, TET2, IDH1,* or *IDH2* vs those who do not have somatic variants in these genes.

14.3 Sample Size

We anticipate initially enrolling 25 patients at Washington University. Once we establish a routine exome analysis with validation and calculation of the rate of patient-specific mutation clearance, we will enroll an additional 100 patients from at least 2 additional centers.

15.0 DATA SUBMISSION SCHEDULE

Case Report Form	Submission Schedule
Original Consent Form	*Prior to registration*
Patient Registration Form *Disease Diagnosis Form* *Inclusion Criteria Worksheet* *Exclusion Criteria Worksheet*	*Prior to starting treatment*
Treatment Record Form	*To be maintained throughout a patient's treatment cycle. The complete record is due at completion of treatment.*
Bone Marrow Sample Collection Form	*To be maintained throughout the course of the study at designated bone marrow sample collections time points (See Appendix 2).*
Pathology: Bone Marrow Biopsy	*To be completed upon the return of pathology report for each bone marrow biopsy.*
Study Calendar Form	*To be completed with the date of each time point upon occurrence.*
Labs Form	*To be completed upon the return of laboratory reports for each study time point (See Appendix 2)*
Dose Modifications	*To be maintained throughout the course of the study if dose modifications are performed.*
Therapy Response Form	*To be filled out upon remission (prior to, and/or on therapy)*
End of Study Form	*Due at the completion of the EOS visit, at the time of death, and/or at the time that the patient is lost to follow-up*
Follow Up Form	• *30 days post date of last decitabine dose.* • *Every 6 months for 2 years after the last dose of study drug.*
Record of Adverse Events	*At the time of any AE*

Any queries generated by Washington University must be responded to within 28 days of receipt by the participating site. The Washington University research team will conduct a regular review of data status at all secondary sites, with appropriate corrective action to be requested as needed.

Appendix 1 ECOG Performance Scale

Activity Status	Description
0	Asymptomatic, fully active, and able to carry on all predisease performance without restrictions.
1	Symptomatic, fully ambulatory but restricted in physically strenuous activity and able to carry out performance of a light or sedentary nature, eg, light housework, office work.
2	Symptomatic, ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours: in bed less than 50% of day.
3	Symptomatic, capable of only limited self-care, confined to bed or chair more than 50% of waking hours, but not bedridden.
4	Completely disabled. Cannot carry on any self-care. Totally bedridden.
5	Dead

Appendix 2 Study Calendar

Study Calendar

Test	Pre-study[1]	Base-line[2]	All Cycles Day 1	All Cycles Days 1-10[3]	Cycle 1 Day 4 ± 1	Cycle 1 Day 10 ± 1 and 28 ± 4	Every week	Every 2 weeks	Even cycles Day 28±4	End of Study
Informed Consent	X									
Medical History		X								
Physical Exam		X[a]	X[b]							X
Transfusion Requirements[c]		X[c]	Ongoing							
Bone Marrow Aspirate and Biopsy	X[d]					X[d]			X[d]	X[d]
Flow Cytometry[e]	X									
Peripheral blood for correlative studies					X					
Pregnancy test		X								
CBC with differential and platelets		X	X				X[f]			X
Serum Chemistries[g]		X	X					X[f]		X
Decitabine administration[h]				X						
Adverse Events			Ongoing							
Post-study follow-up										X[i]

[1] To be performed within 14 days of the first dose of decitabine.
[2] To be performed within 7 days of the first dose of decitabine.
[3] Decitabine may be reduced to Days 1-5 or Days 1-3. See Section 6.3 for details.
[a] To include height, weight, and ECOG.
[b] To include weight.
[c] Care should be given to record the frequency of transfusions from 8 weeks prior to first dose of study drug through the duration of the study.
[d] Bone marrow biopsy and aspirate should be performed at baseline, at Cycle 1 Day 10 ± 1, and at Day 28 ± 4 of Cycles 1, 2, 4 and 6 for clinical disease assessment. Patients who achieve with clinical response (CRi or better) and are receiving cycles every 6 weeks, will undergo bone marrow biopsies on day 42 ± 4. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression. Standard bone marrow procurement procedures will be followed for collection of the tissue. To include: correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics and FISH at all time points. A skin biopsy sample (collected under HRPO# 201011766) is required at pre-study, but may have been collected months or even years prior to first dose of decitabine.
[e] On bone marrow or peripheral blood.
[f] After Cycle 1, these labs may be obtained every other week (CBC) or once a month (Chemistries) at the discretion of the treating physician.
[g] Serum chemistries include bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, and LDH.
[h] Decitabine treatment will be 20 mg/m^2 over a 1-hour IV infusion on consecutive Days 1-10 every 28-day cycle. The dose will be calculated based on actual patient weight at the beginning of each cycle. Patients may be premedicated with lorazepam, prochlorperazine, palonosetron, or ondansetron at the treating physician's discretion.
[i] Patients will be followed for 30 days following the date of the last decitabine dose to monitor for toxicity, survival and response. Thereafter, the investigator or designee will record information on subsequent therapy, disease progression, and death, every 6 months for 2 years after first dose of study drug or until the patient is lost to follow-up.

16.0 REFERENCES

1. Cheson, B.D., et al., *Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia.* J Clin Oncol, 2003. **21**(24): p. 4642-9.
2. Cashen, A.F., et al., *Multicenter, phase II study of decitabine for the first-line treatment of older patients with acute myeloid leukemia.* J Clin Oncol, 2010. **28**(4): p. 556-61.
3. Blum, W., et al., *Clinical response and miR-29b predictive significance in older AML patients treated with a 10-day schedule of decitabine.* Proc Natl Acad Sci U S A, 2010. **107**(16): p. 7473-8.
4. Schlenk, R.F., et al., *Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia.* N Engl J Med, 2008. **358**(18): p. 1909-18.
5. Erba, H.P., *Has there been progress in the treatment of older patients with acute myeloid leukemia?* Best Pract Res Clin Haematol, 2010. **23**(4): p. 495-501.
6. Heaney, M.L. and D.W. Golde, *Myelodysplasia.* N Engl J Med, 1999. **340**(21): p. 1649-60.
7. Bennett, J.M., et al., *Proposals for the classification of the myelodysplastic syndromes.* Br J Haematol, 1982. **51**(2): p. 189-99.
8. Kantarjian, H., et al., *Results of intensive chemotherapy in 998 patients age 65 years or older with acute myeloid leukemia or high-risk myelodysplastic syndrome: predictive prognostic models for outcome.* Cancer, 2006. **106**(5): p. 1090-8.
9. Martin, M.G. and C.N. Abboud, *Induction therapy for elderly patients with acute myeloid leukemia.* Blood Rev, 2008. **22**(6): p. 311-20.
10. Bow, E.J., et al., *Therapy of untreated acute myeloid leukemia in the elderly: remission-induction using a non-cytarabine-containing regimen of mitoxantrone plus etoposide.* J Clin Oncol, 1996. **14**(4): p. 1345-52.
11. Goldstone, A.H., et al., *Attempts to improve treatment outcomes in acute myeloid leukemia (AML) in older patients: the results of the United Kingdom Medical Research Council AML11 trial.* Blood, 2001. **98**(5): p. 1302-11.
12. Stone, R.M., *The difficult problem of acute myeloid leukemia in the older adult.* CA Cancer J Clin, 2002. **52**(6): p. 363-71.
13. Liu Yin, J.A., et al., *Mitozantrone and cytosine arabinoside as first-line therapy in elderly patients with acute myeloid leukaemia.* Br J Haematol, 1991. **79**(3): p. 415-20.
14. Stone, R.M., et al., *Granulocyte-macrophage colony-stimulating factor after initial chemotherapy for elderly patients with primary acute myelogenous leukemia. Cancer and Leukemia Group B.* N Engl J Med, 1995. **332**(25): p. 1671-7.
15. Kantarjian, H., et al., *Decitabine improves patient outcomes in myelodysplastic syndromes.* Cancer, 2006. **106**(8): p. 1794-1803.

16. Lubbert, M., et al., *A multicenter phase II trial of Decitabine as first-line treatment of older AML patients judged unfit for induction chemotherapy.* Haematologica, 2011. **97**(3): p. 393-401.
17. Issa, J.P., et al., *Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies.* Blood, 2004. **103**(5): p. 1635-40.
18. Petti, M.C., et al., *Pilot study of 5-aza-2'-deoxycytidine (Decitabine) in the treatment of poor prognosis acute myelogenous leukemia patients: preliminary results.* Leukemia, 1993. **7 Suppl 1**: p. 36-41.
19. Wijermans, P.W., et al., *Continuous infusion of low-dose 5-Aza-2'-deoxycytidine in elderly patients with high-risk myelodysplastic syndrome.* Leukemia, 1997. **11**(1): p. 1-5.
20. Kantarjian, H., et al., *Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study.* Cancer, 2006. **106**(8): p. 1794-803.
21. Wijermans, P.W., et al., *An epigenetic approach to the treatment of advanced MDS; the experience with the DNA demethylating agent 5-aza-2'-deoxycytidine (decitabine) in 177 patients.* Ann Hematol, 2005. **84 Suppl 1**: p. 9-17.
22. Cashen, A.F., et al., *Pharmacokinetics of decitabine administered as a 3-h infusion to patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS).* Cancer Chemother Pharmacol, 2008. **61**(5): p. 759-66.
23. Chabot, G.G., J. Bouchard, and R.L. Momparler, *Kinetics of deamination of 5-aza-2'-deoxycytidine and cytosine arabinoside by human liver cytidine deaminase and its inhibition by 3-deazauridine, thymidine or uracil arabinoside.* Biochem Pharmacol, 1983. **32**(7): p. 1327-8.
24. Momparler, R.L., et al., *Cell cycle effects and cellular pharmacology of 5-aza-2'-deoxycytidine.* Cancer Chemother Pharmacol, 1984. **13**(3): p. 191-4.
25. Raza, A., et al., *Biological significance of cell cycle kinetics in 128 standard risk newly diagnosed patients with acute myelocytic leukemia.* Br J Haematol, 1991. **79**(1): p. 33-9.
26. Brons, P.P., et al., *Proliferation patterns in acute myeloid leukemia: leukemic clonogenic growth and in vivo cell cycle kinetics.* Ann Hematol, 1993. **66**(5): p. 225-33.
27. Raza, A., et al., *Direct relationship between remission duration in acute myeloid leukemia and cell cycle kinetics: a leukemia intergroup study.* Blood, 1990. **76**(11): p. 2191-7.
28. Shen, L., et al., *DNA methylation predicts survival and response to therapy in patients with myelodysplastic syndromes.* J Clin Oncol, 2010. **28**(4): p. 605-13.
29. Tefferi, A., et al., *IDH1 and IDH2 mutation studies in 1473 patients with chronic-, fibrotic- or blast-phase essential thrombocythemia, polycythemia vera or myelofibrosis.* Leukemia, 2010. **24**(7): p. 1302-9.
30. Ley, T.J., et al., *DNMT3A mutations in acute myeloid leukemia.* N Engl J Med, 2010. **363**(25): p. 2424-33.
31. Mardis, E.R., et al., *Recurring mutations found by sequencing an acute myeloid leukemia genome.* N Engl J Med, 2009. **361**(11): p. 1058-66.

32. Abdel-Wahab, O., J. Patel, and R.L. Levine, *Clinical Implications of Novel Mutations in Epigenetic Modifiers in AML.* Hematol Oncol Clin North Am, 2011. **25**(6): p. 1119-33.
33. Reuther, G.W., *Recurring mutations in myeloproliferative neoplasms alter epigenetic regulation of gene expression.* Am J Cancer Res, 2011. **1**(6): p. 752-62.
34. Paschka, P., et al., *IDH1 and IDH2 mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3 internal tandem duplication.* J Clin Oncol, 2010. **28**(22): p. 3636-43.
35. Shen, Y., et al., *Gene mutation patterns and their prognostic impact in a cohort of 1185 patients with acute myeloid leukemia.* Blood, 2011. **118**(20): p. 5593-603.
36. Tefferi, A., et al., *Detection of mutant TET2 in myeloid malignancies other than myeloproliferative neoplasms: CMML, MDS, MDS/MPN and AML.* Leukemia, 2009. **23**(7): p. 1343-5.
37. Walter, M.J., et al., *Recurrent DNMT3A mutations in patients with myelodysplastic syndromes.* Leukemia, 2011. **25**(7): p. 1153-8.
38. Lin, J., et al., *Recurrent DNMT3A R882 Mutations in Chinese Patients with Acute Myeloid Leukemia and Myelodysplastic Syndrome.* PLoS One, 2011. **6**(10): p. e26906.
39. Bejar, R., et al., *Clinical effect of point mutations in myelodysplastic syndromes.* N Engl J Med, 2011. **364**(26): p. 2496-506.
40. Walter, M.J., et al., *Clonal architecture of secondary acute myeloid leukemia.* N Engl J Med, 2012. **366**(12): p. 1090-8.
41. Kantarjian, H.M., et al., *Survival advantage with decitabine versus intensive chemotherapy in patients with higher risk myelodysplastic syndrome: comparison with historical experience.* Cancer, 2007. **109**(6): p. 1133-7.
42. Lubbert, M., et al., *Continued Low-Dose Decitabine (DAC) Is an Active First-Line Treatment in All Cytogenetic Subgroups of Older AML Patients: Results of the FR00331 Multicenter Phase II Study.* ASH Annual Meeting Abstracts, 2007. **110**(11): p. 300-.
43. Blum, W., et al., *Phase I Study of Decitabine Alone or in Combination With Valproic Acid in Acute Myeloid Leukemia.* J Clin Oncol, 2007. **25**(25): p. 3884-3891.
44. Issa, J.-P.J., et al., *Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies.* Blood, 2004. **103**(5): p. 1635-1640.
45. Garcia-Manero, G., et al., *Phase 1/2 study of the combination of 5-aza-2'-deoxycytidine with valproic acid in patients with leukemia.* Blood, 2006. **108**(10): p. 3271-3279.
46. Issa, J.P., S.B. Baylin, and J.G. Herman, *DNA methylation changes in hematologic malignancies: biologic and clinical implications.* Leukemia, 1997. **11 Suppl 1**: p. S7-11.
47. Ganetzky, R.D., et al., *Differences Between Normal and Leukemic Stem Cell-Specific Methylome Indicates Aberrantly Silenced Genes Involved in the*

Pathogenesis of Malignant Evolution. ASH Annual Meeting Abstracts, 2008. **112**(11): p. 599-.

48. Christman, J.K., *5-Azacytidine and 5-aza-2'-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy.* Oncogene, 2002. **21**(35): p. 5483-95.
49. Lubbert, M., *DNA methylation inhibitors in the treatment of leukemias, myelodysplastic syndromes and hemoglobinopathies: clinical results and possible mechanisms of action.* Curr Top Microbiol Immunol, 2000. **249**: p. 135-64.
50. Kantarjian, H., et al., *Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia.* Blood, 2007. **109**(1): p. 52-57.
51. Steensma, D.P., et al., *Multicenter Study of Decitabine Administered Daily for 5 Days Every 4 Weeks to Adults With Myelodysplastic Syndromes: The Alternative Dosing for Outpatient Treatment (ADOPT) Trial.* J Clin Oncol, 2009: p. JCO.2008.19.6550.
52. Cashen, A.F., et al., *Preliminary Results of a Multicenter Phase II Trial of 5-Day Decitabine as Front-Line Therapy for Elderly Patients with Acute Myeloid Leukemia (AML).* ASH Annual Meeting Abstracts, 2008. **112**(11): p. 560-.
53. Jabbour, E., et al., *Evolution of decitabine development: accomplishments, ongoing investigations, and future strategies.* Cancer, 2008. **112**(11): p. 2341-51.
54. Kantarjian, H., et al., *Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia.* Blood, 2007. **109**(1): p. 52-7.
55. Blum, W.G., Klisovic, R., Liu, S., Kefauver, C., Grever, M. R., Schaaf, L., Chan, K., Byrd, J. C., Villalona-Calero, M., Marcucci, G. *Efficacy of a novel schedule of decitabine in previously untreated AML, age 60 or older*. in *ASCO Annual Meeting*. 2009.
56. van Groeningen, C.J., et al., *Phase I and pharmacokinetic study of 5-aza-2'-deoxycytidine (NSC 127716) in cancer patients.* Cancer Res, 1986. **46**(9): p. 4831-6.
57. Rivard, G.E., et al., *Phase I study on 5-aza-2'-deoxycytidine in children with acute leukemia.* Leuk Res, 1981. **5**(6): p. 453-62.
58. Lin, K.T., R.L. Momparler, and G.E. Rivard, *Sample preparation for the determination of 5-aza-2'-deoxycytidine in plasma by high-performance liquid chromatography.* J Chromatogr, 1985. **345**(1): p. 162-7.
59. Momparler, R.L., G.E. Rivard, and M. Gyger, *Clinical trial on 5-aza-2'-deoxycytidine in patients with acute leukemia.* Pharmacol Ther, 1985. **30**(3): p. 277-86.
60. Raska, K., Jurovcik, M., Sormova., Z., *On the metabolism of 5-azacytidine and 5-aza-2'-deoxycytidine in mice.* Coll Czech Chem Commun, 1965. **30**: p. 3001-3005.
61. Momparler, R.L., et al., *Pilot phase I-II study on 5-aza-2'-deoxycytidine (Decitabine) in patients with metastatic lung cancer.* Anticancer Drugs, 1997. **8**(4): p. 358-68.

62. Aparicio, A., et al., *Phase I trial of continuous infusion 5-aza-2'-deoxycytidine.* Cancer Chemother Pharmacol, 2003. **51**(3): p. 231-9.
63. Bryan, J., et al., *Pharmacokinetic evaluation of decitabine for the treatment of leukemia.* Expert Opin Drug Metab Toxicol, 2011. **7**(5): p. 661-72.
64. Scandura, J.M., et al., *Phase 1 study of epigenetic priming with decitabine prior to standard induction chemotherapy for patients with AML.* Blood, 2011. **118**(6): p. 1472-80.
65. Westfall, P.H. and S.A. Young, *Resampling-based multiple testing: examples and methods for p-value adjustment*. 1993, New York: John Wiley & Sons, Inc.
66. Samlowski, W.E., et al., *Evaluation of a 7-day continuous intravenous infusion of decitabine: inhibition of promoter-specific and global genomic DNA methylation.* J Clin Oncol, 2005. **23**(17): p. 3897-905.
67. Blum, W., et al., *Phase I study of decitabine alone or in combination with valproic acid in acute myeloid leukemia.* J Clin Oncol, 2007. **25**(25): p. 3884-91.
68. Yang, A.S., et al., *DNA methylation changes after 5-aza-2'-deoxycytidine therapy in patients with leukemia.* Cancer Res, 2006. **66**(10): p. 5495-503.
69. Cheson, B.D., et al., *Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia.* Blood, 2006. **108**(2): p. 419-25.

Genomic Predictors of Decitabine Response in AML/MDS

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Genomic Predictors of Decitabine Response in AML/MDS

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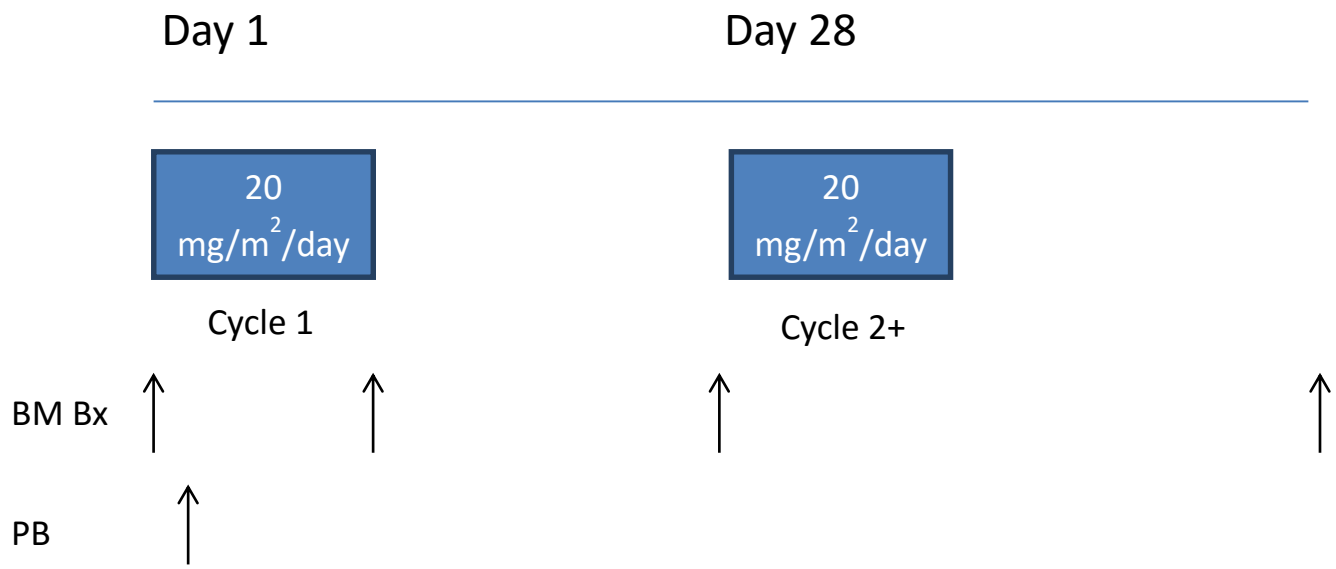
CONFIDENTIAL

Genomic Predictors of Decitabine Response in AML/MDS
Principal Investigator Signature Page

Principal Investigator:	John Welch, M.D., Ph.D.	
	Signature of Investigator	Date
	Printed Name of Investigator	
	By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IRB/HRPO procedures, the Declaration of Helsinki, ICH Good Clinical Practices guidelines, and the applicable parts of the United States Code of Federal Regulations or local regulations governing the conduct of clinical studies.	

Study Schema:



Patients with progressive disease after two cycles (defined as a clear increase in bone marrow blast count, typically a > 50% increase in bone marrow blasts over baseline) should be removed from study.

Patients with stable disease may continue on study and should undergo a diagnostic bone marrow biopsy at the end of even numbered cycles.

Once AML patients achieve morphologic complete remission with incomplete blood count recovery (CRi) or MDS patients achieve a marrow complete response (Marrow CR) as defined in Section 9.0, decitabine administration may be decreased to 20 mg/m2/day for ten consecutive days every 6 weeks, to limit myelosuppressive toxicity and improve count recovery. After 2 cycles at 6 week intervals, the dose may be reduce to 20 mg/m2/day for five consecutive days in 6 week cycles, at the discretion of the treating physcian. After two cycles of five consecutive days of treatment, if the patient meets the definition of CR with complete cytogenetic response as defined in Section 9.0, administration may further be decreased to three consecutive days per cycle at the discretion of the treating physician.

Sample collection for correlative studies:

Bone marrow biopsies should be performed at baseline, at Cycle 1 Day 10 ± 1, and at Day 28 ± 4 of Cycles 1, 2, 4, and 6. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression.

Peripheral blood samples should be collected on Day 4± 1 of Cycle 1. Collection should be obtained 45 ± 15 minutes after decitabine infusion is initiated.

Genomic Predictors of Decitabine Response in AML/MDS

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1.0 INTRODUCTION

1.1 Rationale

Personalized therapy for cancer requires that 1) patient-specific factors (e.g. patient-specific mutations, expression profiles, or pharmacogenomics) influence treatment algorithms, and 2) once therapy is initiated, *in vivo* responsiveness is used to guide further therapy. The modern era of genomics has provided significant insights into cancer pathogenesis by describing patterns of germline and somatic variants, as well as defining disease-specific patterns of gene expression. However, the application and integration of this data with therapeutics remains a difficult task.

Decitabine is a commonly used single-agent therapy for patients with acute myeloid leukemia (AML) and/or myelodysplastic syndrome (MDS). Historically, it has yielded clinical complete responses in approximately 25% of patients across diverse clinical trials, it is well tolerated, and it can be given as in the outpatient setting. However, the majority of patients do not respond to decitabine, the molecular etiology of decitabine sensitivity is unknown, and there are no algorithms to predict patient responsiveness *a priori* or even within one month of starting therapy.

AML and MDS are two closely related hematological disorders characterized by ineffective hematopoiesis and malignant expansion of clonal myeloid cells. Both AML and MDS frequently occur in elderly patients and some cases are secondary and/or related to long-term bone marrow damage after radiation or chemotherapy. Current therapy for AML consists of cytotoxic chemotherapy followed by consolidation chemotherapy or stem cell transplantation. Using these approaches, current cure rates may be as high as 60% for patients younger than 60 with good risk cytogenetics (e.g. t(15;17), t(8;21), inv(16)).[4] Outcomes in patients ≥ 60 years old and with relapsed disease remain poor with 2 year survival of only ~25%.[5] Historically, the available treatment for MDS has been symptomatic and has not been shown to be effective in producing sustained improvement in hematopoiesis or in delaying leukemic evolution[6, 7].

Current AML therapy is poorly tolerated in elderly and pre-treated patients due to hematologic and non-hematologic toxicities; following such therapy, patients typically require 3-4 weeks of hospitalization for treatment of neutropenic fevers and for regular transfusions. Even among the relatively healthy patients enrolled in clinical trials, induction mortality in the elderly can be as high as 20-40% following standard induction therapy,[8-11] and moderate to severe non-hematologic toxicity occurs in at least an additional 30% of patients.[9, 12-14] Consequently, many older AML patients are not offered traditional intensive chemotherapy, but receive supportive care alone.[15] More effective and less toxic therapies are needed to provide durable remissions in a significant proportion of elderly AML in populations.

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We and others have recently used whole genome sequencing to identify recurrent, somatic mutations in AML and MDS patients in four genes that are involved in methylcytosine regulation: *DNMT3A, IDH1, IDH2,* and *TET2.*[29-37] Collectively, nearly half of all AML/MDS patients have a mutation in one of these four genes. *DNMT3A* mutations are associated with an adverse outcome in AML patients treated with cytotoxic chemotherapy.[35, 38] However, it is not yet clear whether the presence of mutations in one or more of these genes is associated with response or resistance to decitabine.

In this study, we will treat patients with the current state-of-the-art decitabine schedule.[3] We will perform exome sequencing on all patients and correlate response with mutation status, rate of mutation clearance, expression profile, plasma decitabine levels, and DNA methylcytosine content reduction. This approach will comprehensively assess genomic and pharmacologic parameters that could clinically predict patient response to decitabine.

In this study, we will combine analysis of patients with AML and MDS. Recent genomic analysis has shown that AML and MDS have shared mutations in many of the same genes, shared patterns of mutation acquisition, and shared patterns of mutation evolution.[39, 40] Furthermore, MDS, like AML, presents with clonal hematopoiesis, even when the morphologic blast count is zero.[36]Because this study will identify and track the rate of clearance of patient specific mutations, and because decitabine has been shown to have efficacy in both AML and MDS patients, [2, 3, 16-19, 41-45] combining analysis of AML and MDS patients is reasonable. Asymptomatic MDS patients are excluded because these patients are typically followed with watchful-waiting rather than with chemotherapy.

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1.3 Correlative Studies

With exception of the peripheral blood sample collected on Cycle 1 Day 4, all correlative studies will be collected under HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Diseases") that all patients will be concurrently enrolled in. The goals of the correlative studies include:

1. To correlate patient-specific mutations with response to decitabine.
   We will perform exome sequencing of each patient's pre-study bone marrow sample and skin sample. We will determine AML/MDS-associated variants found in the bone marrow, but not skin sample. We will correlate the presence of mutations with response (especially mutations in *DNMT3A, IDH1, IDH2,* and *TET2*). This study is powered to identify mutations with >80% sensitivity to predict decitabine response if used as a clinical assay.

2. To correlate the velocity and depth of response with response to decitabine.
   We will assess tumor burden by allele frequency of patient-specific mutations in bone marrow samples collected pre study, Cycle 1 Day 10±1, Cycle 1 Day 28±4, and Cycle 2 Day 28±4. We will assess mutations in at least 24 recurrently mutated genes (including, but not limited to: *FLT3, DNMT3A, NPM1, TET2, WT1, IDH1, IDH2, RUNX1, NRAS, TP53, U2AF1, KRAS, PHF6, MSTP9, PTPN11, KIT, SMC3, PRUNE2, ETV6, CEBPA, SMC1A, ASXL1, STAG2, RAD21*) as well as all patient-

specific mutations discovered during exome sequencing. The leukemic allele frequency will be measured using the Illumnia MySeq or HiSeq platform using DNA capture by gene specific biotinylated-oligonucleotides. Patient bone marrow samples will be assessed pre-study, Cycle 1 Day 10 ± 1, Cycle 1 Day 28 ± 4, and Cycle 2 Day 28 ± 4 and compared with patient specific skin samples (normal control). The velocity of response will be calculated as the natural log rate of patient-specific mutation allele frequency clearance. Depth of response will be defined as the lowest patient-specific mutation allele frequency in a bone marrow sample. The velocity and depth of tumor response will be correlated to:

- Achievement of clinical CR,
- Time to best response,
- Mutational status,
- Time to disease progression.

Because all patient-specific exome-discovered mutations will be assessed a second time in the primary bone marrow sample as well as the Cycle 1 Day 10 ± 1, Cycle 1 Day 28 ± 4, and Cycle 2 Day 28 ± 4 bone marrow samples, this study will also serve to validate all exome-discovered mutations. This is important, because exome sequencing is associated with a 20-50% false-discovery rate depending on type I error tolerated (mutation-calling algorithms are typically set for greater sensitivity than specificity). Thus, many mutations discovered during exome sequencing are incorrect. This second analysis of these mutations will server to exclude these false positive calls.

3. To correlate the bone marrow expression profile from pre-study and Cycle 1 Day 10 ± 1 with response to decitabine.
   We will correlate expression signatures, and changes in expression signatures, with:
   - Achievement of clinical CR,
   - Mutation status.

4. To correlate steady-state plasma decitabine concentration on Cycle 1 Day 4 ± 1 with:
   - Achievement of CR.

5. To correlate the extent of bone marrow DNA hypomethylation on Cycle 1 Day 10 ± 1 with response to decitabine.
   We will assess the percent reduction in bone marrow methylcytosine content using LC-MS. Patients will be evaluated pre-study and Cycle 1 Day 10 ± 1. The change in DNA methylcytosine content will be correlated to:
   - Achievement of clinical CR,
   - Change in expression signature.

   This will determine whether inadequate blast exposure to decitabine correlates with drug resistance.

2.0 OBJECTIVES

2.1 Primary Objective

To determine whether patient-specific mutations correlate with clinical overall response to decitabine in patients with AML or MDS.

2.2 Secondary Objectives

- To determine whether 10 consecutive days per cycle of decitabine improves outcomes compared with historical controls treated with 5 consecutive days per cycle of decitabine.

- To determine whether other biomarkers correlate with clinical overall response to decitabine in patients with AML or MDS. These will include:

    1. The velocity and depth of patient-specific mutation clearance;
    2. The bone marrow expression profile and change in expression profile during decitabine treatment;
    3. The steady-state plasma decitabine concentrations;
    4. The decrease in bone marrow methylcytosine content in response to decitabine.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

One of the following:

- Patient must have non-M3 AML and be ≥ 60 years of age OR
- non-M3 AML with relapsed disease OR
- Symptomatic MDS with one of the following:
  - □ Symptomatic anemia with either hemoglobin < 10.0 g/dL or requiring RBC transfusion
  - □ Thrombocytopenia with a history of two or more platelet counts < 50,000/mcL or a significant hemorrhage requiring platelet transfusions
  - □ Neutropenia with two or more ANC < 1,000/mcL

All of the following:

1. Patient must have an ECOG performance status ≤ 2.
2. Patient must have >10% disease burden measured by cytomorphology, flow cytometry, or cytogenetics.
3. Patient must have peripheral white blood cell count ≤ 50,000/mcl.
4. Patient must have adequate organ function, defined as:
   a. Total bilirubin ≤ 1.5 x ULN
   b. AST/ALT ≤ 2.5 x ULN
   c. Serum creatinine ≤ 2.0 x ULN
5. Patient must have undergone ≤ 2 cycles of prior hypomethylating agent (decitabine or azacitidine).
6. Patient must be enrolled in HRPO# 201011766 (“Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Diseases”).
7. Patient must be ≥ 18 years of age.
8. Patient must be able to understand and willing to sign an IRB-approved written informed consent document.

3.2 Exclusion Criteria

1. Patient must not be pregnant or nursing.
2. Patient must not have known CNS leukemia.
3. Patient must not have a history of positive HIV serology.
4. Patient must not have a history of positive Hepatitis C serology.
5. Patient must not have undergone a prior allogeneic stem cell transplant.
6. Patient must not have any uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, ongoing or active GVHD, congestive heart failure of NYHA class 3 or 4, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situation that would limit compliance with study requirements.
7. Patient must not have had radiation therapy within 14 days of enrollment.
8. Patient must not have received any chemotherapy within 21 days of enrollment, and any acute treatment-related toxicities must have returned to baseline. Patients

may be receiving hydrea at time of enrollment.

3.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4.0 REGISTRATION PROCEDURES

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility by Washington University
2. Registration of patient in the Siteman Cancer Center database
3. Assignment of unique patient number (UPN)

Once the patient has been entered in the Siteman Cancer Center database, the WUSM coordinator will forward verification of enrollment and the UPN via email.

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility collecting the information listed below and scanning and emailing it to the research coordinator listed in the *Siteman Cancer Center Clinical Trials Core Protocol Procedures for Secondary Sites* packet at least one business day prior to registering patient:

1. Your name and contact information (telephone number, fax number, and email address)
2. Your site PI's name, the registering MD's name, and your institution name
3. Patient's race, sex, and DOB
4. Three letters (or two letters and a dash) for the patient's initials
5. Currently approved protocol version date
6. Copy of signed consent form (patient name may be blacked out)
7. Planned date of enrollment
8. Completed eligibility checklist, signed and dated by a member of the study team
9. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center Database

Registrations may be submitted Monday through Friday between 8am and 5pm CT. Urgent late afternoon or early morning enrollments should be planned in advance and coordinated with the Washington University research coordinator. Registration will be confirmed by the research coordinator or his/her delegate by email within one business day. Verification of eligibility and registration should be kept in the patient chart.

All patients at all sites must be registered through the Siteman Cancer Center database at Washington University.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. Patients will also be identified by first, middle, and last initials. If the patient has no middle initial, a dash will be used on the case report forms (CRFs). All data will be recorded with this identification number on the appropriate CRFs.

5.0 INVESTIGATIONAL PLAN

5.1 Summary of Study Design

This is an open-label, single-arm, Phase II, biomarker study of patients with AML or MDS who are treated with 10 consecutive days of decitabine. FAB-M3 AML is excluded from this trial, because other well-tolerated options exist for the treatment of FAB-M3 acute promyelocytic leukemia. AML patients who are < 60 years old and have not received cytotoxic induction chemotherapy are also excluded, because cytotoxic therapy offers a curative possibility for these patients. MDS patients without cytopenias are also excluded because these patients can be followed with watchful-waiting.

All patients will undergo bone marrow biopsies pre-study, on Cycle 1 Day 10 ± 1, and on Day 28 ± 4 of Cycles 1, 2, 4, and 6. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression. Patients will provide a skin biopsy sample pre-study. Patients will also provide peripheral blood samples on Cycle 1 Day 4 ± 1 for pharmacokinetic studies. See Section 8.0 for details on correlative studies.

Patients will receive decitabine 20 mg/m^2/day as a 1-hour infusion on consecutive Days 1-10 in 28-day cycles. The dose will be calculated based on actual patient weight at the beginning of each cycle.

Once AML patients achieve morphologic complete remission with incomplete blood count recovery (CRi) or MDS patients achieve a marrow complete response (Marrow CR) as defined in Section 9.0, decitabine administration may be decreased to five consecutive days to limit myelosuppressive toxicity at the discretion of the treating physician. After two cycles of five consecutive days of treatment, if the patient meets the definition of CR with complete cytogenetic response as defined in Section 9.0, administration may further be decreased to three consecutive days per cycle at the discretion of the treating physician.

It will be a logistical goal of this trial consistently to return preliminary exome results to the treating physician by the end of Cycle 1 and validated results by the end of Cycle 2. However, this logistical goal is not a primary or secondary aim of this study. Given the limited data regarding mutation presence and drug response, we do not anticipate that the results will directly influence therapeutic decisions made by the treating physician. However, the development of comprehensive genomic pipelines and integration of this data into a clinical trial setting will be required before personalized medicine can be realized.

5.2 Study Procedures

5.2.1 Pre-study Procedures

Pre-study procedures must take place no more than 14 days prior to the first dose of decitabine except where noted below.

1. Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics, and FISH.
2. Bone marrow or peripheral blood for flow cytometry
3. Skin biopsy sample (collected under HRPO# 201011766). There is no required time frame for this sample; it may have been collected months or even years prior to the first dose of decitabine.

5.2.2 Baseline Evaluation

Baseline evaluations must take place no more than 7 days prior to the first dose of decitabine.

1. Medical history
2. Physical exam including height, weight, and ECOG performance score.
3. Record transfusion requirements for 8 weeks before first dose of study drug
4. CBC with differential and platelets
5. Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH
6. Urine βHCG pregnancy test if appropriate

5.2.3 Day 1 of Each Cycle

1. Physical exam, including weight
2. CBC with differential and platelets
3. Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH

5.2.4 Cycle 1 Days 1-10

Decitabine administration, 20 mg/m^2 per day given as a 1-hour continuous intravenous infusion. The dose will be calculated based on actual patient weight at the beginning of each cycle. Patients may be premedicated with lorazepam, prochlorperazine, palonosetron, or ondansetron at the treating physician's discretion.

5.2.5 Cycle 1 Day 4 ± 1

Obtain 3 mL peripheral blood for correlative studies. Sample must be obtained 45 minutes ± 15 minutes after beginning the infusion, before the decitabine infusion

is complete, and should be obtained from the opposite arm as the infusion. The sample should be collected in a 3 mL K3EDTA tube pre-loaded with 8 mcL of tetrahydrouridine (THU 500 mcg/mL), an inhibitor of cytidine deaminase. The sample should be processed by the Tissue Procurement Core Facility at the Siteman Cancer Center. Plasma should be separated and stored per protocols.

5.2.6 Cycle 1 Day 10 ± 1

Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics, and FISH.

5.2.7 Weekly

CBC with differential and platelets. After the first cycle, this may be changed to every other week at the discretion of the treating physician.

5.2.8 Every Other Week

Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH. After the first cycle, this may be changed to every month at the discretion of the treating physician.

5.2.9 Cycles 1, 2, 4 and 6 Day 28 ± 4 days

Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include: correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics, and FISH.

Patients who achieve with clinical response (CRi or better) and are receiving cycles every 6 weeks, will undergo bone marrow biopsies on day 42 ± 4.

5.2.10 End-of-Study Procedures

1. Physical exam
2. CBC with differential and platelets.
3. Serum chemistries including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, and LDH.
4. Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include morphologic assessment, cytogenetics, and FISH.

5.2.11 Post-study Follow-up

Patients are to be followed according to standard patient care procedures for 30 days following the date of the last decitabine dose to monitor for toxicity, survival, and response. Thereafter, the investigator or designee will record information on subsequent therapy, disease progression, and/or death every 6 months for 2 years after last dose of study drug or until the patient is lost to follow-up or dies.

5.3 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented in the case report forms.

In the absence of treatment delays due to adverse events, treatment may continue indefinitely or until one of the following criteria applies:

- Documented and confirmed disease progression
- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious non-compliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study

Patients who prematurely discontinue treatment for any reason will be followed as indicated in the study calendar.

5.4 Concomitant Therapy and Supportive Care Guidelines

5.4.1 Chemotherapy

Patients cannot receive any chemotherapy within 21 days of study enrollment and must have recovered from any treatment-related toxicities. However, patients may be receiving hydrea at enrollment and may continue on hydrea through Cycle 2 of decitabine. An indication for hydrea following 2 cycles of decitabine is evidence that the patient is not responding and decitabine should be discontinued. Other than decitabine, patients cannot receive chemotherapy during this trial.

5.4.2 Growth Factors

Erythroid growth factors should not be routinely administered to patients during the study.

Thrombopoietin receptor agonists should not be routinely administered to patients during the study.

Filgrastim (G-CSF) or sargramostim (GM-CSF) may be administered to patients with septic shock or febrile neutropenia at the discretion of the treating physician.

5.4.3 Transfusions

- *Packed red blood cells (RBCs) transfusion*:
  RBC transfusions will be administered at the discretion of the treating physician.

- *Platelet transfusion*:
  Platelet transfusions will be administered at the discretion of the treating physician

5.4.4 Prophylactic Antimicrobial Agents

Antimicrobial agents will be administered at the discretion of the treating physician. Common antimicrobial prophylaxis during cycles 1 and 2 include acyclovir, ciprofloxacin, and fluconazole.

5.4.5 Radiotherapy

Patients cannot receive radiotherapy within 14 days prior to study enrollment or while on study.

6.0 DOSE MODIFICATIONS

A criterion for discontinuation of the study is a delay of > 8 weeks between any two cycles.

6.1 Delay for Cytopenia or Infection

As cytopenias are a defining feature of AML and MDS, their presence alone does not generally warrant dose modifications or delays. Patients will receive four cycles of therapy without adjustment for any cytopenia. Following Cycle 4, dose modifications or delays for cytopenias will be made at the discretion of the investigator.

Patients with febrile neutropenia (temperature ≥ 38.5° with ANC < 1,000/μl) or systemic infections should have treatment held until clinical resolution of the infection.

6.2 Delay for Organ Dysfunction

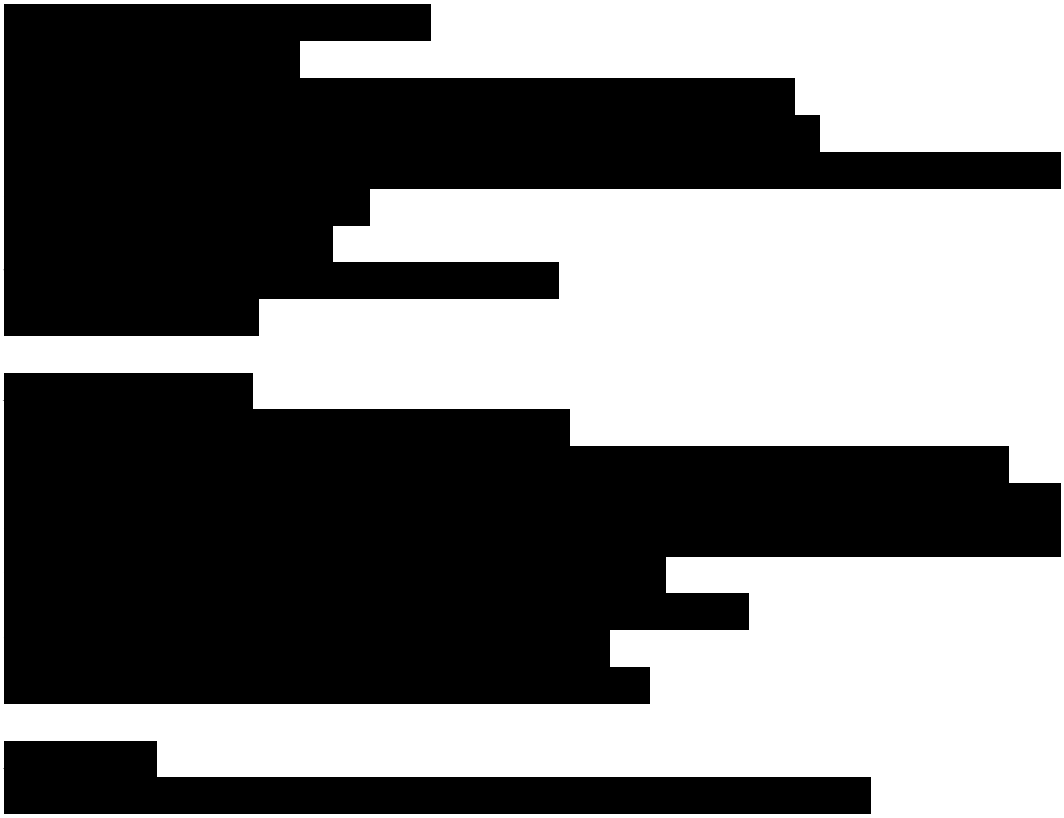
If renal dysfunction (creatinine ≥ 3 X institutional upper limit of normal) or hepatic dysfunction (bilirubin ≥ 2.5 mg/dl or ALT/AST ≥ 3 X institutional upper limit of normal) occurs, therapy may not resume until the abnormalities have resolved to below the limits stated above. If renal or hepatic dysfunction occurs during a treatment course, the next dose of decitabine should be held, and serum chemistries should be checked twice per week. Decitabine can be resumed at full dose when creatinine, bilirubin, ALT and AST are below the limits listed above.

For grade 3-4 non-hematologic toxicities related to decitabine, decitabine treatment should be delayed until recovery to baseline or to grade ≤ 1. Decitabine may be reintroduced with a 50% dose reduction, or at full dose at the discretion of the investigator.

6.3 Modification in Dose or Schedule for Treatment Response

Once AML patients achieve morphologic complete remission with incomplete blood count recovery (CRi) or MDS patients achieve a marrow complete response (Marrow CR) as defined in Section 9.0, decitabine administration may be decreased to 20 mg/m2/day for ten consecutive days, and the cycles may be administered every 6 weeks to limit myelosuppressive toxicity, at the discretion of the treating physician. After two cycles at six week intervals, the dose may be reduced to 20 mg/m2/day for five consecutive days of treatment in six week intervals. If the patient meets the definition of CR with complete cytogenetic response as defined in Section 9.0, administration may further be decreased to three consecutive days per cycle at the discretion of the treating physician.

[redacted]



8.0 CORRELATIVE STUDIES SAMPLE COLLECTIONS

Bone marrow samples will be collected pre-study, on Cycle 1 Day 10 ± 1, and on Day 28 ± 4 of Cycle 1, 2, 4, and 6 for correlative studies. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression. The bone marrow samples will be collected under HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Diseases") that all patients will be concurrently enrolled in.

Skin biopsy sample will be collected as part of the patient's participation in HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Malignancies"). There is no required time frame for this sample, and it may have been collected months or even years prior to the first dose of decitabine.

Peripheral blood sample (3 mL) will be collected on Cycle 1 Day 4 ± 1. Sample must be obtained 45 minutes ± 15 minutes after beginning the infusion, before the decitabine infusion is complete, and should be obtained from the opposite arm as the infusion. The sample should be collected in a 3 mL K3EDTA tube pre-loaded with 8 mcL of tetrahydrouridine (THU 500 mcg/mL), an inhibitor of cytidine deaminase. The sample should be processed by the Tissue Procurement Core Facility at the Siteman Cancer Center. Plasma should be separated and stored per protocols.

9.0 EFFICACY, SAFETY AND CORRELATIVE STUDY MEASURES

A secondary endpoint of this study is to estimate morphologic complete remission rate.

9.1 Response Criteria - AML

AML Patients will be assessed for response according to the IWG criteria:[1]

Morphologic complete remission (CR) – Defined as morphologic leukemia-free state, defined as less than 5% blasts in aspirate with marrow spicules and a count of ≥200 nucleated cells and no blasts with Auer rods or any persistence of extramedullary disease. When erythroid precursors constitute less than 50% of bone marrow nucleated cells, the percentage of blasts is based on all nucleated cells; when there are 50% or more erythroid cells, the percentage blasts should be based on the nonerythroid cells. The presence of mild megaloblastoid changes may be permitted if they are thought to be consistent with treatment effect. However, persistence of pretreatment abnormalities (eg, pseudo-Pelger-Hüet cells, ringed sideroblasts, dysplastic megakaryocytes) are not consistent with CR. In addition, ANC >1000/µl and platelet count of ≥100,000/µl. Patient must be independent of transfusions. No duration requirement for this designation.

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

Morphologic complete remission with incomplete blood count recovery (CRi) – Defined as morphologic complete recovery with the exception of neutropenia <1000/µl or thrombocytopenia <100,000/µl.

Partial remission (PR) – Defined as meeting the above criteria for complete remission, with the following exceptions: decrease of ≥50% in the percentage of blast to 5-25% in the bone marrow aspirate. A value of ≤5% blasts in bone marrow with Auer rods present may also be considered a partial remission.

Stable Disease (SD) – Defined as not meeting the definitions of CR, CRi, PR, PD, or recurrence/morphologic relapse.

Progressive disease (PD) – Defined as a clear increase in bone marrow blast count, typically a greater than 50% increase compared with baseline, or new extramedullary disease. Patients with a > 50% increase in peripheral blast count with an increase of the total peripheral white blood cell count to > 10,000/µl should undergo evaluation by bone marrow biopsy to access for progressive disease.

Recurrence/morphologic relapse **-** Defined as relapse following 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. New dysplastic changes is considered

relapse. 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.

9.2 Response Criteria - MDS

MDS Patients will be assessed for response according to the IWG criteria:[69]

Complete remission (CR) – Defined as ≤ 5% myeloblasts with normal maturation of all cell lines in the bone marrow and peripheral blood values of Hgb ≥ 11g/dL, Platelets ≥ 100 x 10^9/L, Neutrophils ≥ 1.0 x 10^9/L, and 0% blasts. Persistent dysplasia does not exclude CR but will be noted.

Marrow Complete Response (Marrow CR) – Defined as ≤ 5% myeloblasts in the bone marrow and a decrease by ≥ 50% from pretreatment values, but not meeting the definition of CR above.

Partial Remission (PR) – Defined as meeting the definition of CR above with a decrease of myeloeblasts in the bone marrow by ≥ 50% from pretreatment values, but absolute myeloblasts still >5%.

Stable Disease (SD) – Defined as not meeting the definitions of CR, Marrow CR, PR, SD, PD, or recurrence/morphologic relapse.

Progressive Disease//Relapse (PD) – Defined as ≥ 50% increase in blasts to > 5% blasts (for patients with less than 5% blasts at baseline only), ≥ 50% increase to > 10% blasts (for patients with 5-10% blasts at baseline only), ≥ 50% increase to > 20% blasts (for patients with l0-20% blasts at baseline only), ≥ 50% increase to > 30% blasts (for patients with 20-30% blasts at baseline only) or any of the following: At least 50% decrement from maximum remission/response in granulocytes or platelets, reduction in Hgb by ≥ 2 g/dL, or New or worsened transfusion dependence not related to study drug toxicity. Or for patients with a CR, Marrow CR, or PR as defined above and subsequently development of one of the following: Return to pretreatment bone marrow blast percentage, decrement of ≥ 50% from maximum remission/response levels in granulocytes or platelets, or reduction in Hgb concentration by ≥ 1.5 g/dL or transfusion dependence.

9.2.1 Hematologic Improvement (HI)

Progressive disease as defined above nullifies hematologic improvement.

Erythroid response requires all of the following (only required if pretreatment HgB <11 g/dL):

- Hgb increase by ≥ 1.5 g/dL
- Relevant reduction of units of RBC transfusions by an absolute number of at least 4 RBC transfusions/8 wk compared with the pretreatment transfusion

number in the previous 8 wk. Only RBC transfusions given for a Hgb of ≤ 9.0 g/dL pretreatment will count in the RBC transfusion response evaluation

Platelet response requires one of the following (only required if pretreatment platelets < 100 x 10^9/L):

- Absolute increase of ≥ 30 x 10^9/L (for patients starting with > 20 x 10^9/L platelets)
- Increase from < 20 x 10^9/L to > 20 x 10^9/L and absolute increase ≥ 100% (for patients starting with < 20 x 10^9/L)

Neutrophil response requires the following (only required if pretreatment ANC <1.0 x 10^9/L):

- At least 100% increase and an absolute increase > 0.5 x 10^9/L

9.3.1 Cytogenetic Response

Complete Cytogenetic Response – Defined as reversion to a normal karyotype. For this study, reversion of a normal karyotype is defined as no clonal abnormalities detected in a minimum of 20 mitotic cells. Progressive disease as defined above nullifies cytogenetic response.

9.3 Guidelines for Evaluation of Disease

Bone marrow biopsy should be performed on Day 28 ± 4 of cycles 1, 2, 4 and 6 for clinical disease assessment. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression.

9.4 Other Secondary Efficacy Measures

9.4.1 Treatment Failure, Overall Survival and Event-free Survival

Treatment failure is defined as failure to achieve a complete or partial remission following 4 cycles of therapy.

Overall survival is defined as the date of first dose of study drug to the date of death from any cause. Patients still alive at the time of the final analysis will be right-censored.

Event-free survival is defined as the interval from the date of first dose of study drug to date of treatment failure, recurrence, death due to any cause, or loss to follow up.

9.4.2 Duration of Remission and Relapse-free Survival for Patients Having Complete Remission

Duration of complete remission is defined as the interval from the date that complete remission is documented to the date of recurrence, death due to any cause, or loss 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.

9.4.3 Safety

Safety evaluation will include assessments of adverse experiences, medical history, physical examinations, vital signs, and laboratory assessments at baseline and throughout the study period. Local laboratories may be used for this study.

10.0 ADVERSE EVENT REPORTING

10.1 Adverse Events (AEs)

Definition: any unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site: http://ctep.cancer.gov/reporting/ctc.html. Grade 1-2 events will be recorded for the first fifty enrolled patients. Subsequently, only events graded as 3 or greater will be recorded. Fifty patients should be sufficient to estimate the true incidence of any adverse event to within ± 14% with 95% confidence intervals.
Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website: (http://www.hhs.gov/ohrp/policy/AdvEvntGuid.htm).

10.2 Unanticipated Problems

Definition:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

10.3 Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

10.4 Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

10.5 Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

Local IRB Pre-approval of all protocol exceptions must be obtained prior to the event. For secondary sites, the Washington University PI will issue approval of the exception, but it must also be submitted to the local IRB with documentation of approval forwarded to Washington University. HRPO approval is not required for protocol exceptions occurring at secondary sites.

10.6 Reporting to the Human Research Protection Office (HRPO) and the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University:

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH or SLCH institution, or that impacts participants or the conduct of the study.
- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within **10 working days** of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification to the PI of the event.

10.7 Reporting Requirements for Secondary Sites

The research team at each secondary site is required to promptly notify the Washington University PI and research coordinator of all reportable events (as described in Section 7.6) within **1 working day** of the occurrence of the event or notification of the secondary site's PI of the event. This notification may take place via email if there is not yet enough information for a formal written report (using either an FDA MedWatch form if required or an institutional SAE reporting form if not). A formal written report must be sent to the Washington University PI and research coordinator within **10 working days** of the occurrence of the event or notification of the secondary site's PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification of the secondary site's PI of the event.

The research team at a secondary site is responsible for following its site's guidelines for reporting applicable events to its site's IRB according to its own institutional guidelines.

10.8 Reporting to Secondary Sites

The Washington University PI (or designee) will notify the research team at each secondary site of all reportable events that have occurred at other sites (as described in Section 7.6) within **10 working days** of the occurrence of the event or notification of the PI of the event. This includes events that take place both at Washington University and at other secondary sites, if applicable.

10.9 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. **PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC.** It is the responsibility of the investigator to report any unanticipated problem to the FDA as follows:

- Report any unexpected fatal or life-threatening adverse experiences associated with use of the drug (i.e., there is a reasonable possibility that the experience may have been caused by the drug) by telephone or fax no later than **7 calendar days** after initial receipt of the information. A life-threatening adverse experience is defined as any adverse drug experience that places the subject (in the view of the investigator) at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Report any serious, unexpected adverse experiences, as well as results from animal studies that suggest significant clinical risk within **15 calendar days** after initial receipt of this information. A serious adverse drug experience is defined as any adverse drug experience occurring at any dose that results in any of the following outcomes:
    - Death
    - A life-threatening adverse drug experience
    - Inpatient hospitalization or prolongation of existing hospitalization
    - A persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions)
    - A congenital anomaly/birth defect
    - Any other experience which, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

  An unexpected adverse drug experience is defined as any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure (or risk information, if an IB is not required or available).

All MedWatch forms will be sent by the investigator or investigator's team to the FDA at the following address or by fax:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Oncology Drug Products
5901-B Ammendale Rd.
Beltsville, MD 20705-1266
FAX: 1-800-FDA-0178

Secondary sites must submit a completed MedWatch form to the Washington University PI and research coordinator within **4 calendar days** (for fatal or life-threatening adverse experiences) or **11 calendar days** (for serious, unexpected adverse experiences). The Washington University PI will be responsible for submitting all MedWatch forms from secondary sites to the FDA within the timeframes specified above.

10.10 Timeframe for Reporting Required Events:

Reportable adverse events will be tracked for 30 days following the last day of study treatment, or until the start of a new chemotherapy/treatment, whichever comes first.

Deaths	
Any reportable death while on study or within 30 days of study	Immediately, within 24 hours, to PI and the IRB
Any reportable death while off study	Immediately, within 24 hours, to PI and the IRB
Adverse Events/Unanticipated Problems	
Any reportable adverse events as described in Sections 10.1 and 10.2 (other than death) and 10.7	Immediately, within 24 hours to PI and within 10 working days to the IRB, and within 7 or 15 days to the FDA
All adverse events are to be reported per protocol regardless of attribution should be submitted cumulatively	Include in DSM report
Noncompliance and Serious Noncompliance	
All noncompliance and serious noncompliance as described in Sections 10.3 and 10.4	Immediately, within 24 hours, to PI and within 10 working days to the IRB

11.0 DATA SAFETY MONITORING PLAN

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months following activation of the first secondary site.

The Principal Investigator will review all patient data at least every six months, and provide a semi-annual report to the QASMC. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual including numbers from participating sites
- Protocol activation date at each participating site
- Average rate of accrual observed in year 1, year 2, and subsequent years at each participating site
- Expected accrual end date and accrual by site
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities at all participating sites
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

Until such a time as the first secondary site activates this protocol, a semi-annual DSM report to be prepared by the study team will be submitted to the QASM Committee beginning 6 months after study activation at Washington University.

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

12.0 AUDITING

Since Washington University is the coordinating center, each site will be audited annually by Siteman Cancer Center personnel (QASMC) unless the outside institution has an auditing mechanism in place and can provide a report. The outside sites will be asked to send copies of all audit materials, including source documentation. The audit notification will be sent to the Washington University Research Patient Coordinator, who will obtain the audit materials from the participating institution.

Notification of an upcoming audit will be sent to the research team one month ahead of the audit. Once accrual numbers are confirmed, and approximately 30 days prior to the audit, a list of the cases selected for review (up to 10 for each site) will be sent to the research team. However, if during the audit the need arises to review cases not initially selected, the research team will be asked to provide the additional charts within two working days.

Additional details regarding the Auditing Policies and procedures can be found at http://www.siteman.wustl.edu/uploadedFiles/Research_Programs/Clinical_Research_Resources/Protocol_Review_and_Monitoring_Committee/QASMCQualityAssurcance.pdf

13.0 MULTICENTER REGULATORY REQUIREMENTS

Washington University requires that each participating site sends its informed consent document to be reviewed and approved by the Washington University Regulatory Coordinator (or designee) prior to IRB/IEC submission.

Each participating institution must have the following documents on file at Washington University prior to first subject enrollment:

- Documentation of IRB approval of the study in the form of a letter or other official document from the participating institution's IRB. This documentation must show which version of the protocol was approved by the IRB.
- Documentation of IRB approval of an informed consent form. The consent must include a statement that data will be shared with Washington University, including the Quality Assurance and Safety Monitoring Committee (QASMC), the DSMC (if applicable), and the Washington University study team.
- Documentation of FWA, signed FDA Form 1572, and signed and dated CVs of all participating investigators.
- Documentation of training in protection of human subjects by all participating investigators.
- Protocol signature page signed and dated by the investigator at each participating site.

The Principal Investigator is responsible for disseminating to the participating sites all study updates, amendments, reportable adverse events, etc. There will be one current version of the protocol document at any given time and each participating institution will utilize that document. Protocol/consent modifications and IB updates will be forwarded electronically to the secondary sites within 2 weeks of obtaining Washington University IRB approval with acknowledgement of receipt requested. Secondary sites are to submit protocol/consent/IB modifications to their local IRBs within 4 weeks of receipt, and confirmation of submission must be forwarded to the appropriate contact person on the Washington University study team at the time of submission. Upon the secondary sites obtaining local IRB approval, documentation of such shall be sent to the Washington University study team within 2 weeks of receipt of approval.

Documentation of participating sites' IRB approval of annual continuing reviews, protocol amendments or revisions, all SAE reports, and all protocol violations/deviations/exceptions must be kept on file at Washington University.

The investigator or a designee from each institution must participate in a regular conference call to update and inform regarding the progress of the trial.

14.0 STATISTICAL ANALYSIS METHODS AND SAMPLE SIZE

14.1 Analysis Populations

Intent-to-Treat - The intent-to-treat (ITT) population will be defined as all patients who receive at least one dose of decitabine.

Efficacy-Evaluable - The efficacy-evaluable (EE) population will be defined as all patients who receive at least one cycle of treatment.

Safety - The safety population will be defined as all patients with at least one dose of decitabine.

14.2 Statistical Analysis

14.2.1 Descriptive Analyses

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

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.

14.2.2 Primary Endpoint Analysis

The primary end-point of this phase II study is the correlation biomarkers with overall response in AML/MDS patients to decitabine.

We will correlate the presence of patient specific mutations (especially mutations in *DNMT3A, IDH1, IDH2,* and *TET2*) with overall response rates. Mutation rates in these genes and in other patient-specific genes will be correlated with overall response and their respective 95% confidence intervals will be provided.

Statistical considerations. The relationship between AML/MDS-associated mutations and the responsiveness to decitabine will be described by contingency tables and assessed using Chi-square test or Fisher's exact test as appropriate. The differentially expressed genes between responders and non-responders will be determined using the significance analysis of microarray (SAM). Multivariate logistic regression will also be fit to adjust the potential confounding effects of other demographic and clinical characteristics. The resultant critical *P* values will be corrected for multiple testing. Since the Bonferroni correction is known to be too conservative, a permutation analysis (e.g., 10,000 random assignments for

responsive status) will be used to derive an empirical critical *P* value. The new critical p-value is the number of permutation tests which yield a lower p-value than the original test divided by the total number of permutation tests.[61]

A power analysis was performed using Chi-square test for the association between mutation and response to decitabine. The frequency of *DNMT3A, IDH1/2,* and *TET2* mutations in AML and MDS patients are each between 15% and 30% in multiple cohorts.[29-37] **Table 3** shows the minimal detectable differences of response rates between mutant and wild-type cases, assuming an expected overall 45% CR/CRi rate and 80% power. For an average of 20% frequency of a mutated gene, for example, 100 patients will provide 80% power to detect 30% difference at a 2-sided 0.1 alpha error. The purpose of this study is to determine whether any of these mutations result in a clinically significant association that would warrant pre-treatment testing. We believe that a reasonable cut off would be if at least 30% of clinical response could be attributed to the presence of a specific mutation. However, we anticipate that more power could be achieved as we assess patient groups with combined characteristics (e.g. *DNMT3A* mutation or loss of *DNMT3A* expression). In addition, we expect that the majority of the enrolled 125 patients can be used in the analysis (since all of our molecular information will be acquired within 30 days of enrollment, we anticipate a drop-out rate of 20% or less).

Table 3. Detectable mutation associated response rates (80% power, $\alpha = 0.05$)

Total N	% of patients with a given mutation	Detectable response difference	Sensitivity of mutation to predict response at minimum detectable response difference*
100	15%	0.40	80%
	20%	0.35	75%
	30%	0.30	67%
	40%	0.27	61%
	50%	0.26	58%

* Assuming the overall response rate (CR/Cri) of 45%

14.2.3 Secondary Endpoint Analyses

The overall response rate (CR/CRi/PR) and complete response rate (CR/CRi) will be compared with historical controls[2], and the 95% confidence intervals will be provided.

The rate of patient specific mutation clearance after 10, 28, and 56 days of therapy will be determined using an Illumina based platform. We will compare the rate of mutation clearance between the patients who achieve a CR/CRi after 4 cycles and those who do not. We anticipate that we will identify between 6 and 25 exonic SNVs per case (the range obtained with >200 AML samples sequenced to date with whole genome or exome sequencing), and that the expected 95% confidence

interval of the mean variant allele frequency will be ±2.56% and ±1.25%, respectively, if we assume an anticipated standard deviation of 3.2% within the same clone. The change of tumor burden over time and its association with clinical response will be assessed using 2-way ANOVA for repeated measurement data, followed by the ad-hoc multiple comparisons for between-group differences (e.g., responders vs. non-responders) at given time points as well as the between-time differences within each group. The resultant critical *P* values will also be corrected using permutation analysis for multiple testing.

The steady-state plasma decitabine concentration on day 4 ± 1 will be measured and correlated with clinical overall response.

The percent decrease in bone marrow methylcytosine will be calculated on day 10±1. We will compare this reduction with previously published data and between the patients who achieve CR/CRi and those who do not. The correlation of peak plasma levels of decitabine with response will be assessed using 2-sample t-test or Mann-Whitney non-parametric rank-sum test as appropriate. The change of total bone marrow DNA methylcytosine from baseline and its association with both steady-state plasma drug levels and response will be assessed using 2-way ANOVA for repeated measurement data, followed by the ad-hoc multiple comparisons for between-group differences (e.g., responders vs. non-responders) and between-time differences. Similar analyses will be performed for the correlation of expression profiles and SNP analysis with pharmacokinetic data.

Statistical power justification. A power analysis was performed using two-sample t-test for between-group difference and paired t-test for the change over time. For tumor clearance, this will provide > 99% power to detect a 10% difference in the means at two separate time points for clones with 6 or more variants, and > 98% power to detect a 15% difference if there are 3 or more variants per clone with $\alpha = 0.05$ (power will decrease to 91% and 82%, respectively, in cases where the standard deviation is 4.7%, the maximum we have observed thus far). For pharmacologic correlations, we anticipate 85% power to determine a 25 ng/ml difference between the average C_{max} of 45 evaluable responders and 55 evaluable non-responders with an anticipated average C_{max} of 70 ng/ml and a standard deviation of 40 ng/ml within the entire cohort, and with $\alpha = 0.05$.[22] We believe a less significant difference would not be sufficient to warrant application in clinical decision making (i.e. whether to continue therapy for more than 1 cycle). We anticipate 99% power to determine a difference of 20% in the reduction of bone marrow methylcytosine between 45 evaluable responders and 55 evaluable non-responders with an expected standard deviation of 15% and $\alpha = 0.05$.[28, 66-68] The difference in methylcytosine reduction previously observed was 30% between patients achieving CR/PR and those with only hematologic improvement.[28]

Time to relapse and time to death will also be listed for each patient.

14.2.4 Safety Analysis

Extent of Exposure – Duration (number of cycles/infusions) of decitabine doses (cumulative and intensity) will be summarized.

Adverse Events - All adverse events will be coded using NCI CTCAE v. 4.0. 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. In addition, adverse events will be summarized by investigator attribution of relationship to study medication and by grade. Similar summaries will be provided by treatment cycle.

Clinical Laboratory Evaluation – Applicable laboratory parameters will be graded according to NCI CTCAE version 4.0. The incidence of maximum grade (number and percent of patients experiencing the maximum grade during the study) will be summarized for each laboratory parameter. Similar summaries will be provided by treatment cycle. Graphical displays of select hematological parameters will also be provided.

Vital Signs, Physical Findings, and Other Safety Observations – Vital signs, findings from physical examinations, ECOG scores and weights will be presented in listing format.

14.2.5 Description of Planned Subgroup Analyses

The following subgroups (if sufficient numbers of patients) will be explored with respect to the primary and overall survival endpoints.

- Age (60-69 years old vs ≥ 70 years old)
- Cytogenetics
- Patients with AML-associated mutations in *DNMT3A, TET2, IDH1,* or *IDH2* vs those who do not have somatic variants in these genes.

14.3 Sample Size

We anticipate initially enrolling 25 patients at Washington University. Once we establish a routine exome analysis with validation and calculation of the rate of patient-specific mutation clearance, we will enroll an additional 100 patients from at least 2 additional centers.

15.0 DATA SUBMISSION SCHEDULE

Case Report Form	Submission Schedule
Original Consent Form	*Prior to registration*
Patient Registration Form *Disease Diagnosis Form* *Inclusion Criteria Worksheet* *Exclusion Criteria Worksheet*	*Prior to starting treatment*
Treatment Record Form	*To be maintained throughout a patient's treatment cycle. The complete record is due at completion of treatment.*
Bone Marrow Sample Collection Form	*To be maintained throughout the course of the study at designated bone marrow sample collections time points (See Appendix 2).*
Pathology: Bone Marrow Biopsy	*To be completed upon the return of pathology report for each bone marrow biopsy.*
Study Calendar Form	*To be completed with the date of each time point upon occurrence.*
Labs Form	*To be completed upon the return of laboratory reports for each study time point (See Appendix 2)*
Dose Modifications	*To be maintained throughout the course of the study if dose modifications are performed.*
Therapy Response Form	*To be filled out upon remission (prior to, and/or on therapy)*
End of Study Form	*Due at the completion of the EOS visit, at the time of death, and/or at the time that the patient is lost to follow-up*
Follow Up Form	• *30 days post date of last decitabine dose.* • *Every 6 months for 2 years after the last dose of study drug.*
Record of Adverse Events	*At the time of any AE*

Any queries generated by Washington University must be responded to within 28 days of receipt by the participating site. The Washington University research team will conduct a regular review of data status at all secondary sites, with appropriate corrective action to be requested as needed.

Appendix 1 ECOG Performance Scale

Activity Status	Description
0	Asymptomatic, fully active, and able to carry on all predisease performance without restrictions.
1	Symptomatic, fully ambulatory but restricted in physically strenuous activity and able to carry out performance of a light or sedentary nature, eg, light housework, office work.
2	Symptomatic, ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours: in bed less than 50% of day.
3	Symptomatic, capable of only limited self-care, confined to bed or chair more than 50% of waking hours, but not bedridden.
4	Completely disabled. Cannot carry on any self-care. Totally bedridden.
5	Dead

Appendix 2 Study Flow Chart

Study Flow Chart

Test	Pre-study[1]	Base-line[2]	All Cycles Day 1	All Cycles Days 1-10[3]	Cycle 1 Day 4 ± 1	Cycle 1 Day 10 ± 1 and 28 ± 4	Every week	Every 2 weeks	Even cycles Day 28±4	End of Study
Informed Consent	X									
Medical History		X								
Physical Exam		X[a]	X[b]							X
Transfusion Requirements[c]		X[c]	Ongoing							
Bone Marrow Aspirate and Biopsy	X[d]					X[d]			X[d]	X[d]
Flow Cytometry[e]	X									
Peripheral blood for correlative studies					X					
Pregnancy test		X								
CBC with differential and platelets		X	X				X[f]			X
Serum Chemistries[g]		X	X					X[f]		X
Decitabine administration[h]				X						
Adverse Events			Ongoing							
Post-study follow-up										X[i]

[1] To be performed within 14 days of the first dose of decitabine.
[2] To be performed within 7 days of the first dose of decitabine.
[3] Decitabine may be reduced to Days 1-5 or Days 1-3. See Section 6.3 for details.
[a] To include height, weight, and ECOG.
[b] To include weight.
[c] Care should be given to record the frequency of transfusions from 8 weeks prior to first dose of study drug through the duration of the study.
[d] Bone marrow biopsy and aspirate should be performed at baseline, at Cycle 1 Day 10 ± 1, and at Day 28 ± 4 of Cycles 1, 2, 4 and 6 for clinical disease assessment. Patients who achieve with clinical response (CRi or better) and are receiving cycles every 6 weeks, will undergo bone marrow biopsies on day 42 ± 4. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression. Standard bone marrow procurement procedures will be followed for collection of the tissue. To include: correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics and FISH at all time points. A skin biopsy sample (collected under HRPO# 201011766) is required at pre-study, but may have been collected months or even years prior to first dose of decitabine.
[e] On bone marrow or peripheral blood.
[f] After Cycle 1, these labs may be obtained every other week (CBC) or once a month (Chemistries) at the discretion of the treating physician.
[g] Serum chemistries include bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, and LDH.
[h] Decitabine treatment will be 20 mg/m^2 over a 1-hour IV infusion on consecutive Days 1-10 every 28-day cycle. The dose will be calculated based on actual patient weight at the beginning of each cycle. Patients may be premedicated with lorazepam, prochlorperazine, palonosetron, or ondansetron at the treating physician's discretion.
[i] Patients will be followed for 30 days following the date of the last decitabine dose to monitor for toxicity, survival and response. Thereafter, the investigator or designee will record information on subsequent therapy, disease progression, and death, every 6 months for 2 years after first dose of study drug or until the patient is lost to follow-up.

16.0 REFERENCES

1. Cheson, B.D., et al., *Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia.* J Clin Oncol, 2003. **21**(24): p. 4642-9.
2. Cashen, A.F., et al., *Multicenter, phase II study of decitabine for the first-line treatment of older patients with acute myeloid leukemia.* J Clin Oncol, 2010. **28**(4): p. 556-61.
3. Blum, W., et al., *Clinical response and miR-29b predictive significance in older AML patients treated with a 10-day schedule of decitabine.* Proc Natl Acad Sci U S A, 2010. **107**(16): p. 7473-8.
4. Schlenk, R.F., et al., *Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia.* N Engl J Med, 2008. **358**(18): p. 1909-18.
5. Erba, H.P., *Has there been progress in the treatment of older patients with acute myeloid leukemia?* Best Pract Res Clin Haematol, 2010. **23**(4): p. 495-501.
6. Heaney, M.L. and D.W. Golde, *Myelodysplasia.* N Engl J Med, 1999. **340**(21): p. 1649-60.
7. Bennett, J.M., et al., *Proposals for the classification of the myelodysplastic syndromes.* Br J Haematol, 1982. **51**(2): p. 189-99.
8. Kantarjian, H., et al., *Results of intensive chemotherapy in 998 patients age 65 years or older with acute myeloid leukemia or high-risk myelodysplastic syndrome: predictive prognostic models for outcome.* Cancer, 2006. **106**(5): p. 1090-8.
9. Martin, M.G. and C.N. Abboud, *Induction therapy for elderly patients with acute myeloid leukemia.* Blood Rev, 2008. **22**(6): p. 311-20.
10. Bow, E.J., et al., *Therapy of untreated acute myeloid leukemia in the elderly: remission-induction using a non-cytarabine-containing regimen of mitoxantrone plus etoposide.* J Clin Oncol, 1996. **14**(4): p. 1345-52.
11. Goldstone, A.H., et al., *Attempts to improve treatment outcomes in acute myeloid leukemia (AML) in older patients: the results of the United Kingdom Medical Research Council AML11 trial.* Blood, 2001. **98**(5): p. 1302-11.
12. Stone, R.M., *The difficult problem of acute myeloid leukemia in the older adult.* CA Cancer J Clin, 2002. **52**(6): p. 363-71.
13. Liu Yin, J.A., et al., *Mitozantrone and cytosine arabinoside as first-line therapy in elderly patients with acute myeloid leukaemia.* Br J Haematol, 1991. **79**(3): p. 415-20.
14. Stone, R.M., et al., *Granulocyte-macrophage colony-stimulating factor after initial chemotherapy for elderly patients with primary acute myelogenous leukemia. Cancer and Leukemia Group B.* N Engl J Med, 1995. **332**(25): p. 1671-7.
15. Kantarjian, H., et al., *Decitabine improves patient outcomes in myelodysplastic syndromes.* Cancer, 2006. **106**(8): p. 1794-1803.

16. Lubbert, M., et al., *A multicenter phase II trial of Decitabine as first-line treatment of older AML patients judged unfit for induction chemotherapy.* Haematologica, 2011. **97**(3): p. 393-401.
17. Issa, J.P., et al., *Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies.* Blood, 2004. **103**(5): p. 1635-40.
18. Petti, M.C., et al., *Pilot study of 5-aza-2'-deoxycytidine (Decitabine) in the treatment of poor prognosis acute myelogenous leukemia patients: preliminary results.* Leukemia, 1993. **7 Suppl 1**: p. 36-41.
19. Wijermans, P.W., et al., *Continuous infusion of low-dose 5-Aza-2'-deoxycytidine in elderly patients with high-risk myelodysplastic syndrome.* Leukemia, 1997. **11**(1): p. 1-5.
20. Kantarjian, H., et al., *Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study.* Cancer, 2006. **106**(8): p. 1794-803.
21. Wijermans, P.W., et al., *An epigenetic approach to the treatment of advanced MDS; the experience with the DNA demethylating agent 5-aza-2'-deoxycytidine (decitabine) in 177 patients.* Ann Hematol, 2005. **84 Suppl 1**: p. 9-17.
22. Cashen, A.F., et al., *Pharmacokinetics of decitabine administered as a 3-h infusion to patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS).* Cancer Chemother Pharmacol, 2008. **61**(5): p. 759-66.
23. Chabot, G.G., J. Bouchard, and R.L. Momparler, *Kinetics of deamination of 5-aza-2'-deoxycytidine and cytosine arabinoside by human liver cytidine deaminase and its inhibition by 3-deazauridine, thymidine or uracil arabinoside.* Biochem Pharmacol, 1983. **32**(7): p. 1327-8.
24. Momparler, R.L., et al., *Cell cycle effects and cellular pharmacology of 5-aza-2'-deoxycytidine.* Cancer Chemother Pharmacol, 1984. **13**(3): p. 191-4.
25. Raza, A., et al., *Biological significance of cell cycle kinetics in 128 standard risk newly diagnosed patients with acute myelocytic leukemia.* Br J Haematol, 1991. **79**(1): p. 33-9.
26. Brons, P.P., et al., *Proliferation patterns in acute myeloid leukemia: leukemic clonogenic growth and in vivo cell cycle kinetics.* Ann Hematol, 1993. **66**(5): p. 225-33.
27. Raza, A., et al., *Direct relationship between remission duration in acute myeloid leukemia and cell cycle kinetics: a leukemia intergroup study.* Blood, 1990. **76**(11): p. 2191-7.
28. Shen, L., et al., *DNA methylation predicts survival and response to therapy in patients with myelodysplastic syndromes.* J Clin Oncol, 2010. **28**(4): p. 605-13.
29. Tefferi, A., et al., *IDH1 and IDH2 mutation studies in 1473 patients with chronic-, fibrotic- or blast-phase essential thrombocythemia, polycythemia vera or myelofibrosis.* Leukemia, 2010. **24**(7): p. 1302-9.
30. Ley, T.J., et al., *DNMT3A mutations in acute myeloid leukemia.* N Engl J Med, 2010. **363**(25): p. 2424-33.
31. Mardis, E.R., et al., *Recurring mutations found by sequencing an acute myeloid leukemia genome.* N Engl J Med, 2009. **361**(11): p. 1058-66.

32. Abdel-Wahab, O., J. Patel, and R.L. Levine, *Clinical Implications of Novel Mutations in Epigenetic Modifiers in AML.* Hematol Oncol Clin North Am, 2011. **25**(6): p. 1119-33.
33. Reuther, G.W., *Recurring mutations in myeloproliferative neoplasms alter epigenetic regulation of gene expression.* Am J Cancer Res, 2011. **1**(6): p. 752-62.
34. Paschka, P., et al., *IDH1 and IDH2 mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3 internal tandem duplication.* J Clin Oncol, 2010. **28**(22): p. 3636-43.
35. Shen, Y., et al., *Gene mutation patterns and their prognostic impact in a cohort of 1185 patients with acute myeloid leukemia.* Blood, 2011. **118**(20): p. 5593-603.
36. Tefferi, A., et al., *Detection of mutant TET2 in myeloid malignancies other than myeloproliferative neoplasms: CMML, MDS, MDS/MPN and AML.* Leukemia, 2009. **23**(7): p. 1343-5.
37. Walter, M.J., et al., *Recurrent DNMT3A mutations in patients with myelodysplastic syndromes.* Leukemia, 2011. **25**(7): p. 1153-8.
38. Lin, J., et al., *Recurrent DNMT3A R882 Mutations in Chinese Patients with Acute Myeloid Leukemia and Myelodysplastic Syndrome.* PLoS One, 2011. **6**(10): p. e26906.
39. Bejar, R., et al., *Clinical effect of point mutations in myelodysplastic syndromes.* N Engl J Med, 2011. **364**(26): p. 2496-506.
40. Walter, M.J., et al., *Clonal architecture of secondary acute myeloid leukemia.* N Engl J Med, 2012. **366**(12): p. 1090-8.
41. Kantarjian, H.M., et al., *Survival advantage with decitabine versus intensive chemotherapy in patients with higher risk myelodysplastic syndrome: comparison with historical experience.* Cancer, 2007. **109**(6): p. 1133-7.
42. Lubbert, M., et al., *Continued Low-Dose Decitabine (DAC) Is an Active First-Line Treatment in All Cytogenetic Subgroups of Older AML Patients: Results of the FR00331 Multicenter Phase II Study.* ASH Annual Meeting Abstracts, 2007. **110**(11): p. 300-.
43. Blum, W., et al., *Phase I Study of Decitabine Alone or in Combination With Valproic Acid in Acute Myeloid Leukemia.* J Clin Oncol, 2007. **25**(25): p. 3884-3891.
44. Issa, J.-P.J., et al., *Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies.* Blood, 2004. **103**(5): p. 1635-1640.
45. Garcia-Manero, G., et al., *Phase 1/2 study of the combination of 5-aza-2'-deoxycytidine with valproic acid in patients with leukemia.* Blood, 2006. **108**(10): p. 3271-3279.
46. Issa, J.P., S.B. Baylin, and J.G. Herman, *DNA methylation changes in hematologic malignancies: biologic and clinical implications.* Leukemia, 1997. **11 Suppl 1**: p. S7-11.
47. Ganetzky, R.D., et al., *Differences Between Normal and Leukemic Stem Cell-Specific Methylome Indicates Aberrantly Silenced Genes Involved in the*

Pathogenesis of Malignant Evolution. ASH Annual Meeting Abstracts, 2008. **112**(11): p. 599-.

48. Christman, J.K., *5-Azacytidine and 5-aza-2'-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy.* Oncogene, 2002. **21**(35): p. 5483-95.
49. Lubbert, M., *DNA methylation inhibitors in the treatment of leukemias, myelodysplastic syndromes and hemoglobinopathies: clinical results and possible mechanisms of action.* Curr Top Microbiol Immunol, 2000. **249**: p. 135-64.
50. Kantarjian, H., et al., *Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia.* Blood, 2007. **109**(1): p. 52-57.
51. Steensma, D.P., et al., *Multicenter Study of Decitabine Administered Daily for 5 Days Every 4 Weeks to Adults With Myelodysplastic Syndromes: The Alternative Dosing for Outpatient Treatment (ADOPT) Trial.* J Clin Oncol, 2009: p. JCO.2008.19.6550.
52. Cashen, A.F., et al., *Preliminary Results of a Multicenter Phase II Trial of 5-Day Decitabine as Front-Line Therapy for Elderly Patients with Acute Myeloid Leukemia (AML).* ASH Annual Meeting Abstracts, 2008. **112**(11): p. 560-.
53. Jabbour, E., et al., *Evolution of decitabine development: accomplishments, ongoing investigations, and future strategies.* Cancer, 2008. **112**(11): p. 2341-51.
54. Kantarjian, H., et al., *Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia.* Blood, 2007. **109**(1): p. 52-7.
55. Blum, W.G., Klisovic, R., Liu, S., Kefauver, C., Grever, M. R., Schaaf, L., Chan, K., Byrd, J. C., Villalona-Calero, M., Marcucci, G. *Efficacy of a novel schedule of decitabine in previously untreated AML, age 60 or older*. in *ASCO Annual Meeting*. 2009.
56. van Groeningen, C.J., et al., *Phase I and pharmacokinetic study of 5-aza-2'-deoxycytidine (NSC 127716) in cancer patients.* Cancer Res, 1986. **46**(9): p. 4831-6.
57. Rivard, G.E., et al., *Phase I study on 5-aza-2'-deoxycytidine in children with acute leukemia.* Leuk Res, 1981. **5**(6): p. 453-62.
58. Lin, K.T., R.L. Momparler, and G.E. Rivard, *Sample preparation for the determination of 5-aza-2'-deoxycytidine in plasma by high-performance liquid chromatography.* J Chromatogr, 1985. **345**(1): p. 162-7.
59. Momparler, R.L., G.E. Rivard, and M. Gyger, *Clinical trial on 5-aza-2'-deoxycytidine in patients with acute leukemia.* Pharmacol Ther, 1985. **30**(3): p. 277-86.
60. Raska, K., Jurovcik, M., Sormova., Z., *On the metabolism of 5-azacytidine and 5-aza-2'-deoxycytidine in mice.* Coll Czech Chem Commun, 1965. **30**: p. 3001-3005.
61. Momparler, R.L., et al., *Pilot phase I-II study on 5-aza-2'-deoxycytidine (Decitabine) in patients with metastatic lung cancer.* Anticancer Drugs, 1997. **8**(4): p. 358-68.

62. Aparicio, A., et al., *Phase I trial of continuous infusion 5-aza-2'-deoxycytidine.* Cancer Chemother Pharmacol, 2003. **51**(3): p. 231-9.
63. Bryan, J., et al., *Pharmacokinetic evaluation of decitabine for the treatment of leukemia.* Expert Opin Drug Metab Toxicol, 2011. **7**(5): p. 661-72.
64. Scandura, J.M., et al., *Phase 1 study of epigenetic priming with decitabine prior to standard induction chemotherapy for patients with AML.* Blood, 2011. **118**(6): p. 1472-80.
65. Westfall, P.H. and S.A. Young, *Resampling-based multiple testing: examples and methods for p-value adjustment*. 1993, New York: John Wiley & Sons, Inc.
66. Samlowski, W.E., et al., *Evaluation of a 7-day continuous intravenous infusion of decitabine: inhibition of promoter-specific and global genomic DNA methylation.* J Clin Oncol, 2005. **23**(17): p. 3897-905.
67. Blum, W., et al., *Phase I study of decitabine alone or in combination with valproic acid in acute myeloid leukemia.* J Clin Oncol, 2007. **25**(25): p. 3884-91.
68. Yang, A.S., et al., *DNA methylation changes after 5-aza-2'-deoxycytidine therapy in patients with leukemia.* Cancer Res, 2006. **66**(10): p. 5495-503.
69. Cheson, B.D., et al., *Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia.* Blood, 2006. **108**(2): p. 419-25.

Genomic Predictors of Decitabine Response in AML/MDS

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SCHOOL OF MEDICINE

Genomic Predictors of Decitabine Response in AML/MDS

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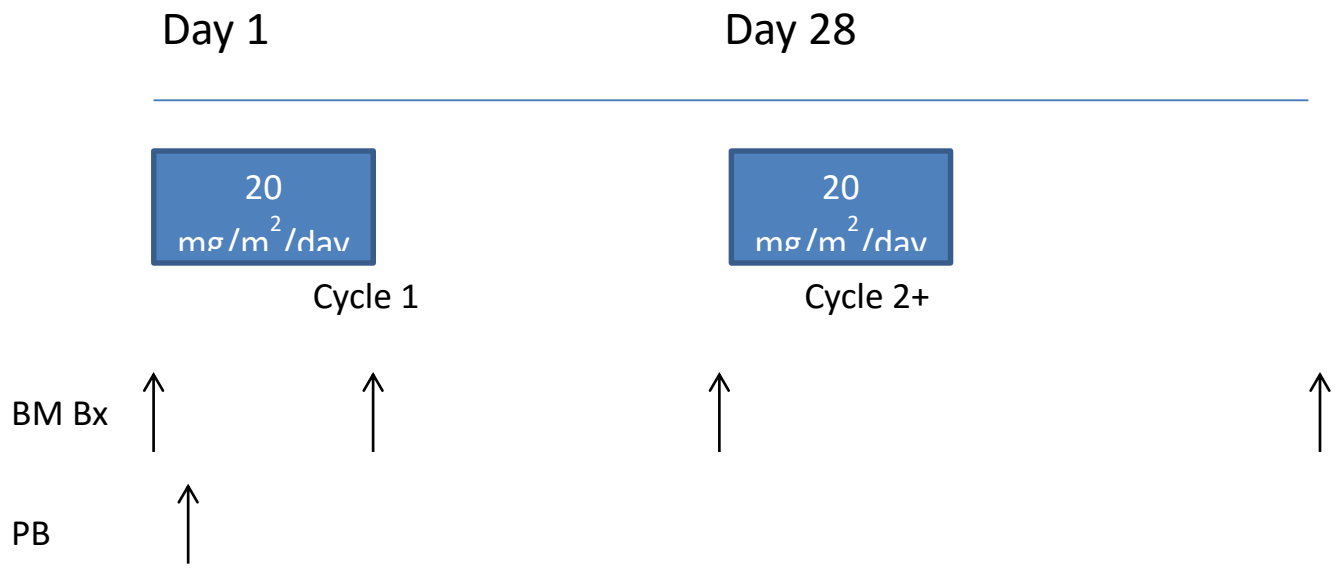
Study Schema:



Patients with progressive disease after two cycles (defined as a clear increase in bone marrow blast count, typically a > 50% increase in bone marrow blasts over baseline) should be removed from study.

Patients with stable disease may continue on study and should undergo a diagnostic bone marrow biopsy at the end of even numbered cycles.

Once AML patients achieve morphologic complete remission with incomplete blood count recovery (CRi) or MDS patients achieve a marrow complete response (Marrow CR) as defined in Section 9.0, decitabine administration may be decreased to five consecutive days to limit myelosuppressive toxicityat the discretion of the treating physician. After two cycles of five consecutive days of treatment, if the patient meets the definition of CR with complete cytogenetic response as defined in Section 9.0, administration may further be decreased to three consecutive days per cycle at the discretion of the treating physician.

Sample collection for correlative studies:

Bone marrow biopsies should be performed at baseline, at Cycle 1 Day 10 ± 1, and at Day 28 ± 4 of Cycles 1, 2, 4, and 6. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression.

Peripheral blood samples should be collected on Day 4± 1 of Cycle 1. Collection should be obtained 45 ± 15 minutes after decitabine infusion is initiated.

Genomic Predictors of Decitabine Response in AML/MDS

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1.0 INTRODUCTION

1.1 Rationale

Personalized therapy for cancer requires that 1) patient-specific factors (e.g. patient-specific mutations, expression profiles, or pharmacogenomics) influence treatment algorithms, and 2) once therapy is initiated, *in vivo* responsiveness is used to guide further therapy. The modern era of genomics has provided significant insights into cancer pathogenesis by describing patterns of germline and somatic variants, as well as defining disease-specific patterns of gene expression. However, the application and integration of this data with therapeutics remains a difficult task.

Decitabine is a commonly used single-agent therapy for patients with acute myeloid leukemia (AML) and/or myelodysplastic syndrome (MDS). Historically, it has yielded clinical complete responses in approximately 25% of patients across diverse clinical trials, it is well tolerated, and it can be given as in the outpatient setting. However, the majority of patients do not respond to decitabine, the molecular etiology of decitabine sensitivity is unknown, and there are no algorithms to predict patient responsiveness *a priori* or even within one month of starting therapy.

AML and MDS are two closely related hematological disorders characterized by ineffective hematopoiesis and malignant expansion of clonal myeloid cells. Both AML and MDS frequently occur in elderly patients and some cases are secondary and/or related to long-term bone marrow damage after radiation or chemotherapy. Current therapy for AML consists of cytotoxic chemotherapy followed by consolidation chemotherapy or stem cell transplantation. Using these approaches, current cure rates may be as high as 60% for patients younger than 60 with good risk cytogenetics (e.g. t(15;17), t(8;21), inv(16)).[4] Outcomes in patients ≥ 60 years old and with relapsed disease remain poor with 2 year survival of only ~25%.[5] Historically, the available treatment for MDS has been symptomatic and has not been shown to be effective in producing sustained improvement in hematopoiesis or in delaying leukemic evolution[6, 7].

Current AML therapy is poorly tolerated in elderly and pre-treated patients due to hematologic and non-hematologic toxicities; following such therapy, patients typically require 3-4 weeks of hospitalization for treatment of neutropenic fevers and for regular transfusions. Even among the relatively healthy patients enrolled in clinical trials, induction mortality in the elderly can be as high as 20-40% following standard induction therapy,[8-11] and moderate to severe non-hematologic toxicity occurs in at least an additional 30% of patients.[9, 12-14] Consequently, many older AML patients are not offered traditional intensive chemotherapy, but receive supportive care alone.[15] More effective and less toxic therapies are needed to provide durable remissions in a significant proportion of elderly AML in populations.

[redacted]

We and others have recently used whole genome sequencing to identify recurrent, somatic mutations in AML and MDS patients in four genes that are involved in methylcytosine regulation: *DNMT3A, IDH1, IDH2,* and *TET2.*[29-37] Collectively, nearly half of all AML/MDS patients have a mutation in one of these four genes. *DNMT3A* mutations are associated with an adverse outcome in AML patients treated with cytotoxic chemotherapy.[35, 38] However, it is not yet clear whether the presence of mutations in one or more of these genes is associated with response or resistance to decitabine.

In this study, we will treat patients with the current state-of-the-art decitabine schedule.[3] We will perform exome sequencing on all patients and correlate response with mutation status, rate of mutation clearance, expression profile, plasma decitabine levels, and DNA methylcytosine content reduction. This approach will comprehensively assess genomic and pharmacologic parameters that could clinically predict patient response to decitabine.

In this study, we will combine analysis of patients with AML and MDS. Recent genomic analysis has shown that AML and MDS have shared mutations in many of the same genes, shared patterns of mutation acquisition, and shared patterns of mutation evolution.[39, 40] Furthermore, MDS, like AML, presents with clonal hematopoiesis, even when the morphologic blast count is zero.[36]Because this study will identify and track the rate of clearance of patient specific mutations, and because decitabine has been shown to have efficacy in both AML and MDS patients, [2, 3, 16-19, 41-45] combining analysis of AML and MDS patients is reasonable. Asymptomatic MDS patients are excluded because these patients are typically followed with watchful-waiting rather than with chemotherapy.

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1.3 Correlative Studies

With exception of the peripheral blood sample collected on Cycle 1 Day 4, all correlative studies will be collected under HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Diseases") that all patients will be concurrently enrolled in. The goals of the correlative studies include:

1. To correlate patient-specific mutations with response to decitabine.
   We will perform exome sequencing of each patient's pre-study bone marrow sample and skin sample. We will determine AML/MDS-associated variants found in the bone marrow, but not skin sample. We will correlate the presence of mutations with response (especially mutations in *DNMT3A, IDH1, IDH2,* and *TET2*). This study is powered to identify mutations with >80% sensitivity to predict decitabine response if used as a clinical assay.

2. To correlate the velocity and depth of response with response to decitabine.
   We will assess tumor burden by allele frequency of patient-specific mutations in bone marrow samples collected pre study, Cycle 1 Day 10±1, Cycle 1 Day 28±4, and Cycle 2 Day 28±4. We will assess mutations in at least 24 recurrently mutated genes (including, but not limited to: *FLT3, DNMT3A, NPM1, TET2, WT1, IDH1, IDH2, RUNX1, NRAS, TP53, U2AF1, KRAS, PHF6, MSTP9, PTPN11, KIT, SMC3, PRUNE2, ETV6, CEBPA, SMC1A, ASXL1, STAG2, RAD21*) as well as all patient-

specific mutations discovered during exome sequencing. The leukemic allele frequency will be measured using the Illumnia MySeq or HiSeq platform using DNA capture by gene specific biotinylated-oligonucleotides. Patient bone marrow samples will be assessed pre-study, Cycle 1 Day 10 ± 1, Cycle 1 Day 28 ± 4, and Cycle 2 Day 28 ± 4 and compared with patient specific skin samples (normal control). The velocity of response will be calculated as the natural log rate of patient-specific mutation allele frequency clearance. Depth of response will be defined as the lowest patient-specific mutation allele frequency in a bone marrow sample. The velocity and depth of tumor response will be correlated to:

- Achievement of clinical CR,
- Time to best response,
- Mutational status,
- Time to disease progression.

Because all patient-specific exome-discovered mutations will be assessed a second time in the primary bone marrow sample as well as the Cycle 1 Day 10 ± 1, Cycle 1 Day 28 ± 4, and Cycle 2 Day 28 ± 4 bone marrow samples, this study will also serve to validate all exome-discovered mutations. This is important, because exome sequencing is associated with a 20-50% false-discovery rate depending on type I error tolerated (mutation-calling algorithms are typically set for greater sensitivity than specificity). Thus, many mutations discovered during exome sequencing are incorrect. This second analysis of these mutations will server to exclude these false positive calls.

3. To correlate the bone marrow expression profile from pre-study and Cycle 1 Day 10 ± 1 with response to decitabine.
   We will correlate expression signatures, and changes in expression signatures, with:
   - Achievement of clinical CR,
   - Mutation status.

4. To correlate steady-state plasma decitabine concentration on Cycle 1 Day 4 ± 1 with:
   - Achievement of CR.

5. To correlate the extent of bone marrow DNA hypomethylation on Cycle 1 Day 10 ± 1 with response to decitabine.
   We will assess the percent reduction in bone marrow methylcytosine content using LC-MS. Patients will be evaluated pre-study and Cycle 1 Day 10 ± 1. The change in DNA methylcytosine content will be correlated to:
   - Achievement of clinical CR,
   - Change in expression signature.

   This will determine whether inadequate blast exposure to decitabine correlates with drug resistance.

2.0 OBJECTIVES

2.1 Primary Objective

To determine whether patient-specific mutations correlate with clinical overall response to decitabine in patients with AML or MDS.

2.2 Secondary Objectives

- To determine whether 10 consecutive days per cycle of decitabine improves outcomes compared with historical controls treated with 5 consecutive days per cycle of decitabine.

- To determine whether other biomarkers correlate with clinical overall response to decitabine in patients with AML or MDS. These will include:

    1. The velocity and depth of patient-specific mutation clearance;
    2. The bone marrow expression profile and change in expression profile during decitabine treatment;
    3. The steady-state plasma decitabine concentrations;
    4. The decrease in bone marrow methylcytosine content in response to decitabine.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

One of the following:

- Patient must have non-M3 AML and be ≥ 60 years of age OR
- non-M3 AML with relapsed disease OR
- Symptomatic MDS with one of the following:
  - □ Symptomatic anemia with either hemoglobin < 10.0 g/dL or requiring RBC transfusion
  - □ Thrombocytopenia with a history of two or more platelet counts < 50,000/mcL or a significant hemorrhage requiring platelet transfusions
  - □ Neutropenia with two or more ANC < 1,000/mcL

All of the following:

1. Patient must have an ECOG performance status ≤ 2.
2. Patient must have >10% disease burden measured by cytomorphology, flow cytometry, or cytogenetics.
3. Patient must have peripheral white blood cell count ≤ 50,000/mcl.
4. Patient must have adequate organ function, defined as:
   a. Total bilirubin ≤ 1.5 x ULN
   b. AST/ALT ≤ 2.5 x ULN
   c. Serum creatinine ≤ 2.0 x ULN
5. Patient must have undergone ≤ 2 cycles of prior hypomethylating agent (decitabine or azacitidine).
6. Patient must be enrolled in HRPO# 201011766 (“Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Diseases”).
7. Patient must be ≥ 18 years of age.
8. Patient must be able to understand and willing to sign an IRB-approved written informed consent document.

3.2 Exclusion Criteria

1. Patient must not be pregnant or nursing.
2. Patient must not have known CNS leukemia.
3. Patient must not have a history of positive HIV serology.
4. Patient must not have a history of positive Hepatitis C serology.
5. Patient must not have undergone a prior allogeneic stem cell transplant.
6. Patient must not have any uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, ongoing or active GVHD, congestive heart failure of NYHA class 3 or 4, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situation that would limit compliance with study requirements.
7. Patient must not have had radiation therapy within 14 days of enrollment.
8. Patient must not have received any chemotherapy within 21 days of enrollment, and any acute treatment-related toxicities must have returned to baseline. Patients

may be receiving hydrea at time of enrollment.

3.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4.0 REGISTRATION PROCEDURES

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility
2. Registration of patient in the Siteman Cancer Center database
3. Assignment of unique patient number (UPN)

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility collecting the information listed below

1. The registering MD's name
2. Patient's race, sex, and DOB
3. Three letters (or two letters and a dash) for the patient's initials
4. Copy of signed consent form
5. Completed eligibility checklist, signed and dated by a member of the study team
6. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center Database

All patients must be registered through the Siteman Cancer Center database.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. Patients will also be identified by first, middle, and last initials. If the patient has no middle initial, a dash will be used on the case report forms (CRFs). All data will be recorded with this identification number on the appropriate CRFs.

5.0 INVESTIGATIONAL PLAN

5.1 Summary of Study Design

This is an open-label, single-arm, Phase II, biomarker study of patients with AML or MDS who are treated with 10 consecutive days of decitabine. FAB-M3 AML is excluded from this trial, because other well-tolerated options exist for the treatment of FAB-M3 acute promyelocytic leukemia. AML patients who are < 60 years old and have not received cytotoxic induction chemotherapy are also excluded, because cytotoxic therapy offers a curative possibility for these patients. MDS patients without cytopenias are also excluded because these patients can be followed with watchful-waiting.

All patients will undergo bone marrow biopsies pre-study, on Cycle 1 Day 10 ± 1, and on Day 28 ± 4 of Cycles 1, 2, 4, and 6. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression. Patients will provide a skin biopsy sample pre-study. Patients will also provide peripheral blood samples on Cycle 1 Day 4 ± 1 for pharmacokinetic studies. See Section 8.0 for details on correlative studies.

Patients will receive decitabine 20 mg/m^2/day as a 1-hour infusion on consecutive Days 1-10 in 28-day cycles. The dose will be calculated based on actual patient weight at the beginning of each cycle.

Once AML patients achieve morphologic complete remission with incomplete blood count recovery (CRi) or MDS patients achieve a marrow complete response (Marrow CR) as defined in Section 9.0, decitabine administration may be decreased to five consecutive days to limit myelosuppressive toxicity at the discretion of the treating physician. After two cycles of five consecutive days of treatment, if the patient meets the definition of CR with complete cytogenetic response as defined in Section 9.0, administration may further be decreased to three consecutive days per cycle at the discretion of the treating physician.

It will be a logistical goal of this trial consistently to return preliminary exome results to the treating physician by the end of Cycle 1 and validated results by the end of Cycle 2. However, this logistical goal is not a primary or secondary aim of this study. Given the limited data regarding mutation presence and drug response, we do not anticipate that the results will directly influence therapeutic decisions made by the treating physician. However, the development of comprehensive genomic pipelines and integration of this data into a clinical trial setting will be required before personalized medicine can be realized.

5.2 Study Procedures

5.2.1 Pre-study Procedures

Pre-study procedures must take place no more than 14 days prior to the first dose of decitabine except where noted below.

1. Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics, and FISH.
2. Bone marrow or peripheral blood for flow cytometry
3. Skin biopsy sample (collected under HRPO# 201011766). There is no required time frame for this sample; it may have been collected months or even years prior to the first dose of decitabine.

5.2.2 Baseline Evaluation

Baseline evaluations must take place no more than 7 days prior to the first dose of decitabine.

1. Medical history
2. Physical exam including height, weight, and ECOG performance score.
3. Record transfusion requirements for 8 weeks before first dose of study drug
4. CBC with differential and platelets
5. Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH
6. Urine βHCG pregnancy test if appropriate

5.2.3 Day 1 of Each Cycle

1. Physical exam, including weight
2. CBC with differential and platelets
3. Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH

5.2.4 Cycle 1 Days 1-10

Decitabine administration, 20 mg/m^2 per day given as a 1-hour continuous intravenous infusion. The dose will be calculated based on actual patient weight at the beginning of each cycle. Patients may be premedicated with lorazepam, prochlorperazine, palonosetron, or ondansetron at the treating physician's discretion.

5.2.5 Cycle 1 Day 4 ± 1

Obtain 3 mL peripheral blood for correlative studies. Sample must be obtained 45 minutes ± 15 minutes after beginning the infusion, before the decitabine infusion

is complete, and should be obtained from the opposite arm as the infusion. The sample should be collected in a 3 mL K3EDTA tube pre-loaded with 8 mcL of tetrahydrouridine (THU 500 mcg/mL), an inhibitor of cytidine deaminase. The sample should be processed by the Tissue Procurement Core Facility at the Siteman Cancer Center. Plasma should be separated and stored per protocols.

5.2.6 Cycle 1 Day 10 ± 1

Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics, and FISH.

5.2.7 Weekly

CBC with differential and platelets. After the first cycle, this may be changed to every other week at the discretion of the treating physician.

5.2.8 Every Other Week

Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH. After the first cycle, this may be changed to every month at the discretion of the treating physician.

5.2.9 Cycles 1, 2, 4 and 6Day 28 ± 4 days

Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include: correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics, and FISH.

5.2.10 End-of-Study Procedures

1. Physical exam
2. CBC with differential and platelets.
3. Serum chemistries including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, and LDH.
4. Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include morphologic assessment, cytogenetics, and FISH.

5.2.11 Post-study Follow-up

Patients are to be followed according to standard patient care procedures for 30 days following the date of the last decitabine dose to monitor for toxicity, survival, and response. Thereafter, the investigator or designee will record information on subsequent therapy, disease progression, and/or death every 6 months for 2 years after last dose of study drug or until the patient is lost to follow-up or dies.

5.3 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented in the case report forms.

In the absence of treatment delays due to adverse events, treatment may continue indefinitely or until one of the following criteria applies:

- Documented and confirmed disease progression
- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious non-compliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study

Patients who prematurely discontinue treatment for any reason will be followed as indicated in the study calendar.

5.4 Concomitant Therapy and Supportive Care Guidelines

5.4.1 Chemotherapy

Patients cannot receive any chemotherapy within 21 days of study enrollment and must have recovered from any treatment-related toxicities. However, patients may be receiving hydrea at enrollment and may continue on hydrea through Cycle 2 of decitabine. An indication for hydrea following 2 cycles of decitabine is evidence that the patient is not responding and decitabine should be discontinued. Other than decitabine, patients cannot receive chemotherapy during this trial.

5.4.2 Growth Factors

Erythroid growth factors should not be routinely administered to patients during the study.

Thrombopoietin receptor agonists should not be routinely administered to patients during the study.

Filgrastim (G-CSF) or sargramostim (GM-CSF) may be administered to patients with septic shock or febrile neutropenia at the discretion of the treating physician.

5.4.3 Transfusions

- *Packed red blood cells (RBCs) transfusion*:
  RBC transfusions will be administered at the discretion of the treating physician.

- *Platelet transfusion*:
  Platelet transfusions will be administered at the discretion of the treating physician

5.4.4 Prophylactic Antimicrobial Agents

Antimicrobial agents will be administered at the discretion of the treating physician.

5.4.5 Radiotherapy

Patients cannot receive radiotherapy within 14 days prior to study enrollment or while on study.

6.0 DOSE MODIFICATIONS

A criterion for discontinuation of the study is a delay of > 8 weeks between any two cycles.

6.1 Delay for Cytopenia or Infection

As cytopenias are a defining feature of AML and MDS, their presence alone does not generally warrant dose modifications or delays. Patients will receive four cycles of therapy without adjustment for any cytopenia. Following Cycle 4, dose modifications or delays for cytopenias will be made at the discretion of the investigator.

Patients with febrile neutropenia (temperature ≥ 38.5° with ANC < 1,000/μl) or systemic infections should have treatment held until clinical resolution of the infection.

6.2 Delay for Organ Dysfunction

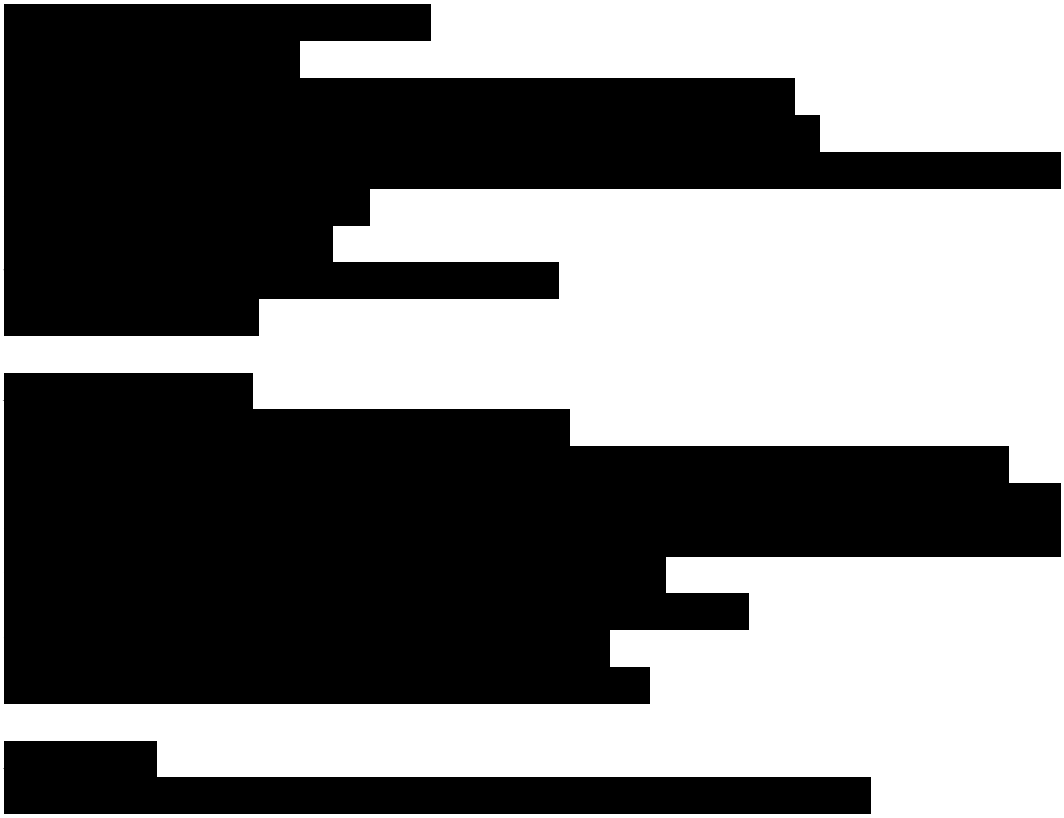
If renal dysfunction (creatinine ≥ 3 X institutional upper limit of normal) or hepatic dysfunction (bilirubin ≥ 2.5 mg/dl or ALT/AST ≥ 3 X institutional upper limit of normal) occurs, therapy may not resume until the abnormalities have resolved to below the limits stated above. If renal or hepatic dysfunction occurs during a treatment course, the next dose of decitabine should be held, and serum chemistries should be checked twice per week. Decitabine can be resumed at full dose when creatinine, bilirubin, ALT and AST are below the limits listed above.

For grade 3-4 non-hematologic toxicities related to decitabine, decitabine treatment should be delayed until recovery to baseline or to grade ≤ 1. Decitabine may be reintroduced with a 50% dose reduction, or at full dose at the discretion of the investigator.

6.3 Modification in Dose or Schedule for Treatment Response

Once AML patients achieve morphologic complete remission with incomplete blood count recovery (CRi) or MDS patients achieve a marrow complete response (Marrow CR) as defined in Section 9.0, decitabine administration may be decreased to 5 consecutive days to limit myelosuppressive toxicity at the discretion of the treating physician. After two cycles of five consecutive days of treatment, if the patient meets the definition of CR with complete cytogenetic response as defined in Section 9.0, administration may further be decreased to three consecutive days per cycle at the discretion of the treating physician.

[redacted]



8.0 CORRELATIVE STUDIES SAMPLE COLLECTIONS

Bone marrow samples will be collected pre-study, on Cycle 1 Day 10 ± 1, and on Day 28 ± 4 of Cycle 1, 2, 4, and 6 for correlative studies. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression. The bone marrow samples will be collected under HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Diseases") that all patients will be concurrently enrolled in.

Skin biopsy sample will be collected as part of the patient's participation in HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Malignancies"). There is no required time frame for this sample, and it may have been collected months or even years prior to the first dose of decitabine.

Peripheral blood sample (3 mL) will be collected on Cycle 1 Day 4 ± 1. Sample must be obtained 45 minutes ± 15 minutes after beginning the infusion, before the decitabine infusion is complete, and should be obtained from the opposite arm as the infusion. The sample should be collected in a 3 mL K3EDTA tube pre-loaded with 8 mcL of tetrahydrouridine (THU 500 mcg/mL), an inhibitor of cytidine deaminase. The sample should be processed by the Tissue Procurement Core Facility at the Siteman Cancer Center. Plasma should be separated and stored per protocols.

9.0 EFFICACY, SAFETY AND CORRELATIVE STUDY MEASURES

A secondary endpoint of this study is to estimate morphologic complete remission rate.

9.1 Response Criteria - AML

AML Patients will be assessed for response according to the IWG criteria:[1]

Morphologic complete remission (CR) – Defined as morphologic leukemia-free state, defined as less than 5% blasts in aspirate with marrow spicules and a count of ≥200 nucleated cells and no blasts with Auer rods or any persistence of extramedullary disease. When erythroid precursors constitute less than 50% of bone marrow nucleated cells, the percentage of blasts is based on all nucleated cells; when there are 50% or more erythroid cells, the percentage blasts should be based on the nonerythroid cells. The presence of mild megaloblastoid changes may be permitted if they are thought to be consistent with treatment effect. However, persistence of pretreatment abnormalities (eg, pseudo-Pelger-Hüet cells, ringed sideroblasts, dysplastic megakaryocytes) are not consistent with CR. In addition, ANC >1000/µl and platelet count of ≥100,000/µl. Patient must be independent of transfusions. No duration requirement for this designation.

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

Morphologic complete remission with incomplete blood count recovery (CRi) – Defined as morphologic complete recovery with the exception of neutropenia <1000/µl or thrombocytopenia <100,000/µl.

Partial remission (PR) – Defined as meeting the above criteria for complete remission, with the following exceptions: decrease of ≥50% in the percentage of blast to 5-25% in the bone marrow aspirate. A value of ≤5% blasts in bone marrow with Auer rods present may also be considered a partial remission.

Stable Disease (SD) – Defined as not meeting the definitions of CR, CRi, PR, PD, or recurrence/morphologic relapse.

Progressive disease (PD) – Defined as a clear increase in bone marrow blast count, typically a greater than 50% increase compared with baseline, or new extramedullary disease. Patients with a > 50% increase in peripheral blast count with an increase of the total peripheral white blood cell count to > 10,000/µl should undergo evaluation by bone marrow biopsy to access for progressive disease.

Recurrence/morphologic relapse **-** Defined as relapse following 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. New dysplastic changes is considered

relapse. 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.

9.2 Response Criteria - MDS

MDS Patients will be assessed for response according to the IWG criteria:[69]

Complete remission (CR) – Defined as ≤ 5% myeloblasts with normal maturation of all cell lines in the bone marrow and peripheral blood values of Hgb ≥ 11g/dL, Platelets ≥ 100 x 10^9/L, Neutrophils ≥ 1.0 x 10^9/L, and 0% blasts. Persistent dysplasia does not exclude CR but will be noted.

Marrow Complete Response (Marrow CR) – Defined as ≤ 5% myeloblasts in the bone marrow and a decrease by ≥ 50% from pretreatment values, but not meeting the definition of CR above.

Partial Remission (PR) – Defined as meeting the definition of CR above with a decrease of myeloeblasts in the bone marrow by ≥ 50% from pretreatment values, but absolute myeloblasts still >5%.

Stable Disease (SD) – Defined as not meeting the definitions of CR, Marrow CR, PR, SD, PD, or recurrence/morphologic relapse.

Progressive Disease//Relapse (PD) – Defined as ≥ 50% increase in blasts to > 5% blasts (for patients with less than 5% blasts at baseline only), ≥ 50% increase to > 10% blasts (for patients with 5-10% blasts at baseline only), ≥ 50% increase to > 20% blasts (for patients with l0-20% blasts at baseline only), ≥ 50% increase to > 30% blasts (for patients with 20-30% blasts at baseline only) or any of the following: At least 50% decrement from maximum remission/response in granulocytes or platelets, reduction in Hgb by ≥ 2 g/dL, or New or worsened transfusion dependence not related to study drug toxicity. Or for patients with a CR, Marrow CR, or PR as defined above and subsequently development of one of the following: Return to pretreatment bone marrow blast percentage, decrement of ≥ 50% from maximum remission/response levels in granulocytes or platelets, or reduction in Hgb concentration by ≥ 1.5 g/dL or transfusion dependence.

9.2.1 Hematologic Improvement (HI)

Progressive disease as defined above nullifies hematologic improvement.

Erythroid response requires all of the following (only required if pretreatment HgB <11 g/dL):

- Hgb increase by ≥ 1.5 g/dL
- Relevant reduction of units of RBC transfusions by an absolute number of at least 4 RBC transfusions/8 wk compared with the pretreatment transfusion

number in the previous 8 wk. Only RBC transfusions given for a Hgb of ≤ 9.0 g/dL pretreatment will count in the RBC transfusion response evaluation

Platelet response requires one of the following (only required if pretreatment platelets < 100 x 10^9/L):

- Absolute increase of ≥ 30 x 10^9/L (for patients starting with > 20 x 10^9/L platelets)
- Increase from < 20 x 10^9/L to > 20 x 10^9/L and absolute increase ≥ 100% (for patients starting with < 20 x 10^9/L)

Neutrophil response requires the following (only required if pretreatment ANC <1.0 x 10^9/L):

- At least 100% increase and an absolute increase > 0.5 x 10^9/L

9.3.1 Cytogenetic Response

Complete Cytogenetic Response – Defined as reversion to a normal karyotype. For this study, reversion of a normal karyotype is defined as no clonal abnormalities detected in a minimum of 20 mitotic cells. Progressive disease as defined above nullifies cytogenetic response.

9.3 Guidelines for Evaluation of Disease

Bone marrow biopsy should be performed on Day 28 ± 4 of cycles 1, 2, 4 and 6 for clinical disease assessment. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression.

9.4 Other Secondary Efficacy Measures

9.4.1 Treatment Failure, Overall Survival and Event-free Survival

Treatment failure is defined as failure to achieve a complete or partial remission following 4 cycles of therapy.

Overall survival is defined as the date of first dose of study drug to the date of death from any cause. Patients still alive at the time of the final analysis will be right-censored.

Event-free survival is defined as the interval from the date of first dose of study drug to date of treatment failure, recurrence, death due to any cause, or loss to follow up.

9.4.2 Duration of Remission and Relapse-free Survival for Patients Having Complete Remission

Duration of complete remission is defined as the interval from the date that complete remission is documented to the date of recurrence, death due to any cause, or loss 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.

9.4.3 Safety

Safety evaluation will include assessments of adverse experiences, medical history, physical examinations, vital signs, and laboratory assessments at baseline and throughout the study period. Local laboratories may be used for this study.

10.0 ADVERSE EVENT REPORTING

10.1 Adverse Events (AEs)

Definition: any unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site: http://ctep.cancer.gov/reporting/ctc.html.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website: (http://www.hhs.gov/ohrp/policy/AdvEvntGuid.htm).

10.2 Unanticipated Problems

Definition:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

10.3 Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

10.4 Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

10.5 Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

Pre-approval of all protocol exceptions must be obtained prior to the event.

10.6 Reporting to the Human Research Protection Office (HRPO) and the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University:

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH or SLCH institution, or that impacts participants or the conduct of the study.
- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within **10 working days** of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification to the PI of the event.

10.7 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. **PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC.** It is the responsibility of the investigator to report any unanticipated problem to the FDA as follows:

- Report any unexpected fatal or life-threatening adverse experiences associated with use of the drug (i.e., there is a reasonable possibility that the experience may have been caused by the drug) by telephone or fax no later than **7 calendar days** after initial receipt of the information. A life-threatening adverse experience is defined as any adverse drug experience that places the subject (in the view of the investigator) at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Report any serious, unexpected adverse experiences, as well as results from animal studies that suggest significant clinical risk within **15 calendar days** after initial receipt of this information. A serious adverse drug experience is defined as

any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions)
- A congenital anomaly/birth defect
- Any other experience which, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

An unexpected adverse drug experience is defined as any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure (or risk information, if an IB is not required or available).

All MedWatch forms will be sent by the investigator or investigator's team to the FDA at the following address or by fax:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Oncology Drug Products
5901-B Ammendale Rd.
Beltsville, MD 20705-1266
FAX: 1-800-FDA-0178

10.8 Timeframe for Reporting Required Events:

Reportable adverse events will be tracked for 30 days following the last day of study treatment, or until the start of a new chemotherapy/treatment, whichever comes first.

Deaths	
Any reportable death while on study or within 30 days of study	Immediately, within 24 hours, to PI and the IRB
Any reportable death while off study	Immediately, within 24 hours, to PI and the IRB
Adverse Events/Unanticipated Problems	
Any reportable adverse events as described in Sections 10.1 and 10.2 (other than death) and 10.7	Immediately, within 24 hours to PI and within 10 working days to the IRB, and within 7 or 15 days to the FDA
All adverse events regardless of grade and attribution should be submitted cumulatively	Include in DSM report
Noncompliance and Serious Noncompliance	
All noncompliance and serious noncompliance as described in Sections 10.3 and 10.4	Immediately, within 24 hours, to PI and within 10 working days to the IRB

11.0 DATA SAFETY MONITORING PLAN

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months after accrual has opened (if at least five patients have been enrolled) or one year after accrual has opened (if fewer than five patients have been enrolled at the six-month mark).

The Principal Investigator will review all patient data at least every six months, and provide a semi-annual report to the QASMC. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual
- Protocol activation date
- Average rate of accrual observed in year 1, year 2, and subsequent years
- Expected accrual end date
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

12.0 STATISTICAL ANALYSIS METHODS AND SAMPLE SIZE

12.1 Analysis Populations

Intent-to-Treat - The intent-to-treat (ITT) population will be defined as all patients who receive at least one dose of decitabine.

Efficacy-Evaluable - The efficacy-evaluable (EE) population will be defined as all patients who receive at least one cycle of treatment.

Safety - The safety population will be defined as all patients with at least one dose of decitabine.

12.2 Statistical Analysis

12.2.1 Descriptive Analyses

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

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.

12.2.2 Primary Endpoint Analysis

The primary end-point of this phase II study is the correlation biomarkers with overall response in AML/MDS patients to decitabine.

We will correlate the presence of patient specific mutations (especially mutations in *DNMT3A, IDH1, IDH2,* and *TET2*) with overall response rates. Mutation rates in these genes and in other patient-specific genes will be correlated with overall response and their respective 95% confidence intervals will be provided.

Statistical considerations. The relationship between AML/MDS-associated mutations and the responsiveness to decitabine will be described by contingency tables and assessed using Chi-square test or Fisher's exact test as appropriate. The differentially expressed genes between responders and non-responders will be determined using the significance analysis of microarray (SAM). Multivariate logistic regression will also be fit to adjust the potential confounding effects of other demographic and clinical characteristics. The resultant critical *P* values will be corrected for multiple testing. Since the Bonferroni correction is known to be too conservative, a permutation analysis (e.g., 10,000 random assignments for

responsive status) will be used to derive an empirical critical *P* value. The new critical p-value is the number of permutation tests which yield a lower p-value than the original test divided by the total number of permutation tests.[61]

A power analysis was performed using Chi-square test for the association between mutation and response to decitabine. The frequency of *DNMT3A, IDH1/2,* and *TET2* mutations in AML and MDS patients are each between 15% and 30% in multiple cohorts.[29-37] **Table 3** shows the minimal detectable differences of response rates between mutant and wild-type cases, assuming an expected overall 45% CR/CRi rate and 80% power. For an average of 20% frequency of a mutated gene, for example, 100 patients will provide 80% power to detect 30% difference at a 2-sided 0.1 alpha error. The purpose of this study is to determine whether any of these mutations result in a clinically significant association that would warrant pre-treatment testing. We believe that a reasonable cut off would be if at least 30% of clinical response could be attributed to the presence of a specific mutation. However, we anticipate that more power could be achieved as we assess patient groups with combined characteristics (e.g. *DNMT3A* mutation or loss of *DNMT3A* expression). In addition, we expect that the majority of the enrolled 125 patients can be used in the analysis (since all of our molecular information will be acquired within 30 days of enrollment, we anticipate a drop-out rate of 20% or less).

Table 3. Detectable mutation associated response rates (80% power, $\alpha = 0.05$)

Total N	% of patients with a given mutation	Detectable response difference	Sensitivity of mutation to predict response at minimum detectable response difference*
100	15%	0.40	80%
	20%	0.35	75%
	30%	0.30	67%
	40%	0.27	61%
	50%	0.26	58%

* Assuming the overall response rate (CR/Cri) of 45%

12.2.3 Secondary Endpoint Analyses

The overall response rate (CR/CRi/PR) and complete response rate (CR/CRi) will be compared with historical controls[2], and the 95% confidence intervals will be provided.

The rate of patient specific mutation clearance after 10, 28, and 56 days of therapy will be determined using an Illumina based platform. We will compare the rate of mutation clearance between the patients who achieve a CR/CRi after 4 cycles and those who do not. We anticipate that we will identify between 6 and 25 exonic SNVs per case (the range obtained with >200 AML samples sequenced to date with whole genome or exome sequencing), and that the expected 95% confidence

interval of the mean variant allele frequency will be ±2.56% and ±1.25%, respectively, if we assume an anticipated standard deviation of 3.2% within the same clone. The change of tumor burden over time and its association with clinical response will be assessed using 2-way ANOVA for repeated measurement data, followed by the ad-hoc multiple comparisons for between-group differences (e.g., responders vs. non-responders) at given time points as well as the between-time differences within each group. The resultant critical *P* values will also be corrected using permutation analysis for multiple testing.

The steady-state plasma decitabine concentration on day 4 ± 1 will be measured and correlated with clinical overall response.

The percent decrease in bone marrow methylcytosine will be calculated on day 10±1. We will compare this reduction with previously published data and between the patients who achieve CR/CRi and those who do not. The correlation of peak plasma levels of decitabine with response will be assessed using 2-sample t-test or Mann-Whitney non-parametric rank-sum test as appropriate. The change of total bone marrow DNA methylcytosine from baseline and its association with both steady-state plasma drug levels and response will be assessed using 2-way ANOVA for repeated measurement data, followed by the ad-hoc multiple comparisons for between-group differences (e.g., responders vs. non-responders) and between-time differences. Similar analyses will be performed for the correlation of expression profiles and SNP analysis with pharmacokinetic data.

Statistical power justification. A power analysis was performed using two-sample t-test for between-group difference and paired t-test for the change over time. For tumor clearance, this will provide > 99% power to detect a 10% difference in the means at two separate time points for clones with 6 or more variants, and > 98% power to detect a 15% difference if there are 3 or more variants per clone with $\alpha = 0.05$ (power will decrease to 91% and 82%, respectively, in cases where the standard deviation is 4.7%, the maximum we have observed thus far). For pharmacologic correlations, we anticipate 85% power to determine a 25 ng/ml difference between the average C_{max} of 45 evaluable responders and 55 evaluable non-responders with an anticipated average C_{max} of 70 ng/ml and a standard deviation of 40 ng/ml within the entire cohort, and with $\alpha = 0.05$.[22] We believe a less significant difference would not be sufficient to warrant application in clinical decision making (i.e. whether to continue therapy for more than 1 cycle). We anticipate 99% power to determine a difference of 20% in the reduction of bone marrow methylcytosine between 45 evaluable responders and 55 evaluable non-responders with an expected standard deviation of 15% and $\alpha = 0.05$.[28, 66-68] The difference in methylcytosine reduction previously observed was 30% between patients achieving CR/PR and those with only hematologic improvement.[28]

Time to relapse and time to death will also be listed for each patient.

12.2.4 Safety Analysis

Extent of Exposure – Duration (number of cycles/infusions) of decitabine doses (cumulative and intensity) will be summarized.

Adverse Events - All adverse events will be coded using NCI CTCAE v. 4.0. 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. In addition, adverse events will be summarized by investigator attribution of relationship to study medication and by grade. Similar summaries will be provided by treatment cycle.

Clinical Laboratory Evaluation – Applicable laboratory parameters will be graded according to NCI CTCAE version 4.0. The incidence of maximum grade (number and percent of patients experiencing the maximum grade during the study) will be summarized for each laboratory parameter. Similar summaries will be provided by treatment cycle. Graphical displays of select hematological parameters will also be provided.

Vital Signs, Physical Findings, and Other Safety Observations – Vital signs, findings from physical examinations, ECOG scores and weights will be presented in listing format.

12.2.5 Description of Planned Subgroup Analyses

The following subgroups (if sufficient numbers of patients) will be explored with respect to the primary and overall survival endpoints.

- Age (60-69 years old vs ≥ 70 years old)
- Cytogenetics
- Patients with AML-associated mutations in *DNMT3A, TET2, IDH1,* or *IDH2* vs those who do not have somatic variants in these genes.

12.3 Sample Size

We anticipate initially enrolling 25 patients at Washington University. Once we establish a routine exome analysis with validation and calculation of the rate of patient-specific mutation clearance, we will enroll an additional 100 patients from at least 2 additional centers.

13.0 DATA SUBMISSION SCHEDULE

Case Report Form	**Submission Schedule**
Original Consent Form	*Prior to registration*
Patient Registration Form *Disease Diagnosis Form* *Inclusion Criteria Worksheet* *Exclusion Criteria Worksheet*	*Prior to starting treatment*
Treatment Record Form	*To be maintained throughout a patient's treatment cycle. The complete record is due at completion of treatment.*
Bone Marrow Sample Collection Form	*To be maintained throughout the course of the study at designated bone marrow sample collections time points (See Appendix 2).*
Pathology: Bone Marrow Biopsy	*To be completed upon the return of pathology report for each bone marrow biopsy.*
Study Calendar Form	*To be completed with the date of each time point upon occurrence.*
Labs Form	*To be completed upon the return of laboratory reports for each study time point (See Appendix 2)*
Dose Modifications	*To be maintained throughout the course of the study if dose modifications are performed.*
Therapy Response Form	*To be filled out upon remission (prior to, and/or on therapy)*
End of Study Form	*Due at the completion of the EOS visit, at the time of death, and/or at the time that the patient is lost to follow-up*
Follow Up Form	• *30 days post date of last decitabine dose.* • *Every 6 months for 2 years after the last dose of study drug.*
Record of Adverse Events	*At the time of any AE*

Appendix 1 ECOG Performance Scale

Activity Status	Description
0	Asymptomatic, fully active, and able to carry on all predisease performance without restrictions.
1	Symptomatic, fully ambulatory but restricted in physically strenuous activity and able to carry out performance of a light or sedentary nature, eg, light housework, office work.
2	Symptomatic, ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours: in bed less than 50% of day.
3	Symptomatic, capable of only limited self-care, confined to bed or chair more than 50% of waking hours, but not bedridden.
4	Completely disabled. Cannot carry on any self-care. Totally bedridden.
5	Dead

Appendix 2 Study Flow Chart

Study Flow Chart

Test	Pre-study[1]	Base-line[2]	All Cycles Day 1	All Cycles Days 1-10[3]	Cycle 1 Day 4 ± 1	Cycle 1 Day 10 ± 1 and 28 ± 4	Every week	Every 2 weeks	Even cycles Day 28±4	End of Study
Informed Consent	X									
Medical History		X								
Physical Exam		X[a]	X[b]							X
Transfusion Requirements[c]		X[c]	Ongoing							
Bone Marrow Aspirate and Biopsy	X[d]					X[d]			X[d]	X[d]
Flow Cytometry[e]	X									
Peripheral blood for correlative studies					X					
Pregnancy test		X								
CBC with differential and platelets		X	X				X[f]			X
Serum Chemistries[g]		X	X					X[f]		X
Decitabine administration[h]				X						
Adverse Events			Ongoing							
Post-study follow-up										X[i]

[1] To be performed within 14 days of the first dose of decitabine.
[2] To be performed within 7 days of the first dose of decitabine.
[3] Decitabine may be reduced to Days 1-5 or Days 1-3. See Section 6.3 for details.
[a] To include height, weight, and ECOG.
[b] To include weight.
[c] Care should be given to record the frequency of transfusions from 8 weeks prior to first dose of study drug through the duration of the study.
[d] Bone marrow biopsy and aspirate should be performed at baseline, at Cycle 1 Day 10 ± 1, and at Day 28 ± 4 of Cycles 1, 2, 4 and 6 for clinical disease assessment. Following Cycle 6, bone marrow biopsies should be performed at the discretion of the treating physician to assess for relapse or progression. Standard bone marrow procurement procedures will be followed for collection of the tissue. To include: correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics and FISH at all time points. A skin biopsy sample (collected under HRPO# 201011766) is required at pre-study, but may have been collected months or even years prior to first dose of decitabine.
[e] On bone marrow or peripheral blood.
[f] After Cycle 1, these labs may be obtained every other week (CBC) or once a month (Chemistries) at the discretion of the treating physician.
[g] Serum chemistries include bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, and LDH.
[h] Decitabine treatment will be 20 mg/m^2 over a 1-hour IV infusion on consecutive Days 1-10 every 28-day cycle. The dose will be calculated based on actual patient weight at the beginning of each cycle. Patients may be premedicated with lorazepam, prochlorperazine, palonosetron, or ondansetron at the treating physician's discretion.
[i] Patients will be followed for 30 days following the date of the last decitabine dose to monitor for toxicity, survival and response. Thereafter, the investigator or designee will record information on subsequent therapy, disease progression, and death, every 6 months for 2 years after first dose of study drug or until the patient is lost to follow-up.

14.0 REFERENCES

1. Cheson, B.D., et al., *Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia.* J Clin Oncol, 2003. **21**(24): p. 4642-9.
2. Cashen, A.F., et al., *Multicenter, phase II study of decitabine for the first-line treatment of older patients with acute myeloid leukemia.* J Clin Oncol, 2010. **28**(4): p. 556-61.
3. Blum, W., et al., *Clinical response and miR-29b predictive significance in older AML patients treated with a 10-day schedule of decitabine.* Proc Natl Acad Sci U S A, 2010. **107**(16): p. 7473-8.
4. Schlenk, R.F., et al., *Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia.* N Engl J Med, 2008. **358**(18): p. 1909-18.
5. Erba, H.P., *Has there been progress in the treatment of older patients with acute myeloid leukemia?* Best Pract Res Clin Haematol, 2010. **23**(4): p. 495-501.
6. Heaney, M.L. and D.W. Golde, *Myelodysplasia.* N Engl J Med, 1999. **340**(21): p. 1649-60.
7. Bennett, J.M., et al., *Proposals for the classification of the myelodysplastic syndromes.* Br J Haematol, 1982. **51**(2): p. 189-99.
8. Kantarjian, H., et al., *Results of intensive chemotherapy in 998 patients age 65 years or older with acute myeloid leukemia or high-risk myelodysplastic syndrome: predictive prognostic models for outcome.* Cancer, 2006. **106**(5): p. 1090-8.
9. Martin, M.G. and C.N. Abboud, *Induction therapy for elderly patients with acute myeloid leukemia.* Blood Rev, 2008. **22**(6): p. 311-20.
10. Bow, E.J., et al., *Therapy of untreated acute myeloid leukemia in the elderly: remission-induction using a non-cytarabine-containing regimen of mitoxantrone plus etoposide.* J Clin Oncol, 1996. **14**(4): p. 1345-52.
11. Goldstone, A.H., et al., *Attempts to improve treatment outcomes in acute myeloid leukemia (AML) in older patients: the results of the United Kingdom Medical Research Council AML11 trial.* Blood, 2001. **98**(5): p. 1302-11.
12. Stone, R.M., *The difficult problem of acute myeloid leukemia in the older adult.* CA Cancer J Clin, 2002. **52**(6): p. 363-71.
13. Liu Yin, J.A., et al., *Mitozantrone and cytosine arabinoside as first-line therapy in elderly patients with acute myeloid leukaemia.* Br J Haematol, 1991. **79**(3): p. 415-20.
14. Stone, R.M., et al., *Granulocyte-macrophage colony-stimulating factor after initial chemotherapy for elderly patients with primary acute myelogenous leukemia. Cancer and Leukemia Group B.* N Engl J Med, 1995. **332**(25): p. 1671-7.
15. Kantarjian, H., et al., *Decitabine improves patient outcomes in myelodysplastic syndromes.* Cancer, 2006. **106**(8): p. 1794-1803.

16. Lubbert, M., et al., *A multicenter phase II trial of Decitabine as first-line treatment of older AML patients judged unfit for induction chemotherapy.* Haematologica, 2011. **97**(3): p. 393-401.
17. Issa, J.P., et al., *Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies.* Blood, 2004. **103**(5): p. 1635-40.
18. Petti, M.C., et al., *Pilot study of 5-aza-2'-deoxycytidine (Decitabine) in the treatment of poor prognosis acute myelogenous leukemia patients: preliminary results.* Leukemia, 1993. **7 Suppl 1**: p. 36-41.
19. Wijermans, P.W., et al., *Continuous infusion of low-dose 5-Aza-2'-deoxycytidine in elderly patients with high-risk myelodysplastic syndrome.* Leukemia, 1997. **11**(1): p. 1-5.
20. Kantarjian, H., et al., *Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study.* Cancer, 2006. **106**(8): p. 1794-803.
21. Wijermans, P.W., et al., *An epigenetic approach to the treatment of advanced MDS; the experience with the DNA demethylating agent 5-aza-2'-deoxycytidine (decitabine) in 177 patients.* Ann Hematol, 2005. **84 Suppl 1**: p. 9-17.
22. Cashen, A.F., et al., *Pharmacokinetics of decitabine administered as a 3-h infusion to patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS).* Cancer Chemother Pharmacol, 2008. **61**(5): p. 759-66.
23. Chabot, G.G., J. Bouchard, and R.L. Momparler, *Kinetics of deamination of 5-aza-2'-deoxycytidine and cytosine arabinoside by human liver cytidine deaminase and its inhibition by 3-deazauridine, thymidine or uracil arabinoside.* Biochem Pharmacol, 1983. **32**(7): p. 1327-8.
24. Momparler, R.L., et al., *Cell cycle effects and cellular pharmacology of 5-aza-2'-deoxycytidine.* Cancer Chemother Pharmacol, 1984. **13**(3): p. 191-4.
25. Raza, A., et al., *Biological significance of cell cycle kinetics in 128 standard risk newly diagnosed patients with acute myelocytic leukemia.* Br J Haematol, 1991. **79**(1): p. 33-9.
26. Brons, P.P., et al., *Proliferation patterns in acute myeloid leukemia: leukemic clonogenic growth and in vivo cell cycle kinetics.* Ann Hematol, 1993. **66**(5): p. 225-33.
27. Raza, A., et al., *Direct relationship between remission duration in acute myeloid leukemia and cell cycle kinetics: a leukemia intergroup study.* Blood, 1990. **76**(11): p. 2191-7.
28. Shen, L., et al., *DNA methylation predicts survival and response to therapy in patients with myelodysplastic syndromes.* J Clin Oncol, 2010. **28**(4): p. 605-13.
29. Tefferi, A., et al., *IDH1 and IDH2 mutation studies in 1473 patients with chronic-, fibrotic- or blast-phase essential thrombocythemia, polycythemia vera or myelofibrosis.* Leukemia, 2010. **24**(7): p. 1302-9.
30. Ley, T.J., et al., *DNMT3A mutations in acute myeloid leukemia.* N Engl J Med, 2010. **363**(25): p. 2424-33.
31. Mardis, E.R., et al., *Recurring mutations found by sequencing an acute myeloid leukemia genome.* N Engl J Med, 2009. **361**(11): p. 1058-66.

32. Abdel-Wahab, O., J. Patel, and R.L. Levine, *Clinical Implications of Novel Mutations in Epigenetic Modifiers in AML.* Hematol Oncol Clin North Am, 2011. **25**(6): p. 1119-33.
33. Reuther, G.W., *Recurring mutations in myeloproliferative neoplasms alter epigenetic regulation of gene expression.* Am J Cancer Res, 2011. **1**(6): p. 752-62.
34. Paschka, P., et al., *IDH1 and IDH2 mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3 internal tandem duplication.* J Clin Oncol, 2010. **28**(22): p. 3636-43.
35. Shen, Y., et al., *Gene mutation patterns and their prognostic impact in a cohort of 1185 patients with acute myeloid leukemia.* Blood, 2011. **118**(20): p. 5593-603.
36. Tefferi, A., et al., *Detection of mutant TET2 in myeloid malignancies other than myeloproliferative neoplasms: CMML, MDS, MDS/MPN and AML.* Leukemia, 2009. **23**(7): p. 1343-5.
37. Walter, M.J., et al., *Recurrent DNMT3A mutations in patients with myelodysplastic syndromes.* Leukemia, 2011. **25**(7): p. 1153-8.
38. Lin, J., et al., *Recurrent DNMT3A R882 Mutations in Chinese Patients with Acute Myeloid Leukemia and Myelodysplastic Syndrome.* PLoS One, 2011. **6**(10): p. e26906.
39. Bejar, R., et al., *Clinical effect of point mutations in myelodysplastic syndromes.* N Engl J Med, 2011. **364**(26): p. 2496-506.
40. Walter, M.J., et al., *Clonal architecture of secondary acute myeloid leukemia.* N Engl J Med, 2012. **366**(12): p. 1090-8.
41. Kantarjian, H.M., et al., *Survival advantage with decitabine versus intensive chemotherapy in patients with higher risk myelodysplastic syndrome: comparison with historical experience.* Cancer, 2007. **109**(6): p. 1133-7.
42. Lubbert, M., et al., *Continued Low-Dose Decitabine (DAC) Is an Active First-Line Treatment in All Cytogenetic Subgroups of Older AML Patients: Results of the FR00331 Multicenter Phase II Study.* ASH Annual Meeting Abstracts, 2007. **110**(11): p. 300-.
43. Blum, W., et al., *Phase I Study of Decitabine Alone or in Combination With Valproic Acid in Acute Myeloid Leukemia.* J Clin Oncol, 2007. **25**(25): p. 3884-3891.
44. Issa, J.-P.J., et al., *Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies.* Blood, 2004. **103**(5): p. 1635-1640.
45. Garcia-Manero, G., et al., *Phase 1/2 study of the combination of 5-aza-2'-deoxycytidine with valproic acid in patients with leukemia.* Blood, 2006. **108**(10): p. 3271-3279.
46. Issa, J.P., S.B. Baylin, and J.G. Herman, *DNA methylation changes in hematologic malignancies: biologic and clinical implications.* Leukemia, 1997. **11 Suppl 1**: p. S7-11.
47. Ganetzky, R.D., et al., *Differences Between Normal and Leukemic Stem Cell-Specific Methylome Indicates Aberrantly Silenced Genes Involved in the*

Pathogenesis of Malignant Evolution. ASH Annual Meeting Abstracts, 2008. **112**(11): p. 599-.

48. Christman, J.K., *5-Azacytidine and 5-aza-2'-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy.* Oncogene, 2002. **21**(35): p. 5483-95.
49. Lubbert, M., *DNA methylation inhibitors in the treatment of leukemias, myelodysplastic syndromes and hemoglobinopathies: clinical results and possible mechanisms of action.* Curr Top Microbiol Immunol, 2000. **249**: p. 135-64.
50. Kantarjian, H., et al., *Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia.* Blood, 2007. **109**(1): p. 52-57.
51. Steensma, D.P., et al., *Multicenter Study of Decitabine Administered Daily for 5 Days Every 4 Weeks to Adults With Myelodysplastic Syndromes: The Alternative Dosing for Outpatient Treatment (ADOPT) Trial.* J Clin Oncol, 2009: p. JCO.2008.19.6550.
52. Cashen, A.F., et al., *Preliminary Results of a Multicenter Phase II Trial of 5-Day Decitabine as Front-Line Therapy for Elderly Patients with Acute Myeloid Leukemia (AML).* ASH Annual Meeting Abstracts, 2008. **112**(11): p. 560-.
53. Jabbour, E., et al., *Evolution of decitabine development: accomplishments, ongoing investigations, and future strategies.* Cancer, 2008. **112**(11): p. 2341-51.
54. Kantarjian, H., et al., *Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia.* Blood, 2007. **109**(1): p. 52-7.
55. Blum, W.G., Klisovic, R., Liu, S., Kefauver, C., Grever, M. R., Schaaf, L., Chan, K., Byrd, J. C., Villalona-Calero, M., Marcucci, G. *Efficacy of a novel schedule of decitabine in previously untreated AML, age 60 or older*. in *ASCO Annual Meeting*. 2009.
56. van Groeningen, C.J., et al., *Phase I and pharmacokinetic study of 5-aza-2'-deoxycytidine (NSC 127716) in cancer patients.* Cancer Res, 1986. **46**(9): p. 4831-6.
57. Rivard, G.E., et al., *Phase I study on 5-aza-2'-deoxycytidine in children with acute leukemia.* Leuk Res, 1981. **5**(6): p. 453-62.
58. Lin, K.T., R.L. Momparler, and G.E. Rivard, *Sample preparation for the determination of 5-aza-2'-deoxycytidine in plasma by high-performance liquid chromatography.* J Chromatogr, 1985. **345**(1): p. 162-7.
59. Momparler, R.L., G.E. Rivard, and M. Gyger, *Clinical trial on 5-aza-2'-deoxycytidine in patients with acute leukemia.* Pharmacol Ther, 1985. **30**(3): p. 277-86.
60. Raska, K., Jurovcik, M., Sormova., Z., *On the metabolism of 5-azacytidine and 5-aza-2'-deoxycytidine in mice.* Coll Czech Chem Commun, 1965. **30**: p. 3001-3005.
61. Momparler, R.L., et al., *Pilot phase I-II study on 5-aza-2'-deoxycytidine (Decitabine) in patients with metastatic lung cancer.* Anticancer Drugs, 1997. **8**(4): p. 358-68.

62. Aparicio, A., et al., *Phase I trial of continuous infusion 5-aza-2'-deoxycytidine.* Cancer Chemother Pharmacol, 2003. **51**(3): p. 231-9.
63. Bryan, J., et al., *Pharmacokinetic evaluation of decitabine for the treatment of leukemia.* Expert Opin Drug Metab Toxicol, 2011. **7**(5): p. 661-72.
64. Scandura, J.M., et al., *Phase 1 study of epigenetic priming with decitabine prior to standard induction chemotherapy for patients with AML.* Blood, 2011. **118**(6): p. 1472-80.
65. Westfall, P.H. and S.A. Young, *Resampling-based multiple testing: examples and methods for p-value adjustment*. 1993, New York: John Wiley & Sons, Inc.
66. Samlowski, W.E., et al., *Evaluation of a 7-day continuous intravenous infusion of decitabine: inhibition of promoter-specific and global genomic DNA methylation.* J Clin Oncol, 2005. **23**(17): p. 3897-905.
67. Blum, W., et al., *Phase I study of decitabine alone or in combination with valproic acid in acute myeloid leukemia.* J Clin Oncol, 2007. **25**(25): p. 3884-91.
68. Yang, A.S., et al., *DNA methylation changes after 5-aza-2'-deoxycytidine therapy in patients with leukemia.* Cancer Res, 2006. **66**(10): p. 5495-503.
69. Cheson, B.D., et al., *Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia.* Blood, 2006. **108**(2): p. 419-25.

Genomic Predictors of Decitabine Response in AML/MDS

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Genomic Predictors of Decitabine Response in AML/MDS

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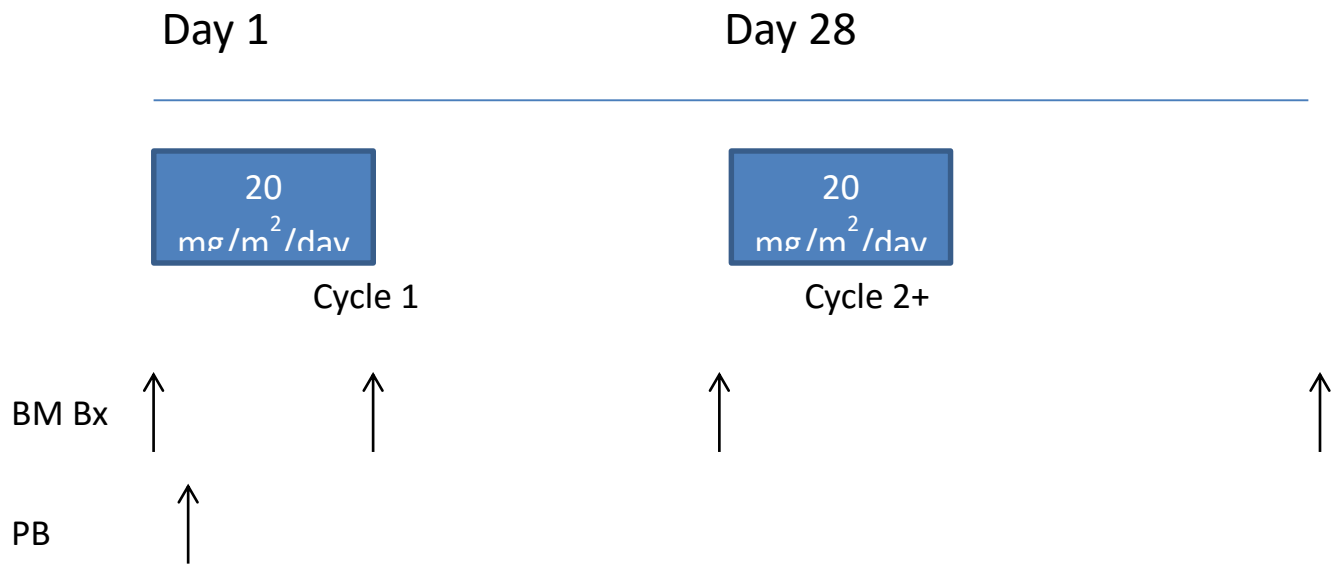
Study Schema:



Patients with progressive disease after two cycles (defined as a clear increase in bone marrow blast count, typically a > 50% increase in bone marrow blasts over baseline) should be removed from study.

Patients with stable disease may continue on study and should undergo a diagnostic bone marrow biopsy at the end of even numbered cycles.

Once AML patients achieve morphologic complete remission with incomplete blood count recovery (CRi) or MDS patients achieve a marrow complete response (Marrow CR) as defined in Section 9.0, decitabine administration may be decreased to five consecutive days to limit myelosuppressive toxicityat the discretion of the treating physician. After two cycles of five consecutive days of treatment, if the patient meets the definition of CR with complete cytogenetic response as defined in Section 9.0, administration may further be decreased to three consecutive days per cycle at the discretion of the treating physician.

Sample collection for correlative studies:

Bone marrow biopsies should be performed at baseline, at Cycle 1 Day 10 ± 1, at Cycle 1Day 28 ± 4, and at the end of even numbered cycles.

Peripheral blood samples should be collected on Day 4± 1 of Cycle 1. Collection should be obtained 45 ± 15 minutes after decitabine infusion is initiated.

Genomic Predictors of Decitabine Response in AML/MDS

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1.0 INTRODUCTION

1.1 Rationale

Personalized therapy for cancer requires that 1) patient-specific factors (e.g. patient-specific mutations, expression profiles, or pharmacogenomics) influence treatment algorithms, and 2) once therapy is initiated, *in vivo* responsiveness is used to guide further therapy. The modern era of genomics has provided significant insights into cancer pathogenesis by describing patterns of germline and somatic variants, as well as defining disease-specific patterns of gene expression. However, the application and integration of this data with therapeutics remains a difficult task.

[redacted]

AML and MDS are two closely related hematological disorders characterized by ineffective hematopoiesis and malignant expansion of clonal myeloid cells. Both AML and MDS frequently occur in elderly patients and some cases are secondary and/or related to long-term bone marrow damage after radiation or chemotherapy. Current therapy for AML consists of cytotoxic chemotherapy followed by consolidation chemotherapy or stem cell transplantation. Using these approaches, current cure rates may be as high as 60% for patients younger than 60 with good risk cytogenetics (e.g. t(15;17), t(8;21), inv(16)).[4] Outcomes in patients ≥ 60 years old and with relapsed disease remain poor with 2 year survival of only ~25%.[5] Historically, the available treatment for MDS has been symptomatic and has not been shown to be effective in producing sustained improvement in hematopoiesis or in delaying leukemic evolution[6, 7].

Current AML therapy is poorly tolerated in elderly and pre-treated patients due to hematologic and non-hematologic toxicities; following such therapy, patients typically require 3-4 weeks of hospitalization for treatment of neutropenic fevers and for regular transfusions. Even among the relatively healthy patients enrolled in clinical trials, induction mortality in the elderly can be as high as 20-40% following standard induction therapy,[8-11] and moderate to severe non-hematologic toxicity occurs in at least an additional 30% of patients.[9, 12-14] Consequently, many older AML patients are not offered traditional intensive chemotherapy, but receive supportive care alone.[15] More effective and less toxic therapies are needed to provide durable remissions in a significant proportion of elderly AML in populations.

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We and others have recently used whole genome sequencing to identify recurrent, somatic mutations in AML and MDS patients in four genes that are involved in methylcytosine regulation: *DNMT3A, IDH1, IDH2,* and *TET2.*[29-37] Collectively, nearly half of all AML/MDS patients have a mutation in one of these four genes. *DNMT3A* mutations are associated with an adverse outcome in AML patients treated with cytotoxic chemotherapy.[35, 38] However, it is not yet clear whether the presence of mutations in one or more of these genes is associated with response or resistance to decitabine.

In this study, we will treat patients with the current state-of-the-art decitabine schedule.[3] We will perform exome sequencing on all patients and correlate response with mutation status, rate of mutation clearance, expression profile, plasma decitabine levels, and DNA methylcytosine content reduction. This approach will comprehensively assess genomic and pharmacologic parameters that could clinically predict patient response to decitabine.

In this study, we will combine analysis of patients with AML and MDS. Recent genomic analysis has shown that AML and MDS have shared mutations in many of the same genes, shared patterns of mutation acquisition, and shared patterns of mutation evolution.[39, 40] Furthermore, MDS, like AML, presents with clonal hematopoiesis, even when the morphologic blast count is zero.[36]Because this study will identify and track the rate of clearance of patient specific mutations, and because decitabine has been shown to have efficacy in both AML and MDS patients, [2, 3, 16-19, 41-45] combining analysis of AML and MDS patients is reasonable. Asymptomatic MDS patients are excluded because these patients are typically followed with watchful-waiting rather than with chemotherapy.

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1.2.2 Experience with extended dosing schedules

Blum *et al* recently published their experience treating elderly AML patients with a 10-day course of decitabine 20 mg/m^2/day as a 1-hour infusion.[3] This

prolonged exposure course was designed to increase the number of AML blast divisions exposed to decitabine per cycle. Toxicities were very similar to a similar regiment given as a 3 and 5 day course,[2, 16] but the overall response (CR/PR) was much improved (64% vs 25%). In all three studies, the toxicities were primarily myeloid suppression and the resultant infectious and bleeding complications associated with leukemic and pharmacologic hematopoietic suppression. Furthermore, Blum *et al* noted that the percent methylcytosine reduction was improved (22% vs 11%) as was the time to blast clearance (50% of patients who ultimately achieved a CR had ≤ 5% bone marrow blasts after 1 cycle vs 0% of patients after 2 cycles). Finally, like Shen *et al*, they observed a correlation between the extent of methylcytosine reduction and response.[28] This study suggests that improvement in response rates and time to response can be achieved by prolonged administration of decitabine. Further, that DNA methylcytosine reduction can be achieved with prolonged exposure to decitabine and that this might be a patient-specific biological marker of adequate dosing.

Scandura *et al.* recently published a study of infusional decitabine prior to cytarabine + daunorubicin induction therapy for AML.[64] They escalated the duration of infusional therapy and reached their planned goal of 20 mg/m^2/day x 7 days of continuous therapy given prior to cytarabine and daunorubicin. They noted similar toxicities (primarily hematopoietic and the associated neutropenic infections), but that these were not different from their experience giving cytarabine + daunorubicin alone.

1.3 Correlative Studies

With exception of the peripheral blood sample collected on Cycle 1 Day 4, all correlative studies will be collected under HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Diseases") that all patients will be concurrently enrolled in. The goals of the correlative studies include:

1. To correlate patient-specific mutations with response to decitabine.
   We will perform exome sequencing of each patient's pre-study bone marrow sample and skin sample. We will determine AML/MDS-associated variants found in the bone marrow, but not skin sample. We will correlate the presence of mutations with response (especially mutations in *DNMT3A, IDH1, IDH2,* and *TET2*). This study is powered to identify mutations with >80% sensitivity to predict decitabine response if used as a clinical assay.

2. To correlate the velocity and depth of response with response to decitabine.
   We will assess tumor burden by allele frequency of patient-specific mutations in bone marrow samples collected pre study, Cycle 1 Day 10±1, Cycle 1 Day 28±4, and Cycle 2 Day 28±4. We will assess mutations in at least 24 recurrently mutated genes (including, but not limited to: *FLT3, DNMT3A, NPM1, TET2, WT1, IDH1, IDH2, RUNX1, NRAS, TP53, U2AF1, KRAS, PHF6, MSTP9, PTPN11, KIT, SMC3, PRUNE2, ETV6, CEBPA, SMC1A, ASXL1, STAG2, RAD21*) as well as all patient-

specific mutations discovered during exome sequencing. The leukemic allele frequency will be measured using the Illumnia MySeq or HiSeq platform using DNA capture by gene specific biotinylated-oligonucleotides. Patient bone marrow samples will be assessed pre-study, Cycle 1 Day 10 ± 1, Cycle 1 Day 28 ± 4, and Cycle 2 Day 28 ± 4 and compared with patient specific skin samples (normal control). The velocity of response will be calculated as the natural log rate of patient-specific mutation allele frequency clearance. Depth of response will be defined as the lowest patient-specific mutation allele frequency in a bone marrow sample. The velocity and depth of tumor response will be correlated to:

- Achievement of clinical CR,
- Time to best response,
- Mutational status,
- Time to disease progression.

Because all patient-specific exome-discovered mutations will be assessed a second time in the primary bone marrow sample as well as the Cycle 1 Day 10 ± 1, Cycle 1 Day 28 ± 4, and Cycle 2 Day 28 ± 4 bone marrow samples, this study will also serve to validate all exome-discovered mutations. This is important, because exome sequencing is associated with a 20-50% false-discovery rate depending on type I error tolerated (mutation-calling algorithms are typically set for greater sensitivity than specificity). Thus, many mutations discovered during exome sequencing are incorrect. This second analysis of these mutations will server to exclude these false positive calls.

3. To correlate the bone marrow expression profile from pre-study and Cycle 1 Day 10 ± 1 with response to decitabine.
   We will correlate expression signatures, and changes in expression signatures, with:
   - Achievement of clinical CR,
   - Mutation status.

4. To correlate steady-state plasma decitabine concentration on Cycle 1 Day 4 ± 1 with:
   - Achievement of CR.

5. To correlate the extent of bone marrow DNA hypomethylation on Cycle 1 Day 10 ± 1 with response to decitabine.
   We will assess the percent reduction in bone marrow methylcytosine content using LC-MS. Patients will be evaluated pre-study and Cycle 1 Day 10 ± 1. The change in DNA methylcytosine content will be correlated to:
   - Achievement of clinical CR,
   - Change in expression signature.

   This will determine whether inadequate blast exposure to decitabine correlates with drug resistance.

2.0 OBJECTIVES

2.1 Primary Objective

To determine whether patient-specific mutations correlate with clinical overall response to decitabine in patients with AML or MDS.

2.2 Secondary Objectives

- To determine whether 10 consecutive days per cycle of decitabine improves outcomes compared with historical controls treated with 5 consecutive days per cycle of decitabine.

- To determine whether other biomarkers correlate with clinical overall response to decitabine in patients with AML or MDS. These will include:

    1. The velocity and depth of patient-specific mutation clearance;
    2. The bone marrow expression profile and change in expression profile during decitabine treatment;
    3. The steady-state plasma decitabine concentrations;
    4. The decrease in bone marrow methylcytosine content in response to decitabine.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

One of the following:

- Patient must have non-M3 AML and be ≥ 60 years of age OR
- non-M3 AML with relapsed disease OR
- Symptomatic MDS with one of the following:
  - □ Symptomatic anemia with either hemoglobin < 10.0 g/dL or requiring RBC transfusion
  - □ Thrombocytopenia with a history of two or more platelet counts < 50,000/mcL or a significant hemorrhage requiring platelet transfusions
  - □ Neutropenia with two or more ANC < 1,000/mcL

All of the following:

1. Patient must have an ECOG performance status ≤ 2.
2. Patient must have >10% disease burden measured by cytomorphology, flow cytometry, or cytogenetics.
3. Patient must have peripheral white blood cell count ≤ 50,000/mcl.
4. Patient must have adequate organ function, defined as:
   a. Total bilirubin ≤ 1.5 x ULN
   b. AST/ALT ≤ 2.5 x ULN
   c. Serum creatinine ≤ 2.0 x ULN
5. Patient must have undergone ≤ 2 cycles of prior hypomethylating agent (decitabine or azacitidine).
6. Patient must be enrolled in HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Diseases").
7. Patient must be ≥ 18 years of age.
8. Patient must be able to understand and willing to sign an IRB-approved written informed consent document.

3.2 Exclusion Criteria

1. Patient must not be pregnant or nursing.
2. Patient must not have known CNS leukemia.
3. Patient must not have a history of positive HIV serology.
4. Patient must not have a history of positive Hepatitis C serology.
5. Patient must not have undergone a prior allogeneic stem cell transplant.
6. Patient must not have any uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, ongoing or active GVHD, congestive heart failure of NYHA class 3 or 4, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situation that would limit compliance with study requirements.
7. Patient must not have had radiation therapy within 14 days of enrollment.
8. Patient must not have received any chemotherapy within 21 days of enrollment, and any acute treatment-related toxicities must have returned to baseline. Patients

may be receiving hydrea at time of enrollment.

3.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4.0 REGISTRATION PROCEDURES

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility
2. Registration of patient in the Siteman Cancer Center database
3. Assignment of unique patient number (UPN)

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility collecting the information listed below

1. The registering MD's name
2. Patient's race, sex, and DOB
3. Three letters (or two letters and a dash) for the patient's initials
4. Copy of signed consent form
5. Completed eligibility checklist, signed and dated by a member of the study team
6. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center Database

All patients must be registered through the Siteman Cancer Center database.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. Patients will also be identified by first, middle, and last initials. If the patient has no middle initial, a dash will be used on the case report forms (CRFs). All data will be recorded with this identification number on the appropriate CRFs.

5.0 INVESTIGATIONAL PLAN

5.1 Summary of Study Design

This is an open-label, single-arm, Phase II, biomarker study of patients with AML or MDS who are treated with 10 consecutive days of decitabine. FAB-M3 AML is excluded from this trial, because other well-tolerated options exist for the treatment of FAB-M3 acute promyelocytic leukemia. AML patients who are < 60 years old and have not received cytotoxic induction chemotherapy are also excluded, because cytotoxic therapy offers a curative possibility for these patients. MDS patients without cytopenias are also excluded because these patients can be followed with watchful-waiting.

All patients will undergo bone marrow biopsies pre-study, on Cycle 1 Day 10 ± 1, and on Day 28 ± 4 of Cycle 1 and all even number cycles. Patients will provide a skin biopsy sample pre-study. Patients will also provide peripheral blood samples on Cycle 1 Day 4 ± 1 for pharmacokinetic studies. See Section 8.0 for details on correlative studies.

Patients will receive decitabine 20 mg/m^2/day as a 1-hour infusion on consecutive Days 1-10 in 28-day cycles. The dose will be calculated based on actual patient weight at the beginning of each cycle.

Once AML patients achieve morphologic complete remission with incomplete blood count recovery (CRi) or MDS patients achieve a marrow complete response (Marrow CR) as defined in Section 9.0, decitabine administration may be decreased to five consecutive days to limit myelosuppressive toxicity at the discretion of the treating physician. After two cycles of five consecutive days of treatment, if the patient meets the definition of CR with complete cytogenetic response as defined in Section 9.0, administration may further be decreased to three consecutive days per cycle at the discretion of the treating physician.

It will be a logistical goal of this trial consistently to return preliminary exome results to the treating physician by the end of Cycle 1 and validated results by the end of Cycle 2. However, this logistical goal is not a primary or secondary aim of this study. Given the limited data regarding mutation presence and drug response, we do not anticipate that the results will directly influence therapeutic decisions made by the treating physician. However, the development of comprehensive genomic pipelines and integration of this data into a clinical trial setting will be required before personalized medicine can be realized.

5.2 Study Procedures

5.2.1 Pre-study Procedures

Pre-study procedures must take place no more than 14 days prior to the first dose of decitabine except where noted below.

1. Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics, and FISH.
2. Bone marrow or peripheral blood for flow cytometry
3. Skin biopsy sample (collected under HRPO# 201011766). There is no required time frame for this sample; it may have been collected months or even years prior to the first dose of decitabine.

5.2.2 Baseline Evaluation

Baseline evaluations must take place no more than 7 days prior to the first dose of decitabine.

1. Medical history
2. Physical exam including height, weight, and ECOG performance score.
3. Record transfusion requirements for 8 weeks before first dose of study drug
4. CBC with differential and platelets
5. Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH
6. Urine βHCG pregnancy test if appropriate

5.2.3 Day 1 of Each Cycle

1. Physical exam, including weight
2. CBC with differential and platelets
3. Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH

5.2.4 Cycle 1 Days 1-10

Decitabine administration, 20 mg/m^2 per day given as a 1-hour continuous intravenous infusion. The dose will be calculated based on actual patient weight at the beginning of each cycle. Patients may be premedicated with lorazepam, prochlorperazine, palonosetron, or ondansetron at the treating physician's discretion.

5.2.5 Cycle 1 Day 4 ± 1

Obtain 3 mL peripheral blood for correlative studies. Sample must be obtained 45 minutes ± 15 minutes after beginning the infusion, before the decitabine infusion is complete, and should be obtained from the opposite arm as the infusion. The sample should be collected in a 3 mL K3EDTA tube pre-loaded with 8 mcL of tetrahydrouridine (THU 500 mcg/mL), an inhibitor of cytidine deaminase. The

sample should be processed by the Tissue Procurement Core Facility at the Siteman Cancer Center. Plasma should be separated and stored per protocols.

5.2.6 Cycle 1 Day 10 ± 1

Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics, and FISH.

5.2.7 Weekly

CBC with differential and platelets. After the first cycle, this may be changed to every other week at the discretion of the treating physician.

5.2.8 Every Other Week

Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH. After the first cycle, this may be changed to every month at the discretion of the treating physician.

5.2.9 Cycle 1 and Even Cycles Day 28 ± 4 days

Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include: correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics, and FISH

5.2.10 End-of-Study Procedures

1. Physical exam
2. CBC with differential and platelets.
3. Serum chemistries including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, and LDH.
4. Bone marrow aspirate and biopsy. Standard bone marrow procurement procedures will be followed for collection of the tissue. Assessments performed on bone marrow will include morphologic assessment, cytogenetics, and FISH.

5.2.11 Post-study Follow-up

Patients are to be followed according to standard patient care procedures for 30 days following the date of the last decitabine dose to monitor for toxicity, survival, and response. Thereafter, the investigator or designee will record

information on subsequent therapy, disease progression, and/or death every 6 months for 2 years after last dose of study drug or until the patient is lost to follow-up or dies.

5.3 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented in the case report forms.

In the absence of treatment delays due to adverse events, treatment may continue indefinitely or until one of the following criteria applies:

- Documented and confirmed disease progression
- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious non-compliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study

Patients who prematurely discontinue treatment for any reason will be followed as indicated in the study calendar.

5.4 Concomitant Therapy and Supportive Care Guidelines

5.4.1 Chemotherapy

Patients cannot receive any chemotherapy within 21 days of study enrollment and must have recovered from any treatment-related toxicities. However, patients may be receiving hydrea at enrollment and may continue on hydrea through Cycle 2 of decitabine. An indication for hydrea following 2 cycles of decitabine is evidence that the patient is not responding and decitabine should be discontinued. Other than decitabine, patients cannot receive chemotherapy during this trial.

5.4.2 Growth Factors

Erythroid growth factors should not be routinely administered to patients during the study.

Thrombopoietin receptor agonists should not be routinely administered to patients during the study.

Filgrastim (G-CSF) or sargramostim (GM-CSF) may be administered to patients with septic shock or febrile neutropenia at the discretion of the treating physician.

5.4.3 Transfusions

- *Packed red blood cells (RBCs) transfusion*:
  RBC transfusions will be administered at the discretion of the treating physician.

- *Platelet transfusion*:
  Platelet transfusions will be administered at the discretion of the treating physician

5.4.4 Prophylactic Antimicrobial Agents

Antimicrobial agents will be administered at the discretion of the treating physician.

5.4.5 Radiotherapy

Patients cannot receive radiotherapy within 14 days prior to study enrollment or while on study.

6.0 DOSE MODIFICATIONS

A criterion for discontinuation of the study is a delay of > 8 weeks between any two cycles.

6.1 Delay for Cytopenia or Infection

As cytopenias are a defining feature of AML and MDS, their presence alone does not generally warrant dose modifications or delays. Patients will receive four cycles of therapy without adjustment for any cytopenia. Following Cycle 4, dose modifications or delays for cytopenias will be made at the discretion of the investigator.

Patients with febrile neutropenia (temperature ≥ 38.5° with ANC < 1,000/μl) or systemic infections should have treatment held until clinical resolution of the infection.

6.2 Delay for Organ Dysfunction

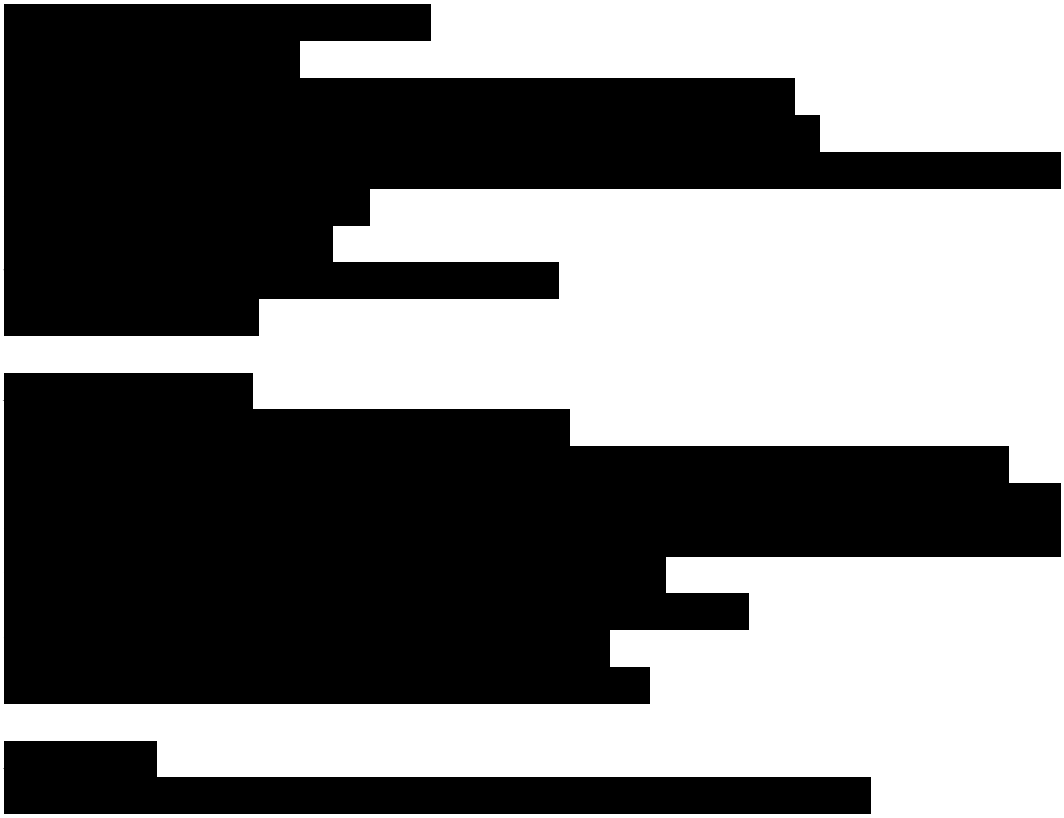
If renal dysfunction (creatinine ≥ 3 X institutional upper limit of normal) or hepatic dysfunction (bilirubin ≥ 2.5 mg/dl or ALT/AST ≥ 3 X institutional upper limit of normal) occurs, therapy may not resume until the abnormalities have resolved to below the limits stated above. If renal or hepatic dysfunction occurs during a treatment course, the next dose of decitabine should be held, and serum chemistries should be checked twice per week. Decitabine can be resumed at full dose when creatinine, bilirubin, ALT and AST are below the limits listed above.

For grade 3-4 non-hematologic toxicities related to decitabine, decitabine treatment should be delayed until recovery to baseline or to grade ≤ 1. Decitabine may be reintroduced with a 50% dose reduction, or at full dose at the discretion of the investigator.

6.3 Modification in Dose or Schedule for Treatment Response

Once AML patients achieve morphologic complete remission with incomplete blood count recovery (CRi) or MDS patients achieve a marrow complete response (Marrow CR) as defined in Section 9.0, decitabine administration may be decreased to 5 consecutive days to limit myelosuppressive toxicity at the discretion of the treating physician. After two cycles of five consecutive days of treatment, if the patient meets the definition of CR with complete cytogenetic response as defined in Section 9.0, administration may further be decreased to three consecutive days per cycle at the discretion of the treating physician.

[redacted]



8.0 CORRELATIVE STUDIES SAMPLE COLLECTIONS

Bone marrow samples will be collected pre-study, on Cycle 1 Day 10 ± 1, and on Day 28 ± 4 of Cycle 1 and of even-numbered cycles for correlative studies. The bone marrow samples will be collected under HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Diseases") that all patients will be concurrently enrolled in.

Skin biopsy sample will be collected as part of the patient's participation in HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Malignancies"). There is no required time frame for this sample, and it may have been collected months or even years prior to the first dose of decitabine.

Peripheral blood sample (3 mL) will be collected on Cycle 1 Day 4 ± 1. Sample must be obtained 45 minutes ± 15 minutes after beginning the infusion, before the decitabine infusion is complete, and should be obtained from the opposite arm as the infusion. The sample should be collected in a 3 mL K3EDTA tube pre-loaded with 8 mcL of tetrahydrouridine (THU 500 mcg/mL), an inhibitor of cytidine deaminase. The sample should be processed by the Tissue Procurement Core Facility at the Siteman Cancer Center. Plasma should be separated and stored per protocols.

9.0 EFFICACY, SAFETY AND CORRELATIVE STUDY MEASURES

A secondary endpoint of this study is to estimate morphologic complete remission rate.

9.1 Response Criteria - AML

AML Patients will be assessed for response according to the IWG criteria:[1]

Morphologic complete remission (CR) – Defined as morphologic leukemia-free state, defined as less than 5% blasts in aspirate with marrow spicules and a count of ≥200 nucleated cells and no blasts with Auer rods or any persistence of extramedullary disease. When erythroid precursors constitute less than 50% of bone marrow nucleated cells, the percentage of blasts is based on all nucleated cells; when there are 50% or more erythroid cells, the percentage blasts should be based on the nonerythroid cells. The presence of mild megaloblastoid changes may be permitted if they are thought to be consistent with treatment effect. However, persistence of pretreatment abnormalities (eg, pseudo-Pelger-Hüet cells, ringed sideroblasts, dysplastic megakaryocytes) are not consistent with CR. In addition, ANC >1000/µl and platelet count of ≥100,000/µl. Patient must be independent of transfusions. No duration requirement for this designation.

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

Morphologic complete remission with incomplete blood count recovery (CRi) – Defined as morphologic complete recovery with the exception of neutropenia <1000/µl or thrombocytopenia <100,000/µl.

Partial remission (PR) – Defined as meeting the above criteria for complete remission, with the following exceptions: decrease of ≥50% in the percentage of blast to 5-25% in the bone marrow aspirate. A value of ≤5% blasts in bone marrow with Auer rods present may also be considered a partial remission.

Stable Disease (SD) – Defined as not meeting the definitions of CR, CRi, PR, PD, or recurrence/morphologic relapse.

Progressive disease (PD) – Defined as a clear increase in bone marrow blast count, typically a greater than 50% increase compared with baseline, or new extramedullary disease. Patients with a > 50% increase in peripheral blast count with an increase of the total peripheral white blood cell count to > 10,000/µl should undergo evaluation by bone marrow biopsy to access for progressive disease.

Recurrence/morphologic relapse - Defined as relapse following 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. New dysplastic changes is considered

relapse. 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.

9.2 Response Criteria - MDS

MDS Patients will be assessed for response according to the IWG criteria:[69]

Complete remission (CR) – Defined as ≤ 5% myeloblasts with normal maturation of all cell lines in the bone marrow and peripheral blood values of Hgb ≥ 11g/dL, Platelets ≥ 100 x 10^9/L, Neutrophils ≥ 1.0 x 10^9/L, and 0% blasts. Persistent dysplasia does not exclude CR but will be noted.

Marrow Complete Response (Marrow CR) – Defined as ≤ 5% myeloblasts in the bone marrow and a decrease by ≥ 50% from pretreatment values, but not meeting the definition of CR above.

Partial Remission (PR) – Defined as meeting the definition of CR above with a decrease of myeloeblasts in the bone marrow by ≥ 50% from pretreatment values, but absolute myeloblasts still >5%.

Stable Disease (SD) – Defined as not meeting the definitions of CR, Marrow CR, PR, SD, PD, or recurrence/morphologic relapse.

Progressive Disease//Relapse (PD) – Defined as ≥ 50% increase in blasts to > 5% blasts (for patients with less than 5% blasts at baseline only), ≥ 50% increase to > 10% blasts (for patients with 5-10% blasts at baseline only), ≥ 50% increase to > 20% blasts (for patients with l0-20% blasts at baseline only), ≥ 50% increase to > 30% blasts (for patients with 20-30% blasts at baseline only) or any of the following: At least 50% decrement from maximum remission/response in granulocytes or platelets, reduction in Hgb by ≥ 2 g/dL, or New or worsened transfusion dependence not related to study drug toxicity. Or for patients with a CR, Marrow CR, or PR as defined above and subsequently development of one of the following: Return to pretreatment bone marrow blast percentage, decrement of ≥ 50% from maximum remission/response levels in granulocytes or platelets, or reduction in Hgb concentration by ≥ 1.5 g/dL or transfusion dependence.

9.2.1 Hematologic Improvement (HI)

Progressive disease as defined above nullifies hematologic improvement.

Erythroid response requires all of the following (only required if pretreatment HgB <11 g/dL):

- Hgb increase by ≥ 1.5 g/dL
- Relevant reduction of units of RBC transfusions by an absolute number of at least 4 RBC transfusions/8 wk compared with the pretreatment transfusion

number in the previous 8 wk. Only RBC transfusions given for a Hgb of ≤ 9.0 g/dL pretreatment will count in the RBC transfusion response evaluation

Platelet response requires one of the following (only required if pretreatment platelets < 100 x 10^9/L):

- Absolute increase of ≥ 30 x 10^9/L (for patients starting with > 20 x 10^9/L platelets)
- Increase from < 20 x 10^9/L to > 20 x 10^9/L and absolute increase ≥ 100% (for patients starting with < 20 x 10^9/L)

Neutrophil response requires the following (only required if pretreatment ANC <1.0 x 10^9/L):

- At least 100% increase and an absolute increase > 0.5 x 10^9/L

9.3.1 Cytogenetic Response

Complete Cytogenetic Response – Defined as reversion to a normal karyotype. For this study, reversion of a normal karyotype is defined as no clonal abnormalities detected in a minimum of 20 mitotic cells. Progressive disease as defined above nullifies cytogenetic response.

9.3 Guidelines for Evaluation of Disease

Bone marrow biopsy should be performed on Day 28 ± 4 of cycle 1 and on Day 28 ± 4 of each even cycle for clinical disease assessment.

9.4 Other Secondary Efficacy Measures

9.4.1 Treatment Failure, Overall Survival and Event-free Survival

Treatment failure is defined as failure to achieve a complete or partial remission following 4 cycles of therapy.

Overall survival is defined as the date of first dose of study drug to the date of death from any cause. Patients still alive at the time of the final analysis will be right-censored.

Event-free survival is defined as the interval from the date of first dose of study drug to date of treatment failure, recurrence, death due to any cause, or loss to follow up.

9.4.2 Duration of Remission and Relapse-free Survival for Patients Having Complete Remission

Duration of complete remission is defined as the interval from the date that

complete remission is documented to the date of recurrence, death due to any cause, or loss 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.

9.4.3 Safety

Safety evaluation will include assessments of adverse experiences, medical history, physical examinations, vital signs, and laboratory assessments at baseline and throughout the study period. Local laboratories may be used for this study.

10.0 ADVERSE EVENT REPORTING

10.1 Adverse Events (AEs)

Definition: any unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site: http://ctep.cancer.gov/reporting/ctc.html.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website: (http://www.hhs.gov/ohrp/policy/AdvEvntGuid.htm).

10.2 Unanticipated Problems

Definition:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

10.3 Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

10.4 Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

10.5 Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

Pre-approval of all protocol exceptions must be obtained prior to the event.

10.6 Reporting to the Human Research Protection Office (HRPO) and the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University:

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH or SLCH institution, or that impacts participants or the conduct of the study.
- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within **10 working days** of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification to the PI of the event.

10.7 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. **PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC.** It is the responsibility of the investigator to report any unanticipated problem to the FDA as follows:

- Report any unexpected fatal or life-threatening adverse experiences associated with use of the drug (i.e., there is a reasonable possibility that the experience may have been caused by the drug) by telephone or fax no later than **7 calendar days** after initial receipt of the information. A life-threatening adverse experience is defined as any adverse drug experience that places the subject (in the view of the investigator) at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Report any serious, unexpected adverse experiences, as well as results from animal studies that suggest significant clinical risk within **15 calendar days** after initial receipt of this information. A serious adverse drug experience is defined as

any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions)
- A congenital anomaly/birth defect
- Any other experience which, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

An unexpected adverse drug experience is defined as any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure (or risk information, if an IB is not required or available).

All MedWatch forms will be sent by the investigator or investigator's team to the FDA at the following address or by fax:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Oncology Drug Products
5901-B Ammendale Rd.
Beltsville, MD 20705-1266
FAX: 1-800-FDA-0178

10.8 Timeframe for Reporting Required Events:

Reportable adverse events will be tracked for 30 days following the last day of study treatment, or until the start of a new chemotherapy/treatment, whichever comes first.

Deaths	
Any reportable death while on study or within 30 days of study	Immediately, within 24 hours, to PI and the IRB
Any reportable death while off study	Immediately, within 24 hours, to PI and the IRB
Adverse Events/Unanticipated Problems	
Any reportable adverse events as described in Sections 10.1 and 10.2 (other than death) and 10.7	Immediately, within 24 hours to PI and within 10 working days to the IRB, and within 7 or 15 days to the FDA
All adverse events regardless of grade and attribution should be submitted cumulatively	Include in DSM report
Noncompliance and Serious Noncompliance	
All noncompliance and serious noncompliance as described in Sections 10.3 and 10.4	Immediately, within 24 hours, to PI and within 10 working days to the IRB

11.0 DATA SAFETY MONITORING PLAN

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months after accrual has opened (if at least five patients have been enrolled) or one year after accrual has opened (if fewer than five patients have been enrolled at the six-month mark).

The Principal Investigator will review all patient data at least every six months, and provide a semi-annual report to the QASMC. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual
- Protocol activation date
- Average rate of accrual observed in year 1, year 2, and subsequent years
- Expected accrual end date
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

12.0 STATISTICAL ANALYSIS METHODS AND SAMPLE SIZE

12.1 Analysis Populations

Intent-to-Treat - The intent-to-treat (ITT) population will be defined as all patients who receive at least one dose of decitabine.

Efficacy-Evaluable - The efficacy-evaluable (EE) population will be defined as all patients who receive at least one cycle of treatment.

Safety - The safety population will be defined as all patients with at least one dose of decitabine.

12.2 Statistical Analysis

12.2.1 Descriptive Analyses

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

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.

12.2.2 Primary Endpoint Analysis

The primary end-point of this phase II study is the correlation biomarkers with overall response in AML/MDS patients to decitabine.

We will correlate the presence of patient specific mutations (especially mutations in *DNMT3A, IDH1, IDH2,* and *TET2*) with overall response rates. Mutation rates in these genes and in other patient-specific genes will be correlated with overall response and their respective 95% confidence intervals will be provided.

Statistical considerations. The relationship between AML/MDS-associated mutations and the responsiveness to decitabine will be described by contingency tables and assessed using Chi-square test or Fisher's exact test as appropriate. The differentially expressed genes between responders and non-responders will be determined using the significance analysis of microarray (SAM). Multivariate logistic regression will also be fit to adjust the potential confounding effects of other demographic and clinical characteristics. The resultant critical *P* values will be corrected for multiple testing. Since the Bonferroni correction is known to be too conservative, a permutation analysis (e.g., 10,000 random assignments for

responsive status) will be used to derive an empirical critical *P* value. The new critical p-value is the number of permutation tests which yield a lower p-value than the original test divided by the total number of permutation tests.[61]

A power analysis was performed using Chi-square test for the association between mutation and response to decitabine. The frequency of *DNMT3A, IDH1/2,* and *TET2* mutations in AML and MDS patients are each between 15% and 30% in multiple cohorts.[29-37] **Table 3** shows the minimal detectable differences of response rates between mutant and wild-type cases, assuming an expected overall 45% CR/CRi rate and 80% power. For an average of 20% frequency of a mutated gene, for example, 100 patients will provide 80% power to detect 30% difference at a 2-sided 0.1 alpha error. The purpose of this study is to determine whether any of these mutations result in a clinically significant association that would warrant pre-treatment testing. We believe that a reasonable cut off would be if at least 30% of clinical response could be attributed to the presence of a specific mutation. However, we anticipate that more power could be achieved as we assess patient groups with combined characteristics (e.g. *DNMT3A* mutation or loss of *DNMT3A* expression). In addition, we expect that the majority of the enrolled 125 patients can be used in the analysis (since all of our molecular information will be acquired within 30 days of enrollment, we anticipate a drop-out rate of 20% or less).

Table 3. Detectable mutation associated response rates (80% power, $\alpha = 0.05$)

Total N	% of patients with a given mutation	Detectable response difference	Sensitivity of mutation to predict response at minimum detectable response difference*
100	15%	0.40	80%
	20%	0.35	75%
	30%	0.30	67%
	40%	0.27	61%
	50%	0.26	58%

* Assuming the overall response rate (CR/Cri) of 45%

12.2.3 Secondary Endpoint Analyses

The overall response rate (CR/CRi/PR) and complete response rate (CR/CRi) will be compared with historical controls[2], and the 95% confidence intervals will be provided.

The rate of patient specific mutation clearance after 10, 28, and 56 days of therapy will be determined using an Illumina based platform. We will compare the rate of mutation clearance between the patients who achieve a CR/CRi after 4 cycles and those who do not. We anticipate that we will identify between 6 and 25 exonic SNVs per case (the range obtained with >200 AML samples sequenced to date with whole genome or exome sequencing), and that the expected 95% confidence

interval of the mean variant allele frequency will be ±2.56% and ±1.25%, respectively, if we assume an anticipated standard deviation of 3.2% within the same clone. The change of tumor burden over time and its association with clinical response will be assessed using 2-way ANOVA for repeated measurement data, followed by the ad-hoc multiple comparisons for between-group differences (e.g., responders vs. non-responders) at given time points as well as the between-time differences within each group. The resultant critical *P* values will also be corrected using permutation analysis for multiple testing.

The steady-state plasma decitabine concentration on day 4 ± 1 will be measured and correlated with clinical overall response.

The percent decrease in bone marrow methylcytosine will be calculated on day 10±1. We will compare this reduction with previously published data and between the patients who achieve CR/CRi and those who do not. The correlation of peak plasma levels of decitabine with response will be assessed using 2-sample t-test or Mann-Whitney non-parametric rank-sum test as appropriate. The change of total bone marrow DNA methylcytosine from baseline and its association with both steady-state plasma drug levels and response will be assessed using 2-way ANOVA for repeated measurement data, followed by the ad-hoc multiple comparisons for between-group differences (e.g., responders vs. non-responders) and between-time differences. Similar analyses will be performed for the correlation of expression profiles and SNP analysis with pharmacokinetic data.

Statistical power justification. A power analysis was performed using two-sample t-test for between-group difference and paired t-test for the change over time. For tumor clearance, this will provide > 99% power to detect a 10% difference in the means at two separate time points for clones with 6 or more variants, and > 98% power to detect a 15% difference if there are 3 or more variants per clone with $\alpha = 0.05$ (power will decrease to 91% and 82%, respectively, in cases where the standard deviation is 4.7%, the maximum we have observed thus far). For pharmacologic correlations, we anticipate 85% power to determine a 25 ng/ml difference between the average C_{max} of 45 evaluable responders and 55 evaluable non-responders with an anticipated average C_{max} of 70 ng/ml and a standard deviation of 40 ng/ml within the entire cohort, and with $\alpha = 0.05$.[22] We believe a less significant difference would not be sufficient to warrant application in clinical decision making (i.e. whether to continue therapy for more than 1 cycle). We anticipate 99% power to determine a difference of 20% in the reduction of bone marrow methylcytosine between 45 evaluable responders and 55 evaluable non-responders with an expected standard deviation of 15% and $\alpha = 0.05$.[28, 66-68] The difference in methylcytosine reduction previously observed was 30% between patients achieving CR/PR and those with only hematologic improvement.[28]

Time to relapse and time to death will also be listed for each patient.

12.2.4 Safety Analysis

Extent of Exposure – Duration (number of cycles/infusions) of decitabine doses (cumulative and intensity) will be summarized.

Adverse Events - All adverse events will be coded using NCI CTCAE v. 4.0. 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. In addition, adverse events will be summarized by investigator attribution of relationship to study medication and by grade. Similar summaries will be provided by treatment cycle.

Clinical Laboratory Evaluation – Applicable laboratory parameters will be graded according to NCI CTCAE version 4.0. The incidence of maximum grade (number and percent of patients experiencing the maximum grade during the study) will be summarized for each laboratory parameter. Similar summaries will be provided by treatment cycle. Graphical displays of select hematological parameters will also be provided.

Vital Signs, Physical Findings, and Other Safety Observations – Vital signs, findings from physical examinations, ECOG scores and weights will be presented in listing format.

12.2.5 Description of Planned Subgroup Analyses

The following subgroups (if sufficient numbers of patients) will be explored with respect to the primary and overall survival endpoints.

- Age (60-69 years old vs ≥ 70 years old)
- Cytogenetics
- Patients with AML-associated mutations in *DNMT3A, TET2, IDH1,* or *IDH2* vs those who do not have somatic variants in these genes.

12.3 Sample Size

We anticipate initially enrolling 25 patients at Washington University. Once we establish a routine exome analysis with validation and calculation of the rate of patient-specific mutation clearance, we will enroll an additional 100 patients from at least 2 additional centers.

13.0 DATA SUBMISSION SCHEDULE

Case Report Form	Submission Schedule
Original Consent Form	*Prior to registration*
Patient Registration Form *Disease Diagnosis Form* *Inclusion Criteria Worksheet* *Exclusion Criteria Worksheet*	*Prior to starting treatment*
Treatment Record Form	*To be maintained throughout a patient's treatment cycle. The complete record is due at completion of treatment.*
Bone Marrow Sample Collection Form	*To be maintained throughout the course of the study at designated bone marrow sample collections time points (See Appendix 2).*
Pathology: Bone Marrow Biopsy	*To be completed upon the return of pathology report for each bone marrow biopsy.*
Study Calendar Form	*To be completed with the date of each time point upon occurrence.*
Labs Form	*To be completed upon the return of laboratory reports for each study time point (See Appendix 2)*
Dose Modifications	*To be maintained throughout the course of the study if dose modifications are performed.*
Therapy Response Form	*To be filled out upon remission (prior to, and/or on therapy)*
End of Study Form	*Due at the completion of the EOS visit, at the time of death, and/or at the time that the patient is lost to follow-up*
Follow Up Form	• *30 days post date of last decitabine dose.* • *Every 6 months for 2 years after the last dose of study drug.*
Record of Adverse Events	*At the time of any AE*

Appendix 1 ECOG Performance Scale

Activity Status	Description
0	Asymptomatic, fully active, and able to carry on all predisease performance without restrictions.
1	Symptomatic, fully ambulatory but restricted in physically strenuous activity and able to carry out performance of a light or sedentary nature, eg, light housework, office work.
2	Symptomatic, ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours: in bed less than 50% of day.
3	Symptomatic, capable of only limited self-care, confined to bed or chair more than 50% of waking hours, but not bedridden.
4	Completely disabled. Cannot carry on any self-care. Totally bedridden.
5	Dead

Appendix 2 Study Flow Chart

Study Flow Chart

Test	Pre-study[1]	Base-line[2]	All Cycles Day 1	All Cycles Days 1-10[3]	Cycle 1 Day 4 ± 1	Cycle 1 Day 10 ± 1 and 28 ± 4	Every week	Every 2 weeks	Even cycles Day 28±4	End of Study
Informed Consent	X									
Medical History		X								
Physical Exam		X[a]	X[b]							X
Transfusion Requirements[c]		X[c]	Ongoing							
Bone Marrow Aspirate and Biopsy	X[d]					X[d]			X[d]	X[d]
Flow Cytometry[e]	X									
Peripheral blood for correlative studies					X					
Pregnancy test		X								
CBC with differential and platelets		X	X				X[f]			X
Serum Chemistries[g]		X	X					X[f]		X
Decitabine administration[h]				X						
Adverse Events			Ongoing							
Post-study follow-up										X[i]

[1] To be performed within 14 days of the first dose of decitabine.
[2] To be performed within 7 days of the first dose of decitabine.
[3] Decitabine may be reduced to Days 1-5 or Days 1-3. See Section 6.3 for details.
[a] To include height, weight, and ECOG.
[b] To include weight.
[c] Care should be given to record the frequency of transfusions from 8 weeks prior to first dose of study drug through the duration of the study.
[d] Standard bone marrow procurement procedures will be followed for collection of the tissue. To include: correlative studies (collected under HRPO# 201011766), morphologic assessment, cytogenetics and FISH at all time points. A skin biopsy sample (collected under HRPO# 201011766) is required at pre-study, but may have been collected months or even years prior to first dose of decitabine.
[e] On bone marrow or peripheral blood.
[f] After Cycle 1, these labs may be obtained every other week (CBC) or once a month (Chemistries) at the discretion of the treating physician.
[g] Serum chemistries include bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, and LDH.
[h] Decitabine treatment will be 20 mg/m^2 over a 1-hour IV infusion on consecutive Days 1-10 every 28-day cycle. The dose will be calculated based on actual patient weight at the beginning of each cycle. Patients may be premedicated with lorazepam, prochlorperazine, palonosetron, or ondansetron at the treating physician's discretion.
[i] Patients will be followed for 30 days following the date of the last decitabine dose to monitor for toxicity, survival and response. Thereafter, the investigator or designee will record information on subsequent therapy, disease progression, and death, every 6 months for 2 years after first dose of study drug or until the patient is lost to follow-up.

14.0 REFERENCES

1. Cheson, B.D., et al., *Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia.* J Clin Oncol, 2003. **21**(24): p. 4642-9.
2. Cashen, A.F., et al., *Multicenter, phase II study of decitabine for the first-line treatment of older patients with acute myeloid leukemia.* J Clin Oncol, 2010. **28**(4): p. 556-61.
3. Blum, W., et al., *Clinical response and miR-29b predictive significance in older AML patients treated with a 10-day schedule of decitabine.* Proc Natl Acad Sci U S A, 2010. **107**(16): p. 7473-8.
4. Schlenk, R.F., et al., *Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia.* N Engl J Med, 2008. **358**(18): p. 1909-18.
5. Erba, H.P., *Has there been progress in the treatment of older patients with acute myeloid leukemia?* Best Pract Res Clin Haematol, 2010. **23**(4): p. 495-501.
6. Heaney, M.L. and D.W. Golde, *Myelodysplasia.* N Engl J Med, 1999. **340**(21): p. 1649-60.
7. Bennett, J.M., et al., *Proposals for the classification of the myelodysplastic syndromes.* Br J Haematol, 1982. **51**(2): p. 189-99.
8. Kantarjian, H., et al., *Results of intensive chemotherapy in 998 patients age 65 years or older with acute myeloid leukemia or high-risk myelodysplastic syndrome: predictive prognostic models for outcome.* Cancer, 2006. **106**(5): p. 1090-8.
9. Martin, M.G. and C.N. Abboud, *Induction therapy for elderly patients with acute myeloid leukemia.* Blood Rev, 2008. **22**(6): p. 311-20.
10. Bow, E.J., et al., *Therapy of untreated acute myeloid leukemia in the elderly: remission-induction using a non-cytarabine-containing regimen of mitoxantrone plus etoposide.* J Clin Oncol, 1996. **14**(4): p. 1345-52.
11. Goldstone, A.H., et al., *Attempts to improve treatment outcomes in acute myeloid leukemia (AML) in older patients: the results of the United Kingdom Medical Research Council AML11 trial.* Blood, 2001. **98**(5): p. 1302-11.
12. Stone, R.M., *The difficult problem of acute myeloid leukemia in the older adult.* CA Cancer J Clin, 2002. **52**(6): p. 363-71.
13. Liu Yin, J.A., et al., *Mitozantrone and cytosine arabinoside as first-line therapy in elderly patients with acute myeloid leukaemia.* Br J Haematol, 1991. **79**(3): p. 415-20.
14. Stone, R.M., et al., *Granulocyte-macrophage colony-stimulating factor after initial chemotherapy for elderly patients with primary acute myelogenous leukemia. Cancer and Leukemia Group B.* N Engl J Med, 1995. **332**(25): p. 1671-7.
15. Kantarjian, H., et al., *Decitabine improves patient outcomes in myelodysplastic syndromes.* Cancer, 2006. **106**(8): p. 1794-1803.

16. Lubbert, M., et al., *A multicenter phase II trial of Decitabine as first-line treatment of older AML patients judged unfit for induction chemotherapy.* Haematologica, 2011. **97**(3): p. 393-401.
17. Issa, J.P., et al., *Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies.* Blood, 2004. **103**(5): p. 1635-40.
18. Petti, M.C., et al., *Pilot study of 5-aza-2'-deoxycytidine (Decitabine) in the treatment of poor prognosis acute myelogenous leukemia patients: preliminary results.* Leukemia, 1993. **7 Suppl 1**: p. 36-41.
19. Wijermans, P.W., et al., *Continuous infusion of low-dose 5-Aza-2'-deoxycytidine in elderly patients with high-risk myelodysplastic syndrome.* Leukemia, 1997. **11**(1): p. 1-5.
20. Kantarjian, H., et al., *Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study.* Cancer, 2006. **106**(8): p. 1794-803.
21. Wijermans, P.W., et al., *An epigenetic approach to the treatment of advanced MDS; the experience with the DNA demethylating agent 5-aza-2'-deoxycytidine (decitabine) in 177 patients.* Ann Hematol, 2005. **84 Suppl 1**: p. 9-17.
22. Cashen, A.F., et al., *Pharmacokinetics of decitabine administered as a 3-h infusion to patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS).* Cancer Chemother Pharmacol, 2008. **61**(5): p. 759-66.
23. Chabot, G.G., J. Bouchard, and R.L. Momparler, *Kinetics of deamination of 5-aza-2'-deoxycytidine and cytosine arabinoside by human liver cytidine deaminase and its inhibition by 3-deazauridine, thymidine or uracil arabinoside.* Biochem Pharmacol, 1983. **32**(7): p. 1327-8.
24. Momparler, R.L., et al., *Cell cycle effects and cellular pharmacology of 5-aza-2'-deoxycytidine.* Cancer Chemother Pharmacol, 1984. **13**(3): p. 191-4.
25. Raza, A., et al., *Biological significance of cell cycle kinetics in 128 standard risk newly diagnosed patients with acute myelocytic leukemia.* Br J Haematol, 1991. **79**(1): p. 33-9.
26. Brons, P.P., et al., *Proliferation patterns in acute myeloid leukemia: leukemic clonogenic growth and in vivo cell cycle kinetics.* Ann Hematol, 1993. **66**(5): p. 225-33.
27. Raza, A., et al., *Direct relationship between remission duration in acute myeloid leukemia and cell cycle kinetics: a leukemia intergroup study.* Blood, 1990. **76**(11): p. 2191-7.
28. Shen, L., et al., *DNA methylation predicts survival and response to therapy in patients with myelodysplastic syndromes.* J Clin Oncol, 2010. **28**(4): p. 605-13.
29. Tefferi, A., et al., *IDH1 and IDH2 mutation studies in 1473 patients with chronic-, fibrotic- or blast-phase essential thrombocythemia, polycythemia vera or myelofibrosis.* Leukemia, 2010. **24**(7): p. 1302-9.
30. Ley, T.J., et al., *DNMT3A mutations in acute myeloid leukemia.* N Engl J Med, 2010. **363**(25): p. 2424-33.
31. Mardis, E.R., et al., *Recurring mutations found by sequencing an acute myeloid leukemia genome.* N Engl J Med, 2009. **361**(11): p. 1058-66.

32. Abdel-Wahab, O., J. Patel, and R.L. Levine, *Clinical Implications of Novel Mutations in Epigenetic Modifiers in AML.* Hematol Oncol Clin North Am, 2011. **25**(6): p. 1119-33.
33. Reuther, G.W., *Recurring mutations in myeloproliferative neoplasms alter epigenetic regulation of gene expression.* Am J Cancer Res, 2011. **1**(6): p. 752-62.
34. Paschka, P., et al., *IDH1 and IDH2 mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3 internal tandem duplication.* J Clin Oncol, 2010. **28**(22): p. 3636-43.
35. Shen, Y., et al., *Gene mutation patterns and their prognostic impact in a cohort of 1185 patients with acute myeloid leukemia.* Blood, 2011. **118**(20): p. 5593-603.
36. Tefferi, A., et al., *Detection of mutant TET2 in myeloid malignancies other than myeloproliferative neoplasms: CMML, MDS, MDS/MPN and AML.* Leukemia, 2009. **23**(7): p. 1343-5.
37. Walter, M.J., et al., *Recurrent DNMT3A mutations in patients with myelodysplastic syndromes.* Leukemia, 2011. **25**(7): p. 1153-8.
38. Lin, J., et al., *Recurrent DNMT3A R882 Mutations in Chinese Patients with Acute Myeloid Leukemia and Myelodysplastic Syndrome.* PLoS One, 2011. **6**(10): p. e26906.
39. Bejar, R., et al., *Clinical effect of point mutations in myelodysplastic syndromes.* N Engl J Med, 2011. **364**(26): p. 2496-506.
40. Walter, M.J., et al., *Clonal architecture of secondary acute myeloid leukemia.* N Engl J Med, 2012. **366**(12): p. 1090-8.
41. Kantarjian, H.M., et al., *Survival advantage with decitabine versus intensive chemotherapy in patients with higher risk myelodysplastic syndrome: comparison with historical experience.* Cancer, 2007. **109**(6): p. 1133-7.
42. Lubbert, M., et al., *Continued Low-Dose Decitabine (DAC) Is an Active First-Line Treatment in All Cytogenetic Subgroups of Older AML Patients: Results of the FR00331 Multicenter Phase II Study.* ASH Annual Meeting Abstracts, 2007. **110**(11): p. 300-.
43. Blum, W., et al., *Phase I Study of Decitabine Alone or in Combination With Valproic Acid in Acute Myeloid Leukemia.* J Clin Oncol, 2007. **25**(25): p. 3884-3891.
44. Issa, J.-P.J., et al., *Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies.* Blood, 2004. **103**(5): p. 1635-1640.
45. Garcia-Manero, G., et al., *Phase 1/2 study of the combination of 5-aza-2'-deoxycytidine with valproic acid in patients with leukemia.* Blood, 2006. **108**(10): p. 3271-3279.
46. Issa, J.P., S.B. Baylin, and J.G. Herman, *DNA methylation changes in hematologic malignancies: biologic and clinical implications.* Leukemia, 1997. **11 Suppl 1**: p. S7-11.
47. Ganetzky, R.D., et al., *Differences Between Normal and Leukemic Stem Cell-Specific Methylome Indicates Aberrantly Silenced Genes Involved in the*

Pathogenesis of Malignant Evolution. ASH Annual Meeting Abstracts, 2008. **112**(11): p. 599-.

48. Christman, J.K., *5-Azacytidine and 5-aza-2'-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy.* Oncogene, 2002. **21**(35): p. 5483-95.
49. Lubbert, M., *DNA methylation inhibitors in the treatment of leukemias, myelodysplastic syndromes and hemoglobinopathies: clinical results and possible mechanisms of action.* Curr Top Microbiol Immunol, 2000. **249**: p. 135-64.
50. Kantarjian, H., et al., *Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia.* Blood, 2007. **109**(1): p. 52-57.
51. Steensma, D.P., et al., *Multicenter Study of Decitabine Administered Daily for 5 Days Every 4 Weeks to Adults With Myelodysplastic Syndromes: The Alternative Dosing for Outpatient Treatment (ADOPT) Trial.* J Clin Oncol, 2009: p. JCO.2008.19.6550.
52. Cashen, A.F., et al., *Preliminary Results of a Multicenter Phase II Trial of 5-Day Decitabine as Front-Line Therapy for Elderly Patients with Acute Myeloid Leukemia (AML).* ASH Annual Meeting Abstracts, 2008. **112**(11): p. 560-.
53. Jabbour, E., et al., *Evolution of decitabine development: accomplishments, ongoing investigations, and future strategies.* Cancer, 2008. **112**(11): p. 2341-51.
54. Kantarjian, H., et al., *Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia.* Blood, 2007. **109**(1): p. 52-7.
55. Blum, W.G., Klisovic, R., Liu, S., Kefauver, C., Grever, M. R., Schaaf, L., Chan, K., Byrd, J. C., Villalona-Calero, M., Marcucci, G. *Efficacy of a novel schedule of decitabine in previously untreated AML, age 60 or older*. in *ASCO Annual Meeting*. 2009.
56. van Groeningen, C.J., et al., *Phase I and pharmacokinetic study of 5-aza-2'-deoxycytidine (NSC 127716) in cancer patients.* Cancer Res, 1986. **46**(9): p. 4831-6.
57. Rivard, G.E., et al., *Phase I study on 5-aza-2'-deoxycytidine in children with acute leukemia.* Leuk Res, 1981. **5**(6): p. 453-62.
58. Lin, K.T., R.L. Momparler, and G.E. Rivard, *Sample preparation for the determination of 5-aza-2'-deoxycytidine in plasma by high-performance liquid chromatography.* J Chromatogr, 1985. **345**(1): p. 162-7.
59. Momparler, R.L., G.E. Rivard, and M. Gyger, *Clinical trial on 5-aza-2'-deoxycytidine in patients with acute leukemia.* Pharmacol Ther, 1985. **30**(3): p. 277-86.
60. Raska, K., Jurovcik, M., Sormova., Z., *On the metabolism of 5-azacytidine and 5-aza-2'-deoxycytidine in mice.* Coll Czech Chem Commun, 1965. **30**: p. 3001-3005.
61. Momparler, R.L., et al., *Pilot phase I-II study on 5-aza-2'-deoxycytidine (Decitabine) in patients with metastatic lung cancer.* Anticancer Drugs, 1997. **8**(4): p. 358-68.

62. Aparicio, A., et al., *Phase I trial of continuous infusion 5-aza-2'-deoxycytidine.* Cancer Chemother Pharmacol, 2003. **51**(3): p. 231-9.
63. Bryan, J., et al., *Pharmacokinetic evaluation of decitabine for the treatment of leukemia.* Expert Opin Drug Metab Toxicol, 2011. **7**(5): p. 661-72.
64. Scandura, J.M., et al., *Phase 1 study of epigenetic priming with decitabine prior to standard induction chemotherapy for patients with AML.* Blood, 2011. **118**(6): p. 1472-80.
65. Westfall, P.H. and S.A. Young, *Resampling-based multiple testing: examples and methods for p-value adjustment*. 1993, New York: John Wiley & Sons, Inc.
66. Samlowski, W.E., et al., *Evaluation of a 7-day continuous intravenous infusion of decitabine: inhibition of promoter-specific and global genomic DNA methylation.* J Clin Oncol, 2005. **23**(17): p. 3897-905.
67. Blum, W., et al., *Phase I study of decitabine alone or in combination with valproic acid in acute myeloid leukemia.* J Clin Oncol, 2007. **25**(25): p. 3884-91.
68. Yang, A.S., et al., *DNA methylation changes after 5-aza-2'-deoxycytidine therapy in patients with leukemia.* Cancer Res, 2006. **66**(10): p. 5495-503.
69. Cheson, B.D., et al., *Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia.* Blood, 2006. **108**(2): p. 419-25.

Genomic Predictors of Decitabine Response in AML/MDS

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Genomic Predictors of Decitabine Response in AML/MDS

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Study Synopsis

Title of study	**Genomic Predictors of Decitabine Response in AML**
Primary investigator/study center location	John S. Welch, M.D./PhD Washington University School of Medicine, Division of Oncology St. Louis, MO Timothy J. Ley, MD Washington University School of Medicine, Division of Oncology St. Louis, MO
Phase of development	Phase II
Study objectives	**Primary:** - To determine whether patient-specific mutations correlate with clinical overall response to decitabine in patients with AML or MDS. **Secondary:** - To determine whether 10 consecutive days per cycle of decitabine improves outcomes compared with historical controls treated with 5 consecutive days per cycle of decitabine. - To determine whether other biomarkers correlate with clinical overall response to decitabine in patients with AML or MDS. These will include: velocity and depth of patient-specific mutation clearance, bone marrow expression profile and change in expression profile during decitabine treatment, steady-state serum decitabine concentrations, and decrease in bone marrow methylcytosine content in response to decitabine.
Study design	Non-randomized, open-label, Phase II study of decitabine in patients with AML or MDS.
Study criteria	**Inclusion Criteria** <u>One of the following:</u> 1. Patient must have non-M3 AML and be > 60 years of age OR 2. non-M3 AML with relapsed disease OR 3. Symptomatic MDS with one of the following: o Symptomatic anemia with either hemoglobin <10.0 g/dL or requiring RBC transfusion o Thrombocytopenia with a history of two or more platelet counts < 50,000 / μL or a significant hemorrhage requiring platelet transfusions, or o Neutropenia with two or more absolute neutrophil counts < 1,000 /μL. <u>All of the following:</u> 1. Patient must have an ECOG performance status ≤ 2. 2. Patient must have >10% disease burden measured by cytomorphology, flow cytometry, or cytogenetics. 3. Patient must have peripheral white blood cell count < 50,000/mcl. 4. Patient must have adequate organ function, defined as: a. Total bilirubin < 1.5 x ULN b. AST/ALT < 2.5 x ULN c. Serum creatinine < 2.0 x ULN 5. Patient must have undergone ≤ 2 cycles of prior hypomethylating agent (decitabine or azacitidine).

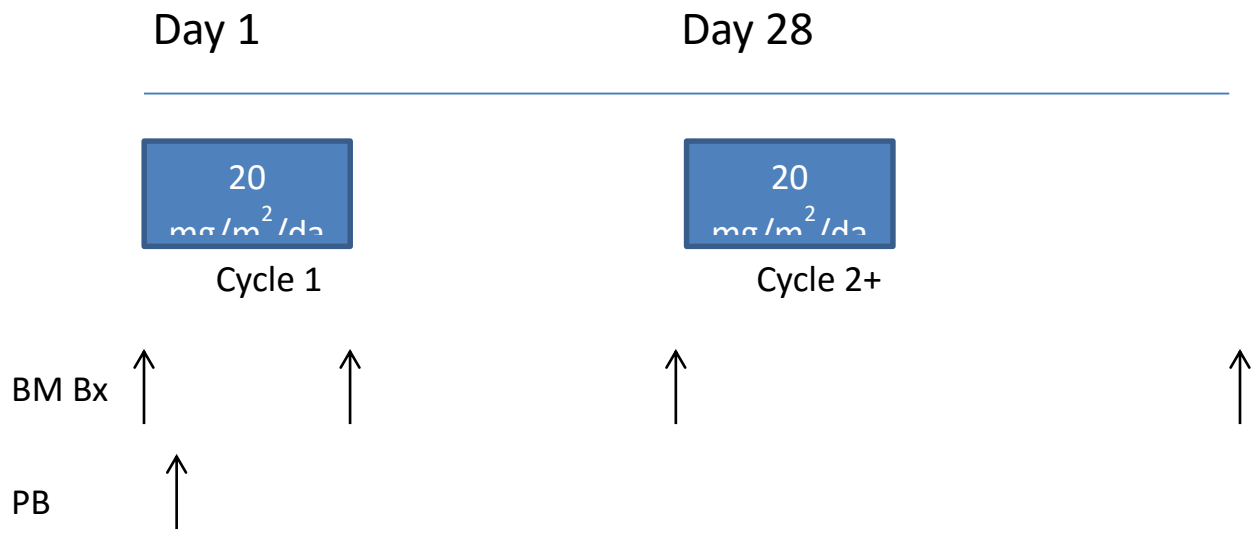
	6. Patient must be able to understand and willing to sign an IRB-approved written informed consent document. 7. Patient must be enrolled in HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Malignancies"). 8. Patient must be ≥ 18 years of age.
	Exclusion Criteria 1. Patient must not be pregnant or nursing. 2. Patient must not have known CNS leukemia. 3. Patient must not have a history of positive HIV serology. 4. Patient must not have a history of positive Hepatitis C serology. 5. Patient must not have undergone prior allogeneic stem cell transplant. 6. Patient must not have any uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, ongoing or active GVHD, congestive heart failure of NYHA class 3 or 4, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situation that would limit compliance with study requirements. 7. Patient must not have had radiation therapy within 14 days of enrollment.
Expected number of patients	Total: 125. Evaluable for all molecular studies: 100. Enrollment time-table: 25 patients will be enrolled initially at Washington University. Once the molecular pipeline for routine exome analysis is established, at least 2 additional sites will be added.
Study drug formulation and administration	[redacted]
Duration of treatment	Treatment may continue until one of the following criteria applies: • Death, • Disease progression (as defined in 3.7.1.3), • Intercurrent illness that prevents further administration of treatment,

	• Unacceptable adverse event(s), • Patient decides to withdraw from the study, or • General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the treating physician.
Correlative studies	Bone marrow samples for correlative studies will be collected prestudy, on Cycle 1 day 10 ± 1 and day 28 ± 4, and on day 28 ± 4 of subsequent even numbered cycles. Peripheral blood samples for correlative studies will be collected on Cycle 1 day 4 ± 1. 1. Identify Patient-specific mutations We will perform exome sequencing of each patient's day 0 bone marrow sample and skin sample. We will determine AML/MDS-associated variants, defined as variants found in the bone marrow sample and not in the skin sample. We will correlate the presence of mutations with overall response. 2. Velocity and depth of response. We will assess the rate of tumor clearance by measuring the allele frequency of patient-specific mutations in bone marrow samples during cycles 1 and 2. We will assess mutations in at least 24 recurrently mutated genes (including, but not limited to: *FLT3, DNMT3A, NPM1, TET2, WT1, IDH1, IDH2, RUNX1, NRAS, TP53, U2AF1, KRAS, PHF6, MSTP9, PTPN11, KIT, SMC3, PRUNE2, ETV6, CEBPA, SMC1A, ASXL1, STAG2, RAD21*) as well as all patient-specific mutations discovered during exome sequencing. The leukemic allele frequency will be measured using the Illumnia MySeq or HiSeq platform using DNA capture by gene specific biotinylated-oligonucleotides, or by a comparable technique. Patient bone marrow samples will be assessed on day 0, day 10 ± 1, and day 28 ± 4 of cycle 1, and on day 28 ± 4 of cycle 2 and compared with patient-specific skin samples (normal control). The velocity of response will be calculated as the natural log rate of patient-specific mutation allele frequency clearance. Depth of response will be defined as the lowest patient-specific mutation allele frequency in a bone marrow sample. The velocity and depth of tumor response will be correlated to: • Overall response, • Time to best response, • Mutation status, • Time to disease progression. 3. Expression profile. Expression array profiling will be performed on bone marrow samples from day 0 and day 10 ± 1. We will correlate expression signatures, and changes in expression signatures, with: • Overall response, • Mutational status. 4. Steady-state serum decitabine concentration. We will assess serum decitabine concentration on day 4 ± 1 and correlation with: • Overall response.

	5. Bone marrow DNA hypomethylation. We will assess the percent reduction in bone marrow methylcytosine content using LC-MS. This may serve as a better biomarker of adequate dosing than serum drug concentration. Patients will be evaluated on day 0 and day 10 ± 1 of cycle 1. The change in DNA methylcytosine content will be correlated to: • Overall response, • Change in expression signatures.
Criteria for evaluation	<u>Efficacy</u>- Bone marrow biopsies should be performed at baseline and at day 10 ± 1 and day 28 ± 4 of cycle 1 and at the end of subsequent even numbered cycles. Standard bone marrow procurement procedures will be followed for collection of the tissue. Jeffrey Klco, MD/PhD will act as a central reviewer for all bone marrow biopsies. Peripheral blood counts will be followed by weekly CBC with differential. After cycle 1, the frequency of routine CBC analysis may be decreased by the treating physician as clinically indicated. Patients will be assessed for response according to the IWG criteria:[1] *Morphologic complete remission (CR)*– Defined as morphologic leukemia-free state, defined as less than 5% blasts in aspirate with marrow spicules and a count of ≥ 200 nucleated cells and no blasts with Auer rods or any persistence of extramedullary disease, and no evidence for dysplasia. When erythroid precursors constitute less than 50% of bone marrow nucleated cells, the percentage of blasts is based on all nucleated cells; when there are 50% or more erythroid cells, the percentage blasts should be based on the nonerythroid cells. The presence of mild megaloblastoid changes may be permitted if they are thought to be consistent with treatment effect. However, persistence of pretreatment abnormalities (eg, pseudo-Pelger-Hüet cells, ringed sideroblasts, dysplastic megakaryocytes) are not consistent with CR. In addition, ANC > 1000/µl and platelet count of ≥ 100,000/µl. Patient must be independent of transfusions. No duration requirement for this designation. *Cytogenetic complete remission (CCR)* – 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. Fluorescent in situ hybridization (FISH) may be used as a supplement to follow a specifically defined cytogenetic abnormality. *Morphologic complete remission with incomplete blood count recovery (CRi)* – Defined as morphologic complete recovery with the exception of neutropenia <1000/µl or thrombocytopenia <100,000/µl. *Partial remission (PR)* – Requires that the above criteria for complete remission be met, with the following exceptions: decrease of ≥50% in the percentage of blasts to 5-25% in the bone marrow aspirate. A value of ≤5% blasts in bone marrow with Auer rods present may also be considered a partial remission.

	Treatment failure – Includes all patients who did not achieve a complete or partial remission. *Recurrence/morphologic relapse* - Relapse following 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. New dysplastic changes are considered a relapse. 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. Safety-- All patients receiving at least one dose of decitabine will be evaluated for safety.
Statistical considerations	This trial will be conducted as a phase II biomarker trial. This trial is designed to detect the most clinically meaningful mutational predictors of response. These are mutations that have sufficient positive predictive value to be used as a clinical assay. A power analysis was performed with Fisher's exact test for mutation status and response to decitabine, assuming an expected 45% CR/CRi. With alpha = 0.05, and a total anticipated enrollment of 100 evaluable patients, we anticipate 80% power to detect a 27% and 40% response difference between patients with and without a mutation, if the mutation occurs with a respective frequency of 30% and 15%. Such mutations would provide 80% sensitivity to predict response to decitabine. These are values that would warrant diagnostic clinical testing prior to therapy. This trial is also designed to detect an improvement in clinical response compared to historical controls treated with 5 consecutive days of decitabine (20 mg/m^2/day every 28 days)[2]. A power analysis was performed with Fisher's exact test for response to decitabine compared to a historical rate of 24% observed in 55 patients. With 100 evaluable patients enrolled, we anticipate 90% power with alpha = 0.1 to detect a 21% improvement in response (CR/ PR ≥ 45%). The reported CR/PR rate of the proposed treatment schedule (10 consecutive days of decitabine 20 mg/m^2/day) is 64%[3]; the expected CR/PR rate of 45% is a conservative estimation of expected outcomes.

Study Schema:



Patients with progressive disease after two cycles (defined as a clear increase in bone marrow blast count, typically a > 50% increase in bone marrow blasts over baseline) should be removed from study.

Patients with stable disease may continue on study and should undergo a diagnostic bone marrow biopsy at the end of even numbered cycles.

After 2 cycles, if patients achieve morphologic CRi, administration will be decreased to 5 days to limit myelosuppressive toxicity (20 mg/m^2 over 1 hour on Days 1-5 every 28 ± 4 days). After two cycles with morphologic CRi and no evidence of disease by cytogenetics or immunophenotype, patients may further decrease therapy to 3 days per cycle (20 mg/m^2 over 1 hour on Days 1-3 every 28 ± 4 days).

Sample collection for correlative studies:

Bone marrow biopsies should be performed at baseline and at Day 10 ± 1 and Day 28 ± 4 of Cycle 1 and at the end of even numbered cycles.

Peripheral blood samples should be collected on Day 4± 1 of Cycle 1. Collection should be obtained 45 ± 15 minutes after decitabine infusion is initiated.

Genomic Predictors of Decitabine Response in AML/MDS

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1.0 INTRODUCTION

1.1 Rationale

Personalized therapy for cancer requires that 1) patient-specific factors (e.g. patient-specific mutations, expression profiles, or pharmacogenomics) influence treatment algorithms, and 2) once therapy is initiated, *in vivo* responsiveness is used to guide further therapy. The modern era of genomics has provided significant insights into cancer pathogenesis by describing patterns of germline and somatic variants, as well as defining disease-specific patterns of gene expression. However, the application and integration of this data with therapeutics remains a difficult task.

Decitabine is a commonly used single-agent therapy for patients with acute myeloid leukemia (AML) and/or myelodysplastic syndrome (MDS). Historically, it has yielded clinical complete responses in approximately 25% of patients across diverse clinical trials, it is well tolerated, and it can be given as in the outpatient setting. However, the majority of patients do not respond to decitabine, the molecular etiology of decitabine sensitivity is unknown, and there are no algorithms to predict patient responsiveness *a priori* or even within one month of starting therapy.

AML and MDS are two closely related hematological disorders characterized by ineffective hematopoiesis and malignant expansion of clonal myeloid cells. Both AML and MDS frequently occur in elderly patients and some cases are secondary and/or related to long-term bone marrow damage after radiation or chemotherapy. Current therapy for AML consists of cytotoxic chemotherapy followed by consolidation chemotherapy or stem cell transplantation. Using these approaches, current cure rates may be as high as 60% for patients younger than 60 with good risk cytogenetics (e.g. t(15;17), t(8;21), inv(16)).[4] Outcomes in patients ≥ 60 years old and with relapsed disease remain poor with 2 year survival of only ~25%.[5] Historically, the available treatment for MDS has been symptomatic and has not been shown to be effective in producing sustained improvement in hematopoiesis or in delaying leukemic evolution[6, 7].

Current AML therapy is poorly tolerated in elderly and pre-treated patients due to hematologic and non-hematologic toxicities; following such therapy, patients typically require 3-4 weeks of hospitalization for treatment of neutropenic fevers and for regular transfusions. Even among the relatively healthy patients enrolled in clinical trials, induction mortality in the elderly can be as high as 20-40% following standard induction therapy,[8-11] and moderate to severe non-hematologic toxicity occurs in at least an additional 30% of patients.[9, 12-14] Consequently, many older AML patients are not offered traditional intensive chemotherapy, but receive supportive care alone.[15] More effective and less toxic therapies are needed to provide durable remissions in a significant proportion of elderly AML in populations.

[redacted]

We and others have recently used whole genome sequencing to identify recurrent, somatic mutations in AML and MDS patients in four genes that are involved in methylcytosine regulation: *DNMT3A, IDH1, IDH2,* and *TET2.*[29-37] Collectively, nearly half of all AML/MDS patients have a mutation in one of these four genes. *DNMT3A* mutations are associated with an adverse outcome in AML patients treated with cytotoxic chemotherapy.[35, 38] However, it is not yet clear whether the presence of mutations in one or more of these genes is associated with response or resistance to decitabine.

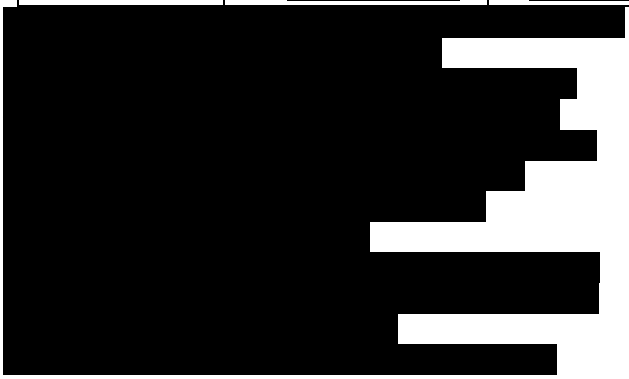
In this study, we will treat patients with the current state-of-the-art decitabine schedule.[3] We will perform exome sequencing on all patients and correlate response with mutation status, rate of mutation clearance, expression profile, serum decitabine levels, and DNA

methylcytosine content reduction. This approach will comprehensively assess genomic and pharmacologic parameters that could clinically predict patient response to decitabine.

In this study, we will combine analysis of patients with AML and MDS. Recent genomic analysis has shown that AML and MDS have shared mutations in many of the same genes, shared patterns of mutation acquisition, and shared patterns of mutation evolution.[39, 40] Furthermore, MDS, like AML, presents with clonal hematopoiesis, even when the morphologic blast count is zero.[36]Because this study will identify and track the rate of clearance of patient specific mutations, and because decitabine has been shown to have efficacy in both AML and MDS patients, [2, 3, 16-19, 41-45] combining analysis of AML and MDS patients is reasonable. Asymptomatic MDS patients are excluded because these patients are typically followed with watchful-waiting rather than with chemotherapy.

[redacted]

[redacted]



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1.3 Correlative Studies

The goals of the correlative studies include:

1. To correlate patient-specific mutations with response to decitabine.
   We will perform exome sequencing of each patient's Day 0 bone marrow sample and skin sample. We will determine AML/MDS-associated variants found in the bone marrow, but not skin sample. We will correlate the presence of mutations with response (especially mutations in *DNMT3A, IDH1, IDH2,* and *TET2*). This study is powered to identify mutations with >80% sensitivity to predict decitabine response if used as a clinical assay.

2. To correlate the velocity and depth of response with response to decitabine.
   We will assess tumor burden by allele frequency of patient-specific mutations in bone marrow samples collected on day 0, 10±1, 28±4, and 56±4. We will assess mutations in at least 24 recurrently mutated genes (including, but not limited to: *FLT3, DNMT3A, NPM1, TET2, WT1, IDH1, IDH2, RUNX1, NRAS, TP53, U2AF1, KRAS, PHF6, MSTP9, PTPN11, KIT, SMC3, PRUNE2, ETV6, CEBPA, SMC1A, ASXL1, STAG2, RAD21*) as well as all patient-specific mutations discovered during exome sequencing. The leukemic allele frequency will be measured using the Illumnia MySeq or HiSeq platform using DNA capture by gene specific biotinylated-oligonucleotides. Patient bone marrow samples will be assessed on day 0, day 10 ± 1, and day 28 ± 4 of cycle 1, and day 28 ± 4 of cycle 2 and compared with patient specific skin samples (normal control). The velocity of response will be calculated as the natural log rate of patient-specific mutation allele frequency clearance. Depth of response will be defined as the lowest patient-specific mutation allele frequency in a bone marrow sample. The velocity and depth of tumor response will be correlated to:
   - Achievement of clinical CR,
   - Time to best response,

- Mutational status,
- Time to disease progression.

Because all patient-specific exome-discovered mutations will be assessed a second time in the primary bone marrow sample as well as the Day 10 ± 1, 28 ± 4, and 56 ± 4 bone marrow samples, this study will also serve to validate all exome-discovered mutations. This is important, because exome sequencing is associated with a 20-50% false-discovery rate depending on type I error tolerated (mutation-calling algorithms are typically set for greater sensitivity than specificity). Thus, many mutations discovered during exome sequencing are incorrect. This second analysis of these mutations will server to exclude these false positive calls.

3. To correlate the bone marrow expression profile on Day 0 and Day 10 ± 1 with response to decitabine.
   We will correlate expression signatures, and changes in expression signatures, with:
   - Achievement of clinical CR,
   - Mutation status.

4. To correlate steady-state serum decitabine concentration on Day 4 ± 1 with:
   - Achievement of CR.

5. To correlate the extent of bone marrow DNA hypomethylation on Day 10 ± 1 with response to decitabine.
   We will assess the percent reduction in bone marrow methylcytosine content using LC-MS. Patients will be evaluated on Day 0 and Day 10 ± 1 of Cycle 1. The change in DNA methylcytosine content will be correlated to:
   - Achievement of clinical CR,
   - Change in expression signature.

   This will determine whether inadequate blast exposure to decitabine correlates with drug resistance.

2.0 OBJECTIVES

2.1 Primary Objective

To determine whether patient-specific mutations correlate with clinical overall response to decitabine in patients with AML or MDS.

2.2 Secondary Objectives

- To determine whether 10 consecutive days per cycle of decitabine improves outcomes compared with historical controls treated with 5 consecutive days per cycle of decitabine.

- To determine whether other biomarkers correlate with clinical overall response to decitabine in patients with AML or MDS. These will include:

    1. The velocity and depth of patient-specific mutation clearance;
    2. The bone marrow expression profile and change in expression profile during decitabine treatment;
    3. The steady-state serum decitabine concentrations;
    4. The decrease in bone marrow methylcytosine content in response to decitabine.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

One of the following:

- Patient must have non-M3 AML and be ≥ 60 years of age OR
- non-M3 AML with relapsed disease OR
- Symptomatic MDS with one of the following:
  - □ Symptomatic anemia with either hemoglobin < 10.0 g/dL or requiring RBC transfusion
  - □ Thrombocytopenia with a history of two or more platelet counts < 50,000/mcL or a significant hemorrhage requiring platelet transfusions
  - □ Neutropenia with two or more ANC < 1,000/mcL

All of the following:

1. Patient must have an ECOG performance status ≤ 2.
2. Patient must have >10% disease burden measured by cytomorphology, flow cytometry, or cytogenetics.
3. Patient must have peripheral white blood cell count ≤ 50,000/mcl.
4. Patient must have adequate organ function, defined as:
   a. Total bilirubin ≤ 1.5 x ULN
   b. AST/ALT ≤ 2.5 x ULN
   c. Serum creatinine ≤ 2.0 x ULN
5. Patient must have undergone ≤ 2 cycles of prior hypomethylating agent (decitabine or azacitidine).
6. Patient must be enrolled in HRPO# 201011766 (“Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Malignancies”).
7. Patient must be ≥ 18 years of age.
8. Patient must be able to understand and willing to sign an IRB-approved written informed consent document.

3.2 Exclusion Criteria

1. Patient must not be pregnant or nursing.
2. Patient must not have known CNS leukemia.
3. Patient must not have a history of positive HIV serology.
4. Patient must not have a history of positive Hepatitis C serology.
5. Patient must not have undergone a prior allogeneic stem cell transplant.
6. Patient must not have any uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, ongoing or active GVHD, congestive heart failure of NYHA class 3 or 4, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situation that would limit compliance with study requirements.
7. Patient must not have had radiation therapy within 14 days of enrollment.

3.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4.0 REGISTRATION PROCEDURES

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility
2. Registration of patient in the Siteman Cancer Center database
3. Assignment of unique patient number (UPN)

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility collecting the information listed below

1. The registering MD's name
2. Patient's race, sex, and DOB
3. Three letters (or two letters and a dash) for the patient's initials
4. Copy of signed consent form
5. Completed eligibility checklist, signed and dated by a member of the study team
6. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center Database

All patients must be registered through the Siteman Cancer Center database.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. Patients will also be identified by first, middle, and last initials. If the patient has no middle initial, a dash will be used on the case report forms (CRFs). All data will be recorded with this identification number on the appropriate CRFs.

5.0 INVESTIGATIONAL PLAN

5.1 Summary of Study Design

This is an open-label, single-arm, Phase II, biomarker study of patients with AML or MDS who are treated with 10 consecutive days of decitabine. FAB-M3 AML is excluded from this trial, because other well-tolerated options exist for the treatment of FAB-M3 acute promyelocytic leukemia. AML patients who are < 60 years old and have not received cytotoxic induction chemotherapy are also excluded, because cytotoxic therapy offers a curative possibility for these patients. MDS patients without cytopenias are also excluded because these patients can be followed with watchful-waiting.

All patients will undergo bone marrow biopsies prior to therapy, on Day 10 ± 1, and on Day 28 ± 4 of Cycles 1 and 2 for comprehensive genomic correlative studies. Patients will also provide peripheral blood samples on Day 4 ± 1 for pharmacokinetic studies.

Patients will receive decitabine 20 mg/m^2/day as a 1-hour infusion on consecutive Days 1-10 in 28-day cycles. The dose will be calculated based on actual patient weight at the beginning of each cycle.

After 2 cycles, if patients achieve CRi, the administration of decitabine will be decreased to 5 days. After two cycles with CRi and no evidence of disease by cytogenetics or immunophenotype, patients will further decrease therapy 3 consecutive days. The dose of decitabine will remain 20 mg/m^2/day over 1 hour regardless of decreases in the number of days per cycle of administration.

Patients who achieve stable disease, partial response, or complete response will continue on decitabine therapy (receiving cycles of 3-10 days every 28 days (i.e., Days 1-10, Days 1-5, or Days 1-3), depending on response) until time of progression, unacceptable toxicity, or patient decision to discontinue study drugs.

It will be a logistical goal of this trial consistently to return preliminary exome results to the treating physician by the end of Cycle 1 and validated results by the end of Cycle 2. However, this logistical goal is not a primary or secondary aim of this study. Given the limited data regarding mutation presence and drug response, we do not anticipate that the results will directly influence therapeutic decisions made by the treating physician. However, the development of comprehensive genomic pipelines and integration of this data into a clinical trial setting will be required before personalized medicine can be realized.

5.2 Study Procedures

5.2.1 Pre-study Procedures

Pre-study procedures must take place no more than 14 days prior to the first dose of decitabine.

- Bone marrow morphologic assessment[a]
- Bone marrow or peripheral blood for flow cytometry
- Bone marrow for cytogenetics.

[a] Standard bone marrow procurement procedures will be followed for collection of the tissue.

5.2.2 Baseline Evaluation

Baseline evaluations must take place no more than 7 days prior to the first dose of decitabine and include:

1. Medical history
2. Physical exam including height, weight, vital signs, and ECOG performance score.
3. Concomitant medications
4. Record transfusion requirements for 8 weeks before first dose of study drug
5. CBC with differential and platelets
6. Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH
7. Urine βHCG pregnancy test if appropriate

5.2.3 Day 1 of Each Cycle

1. Record adverse events
2. Physical exam, including weight and ECOG performance score
3. Vital signs, including blood pressure, pulse and temperature
4. CBC with differential and platelets
5. Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH

5.2.4 Cycle 1 Days 1-10

Decitabine administration, 20 mg/m^2 per day given as a 1-hour continuous intravenous infusion. The dose will be calculated based on actual patient weight at the beginning of each cycle. Patients may be premedicated with lorazepam, prochlorperazine, palonosetron, or ondansetron at the treating physician's discretion.

5.2.5 Cycle 1 Day 4 ± 1

Obtain 3 mL peripheral blood for correlative studies. Sample must be obtained 45 minutes ± 15 minutes after beginning the infusion, before the decitabine infusion is complete, and should be obtained from the opposite arm as the infusion. The sample should be collected in a 3 mL K3EDTA tube pre-loaded with 8 mcL of tetrahydrouridine (THU 500 mcg/mL), an inhibitor of cytidine deaminase. The

sample should be processed by the Tissue Procurement Core Facility at the Siteman Cancer Center. Serum should be separated and stored per protocols.

5.2.6 Cycle 1 Day 10 ± 1

Obtain bone marrow biopsy for response and correlative studies. [a]

[a] Standard bone marrow procurement procedures will be followed for collection of the tissue.

5.2.7 Weekly

1. CBC with differential and platelets. After the first cycle, this may be changed to every other week at the discretion of the treating physician.
2. Record adverse events.

5.2.8 Every Other Week

Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH. After the first cycle, this may be changed to every month at the discretion of the treating physician.

5.2.9 Cycle 1 and Even Cycles Day 28 ± 4 days

1. Bone marrow biopsy and aspirate collection.[a]
2. Clinical samples for response determination should be obtained at the end of Cycles 1, 2 and 4. Patients who receive > 4 cycles should continue to undergo bone marrow biopsies on even cycles for clinical evaluation (or as clinically indicated).

[a] Standard bone marrow procurement procedures will be followed for collection of the tissue.

5.2.10 End-of-Study Procedures

1. CBC with differential and platelets.
2. Record adverse events.
3. Physical examination including weight, vital signs, and ECOG performance score.
4. Serum chemistries including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, and LDH.
5. Bone marrow collection, with samples sent for routine pathology.[a]

[a] Standard bone marrow procurement procedures will be followed for collection of the tissue.

5.2.11 Post-study Follow-up

Patients are to be followed according to standard patient care procedures for 6 weeks following the date of the last decitabine dose to monitor for toxicity, survival,

and response. Thereafter, the investigator or designee will record information on subsequent therapy, disease progression, and/or death every 2 months for 2 years after first dose of study drug or until the patient is lost to follow-up or dies.

5.3 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented in the case report forms.

In the absence of treatment delays due to adverse events, treatment may continue indefinitely or until one of the following criteria applies:

- Documented and confirmed disease progression
- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious non-compliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study

Patients who prematurely discontinue treatment for any reason will be followed as indicated in the study calendar.

5.4 Concomitant Therapy and Supportive Care Guidelines

5.4.1 Chemotherapy

Patients cannot receive any chemotherapy within 21 days of study enrollment and must have recovered from any treatment-related toxicities. Patients may have received hydrea prior to enrollment for a WBC > 50,000/μl but must stop hydrea at least one day prior to initiating decitabine. Other than decitabine, patients cannot receive chemotherapy during this trial.

5.4.2 Growth Factors

Erythroid growth factors should not be routinely administered to patients during the study.

Thrombopoietin receptor agonists should not be routinely administered to patients during the study.

Filgrastim (G-CSF) or sargramostim (GM-CSF) may be administered to patients with septic shock or febrile neutropenia at the discretion of the treating physician.

5.4.3 Transfusions

- *Packed red blood cells transfusion*:
  Two units of packed RBCs are to be administered when the hemoglobin drops under 8 g/dL. Transfusion administration when the patient's hemoglobin is 8-10 g/dL may be done according to the treating physician's clinical judgment.

- *Platelet transfusion*:
  One unit of single-donor platelets will be administered when platelets are < 20,000/μL.

5.4.4 Prophylactic Antimicrobial Agents

Antimicrobial agents will be administered at the discretion of the treating physician.

5.4.5 Radiotherapy

Patients cannot receive radiotherapy within 14 days prior to study enrollment or while on study.

6.0 DOSE MODIFICATIONS

6.1 Delay for Cytopenia or Infection

As cytopenias are a defining feature of AML and MDS, their presence alone does not generally warrant dose modifications or delays. Patients will receive four cycles of therapy without adjustment for any cytopenia.

Patients with febrile neutropenia (temperature ≥ 38.5° with ANC < 1,000/μl) or systemic infections should have treatment held until clinical resolution of the infection. A patient will meet criterion for discontinuation of the study if the patient is observed to have a delay of > 8 weeks between any two cycles.

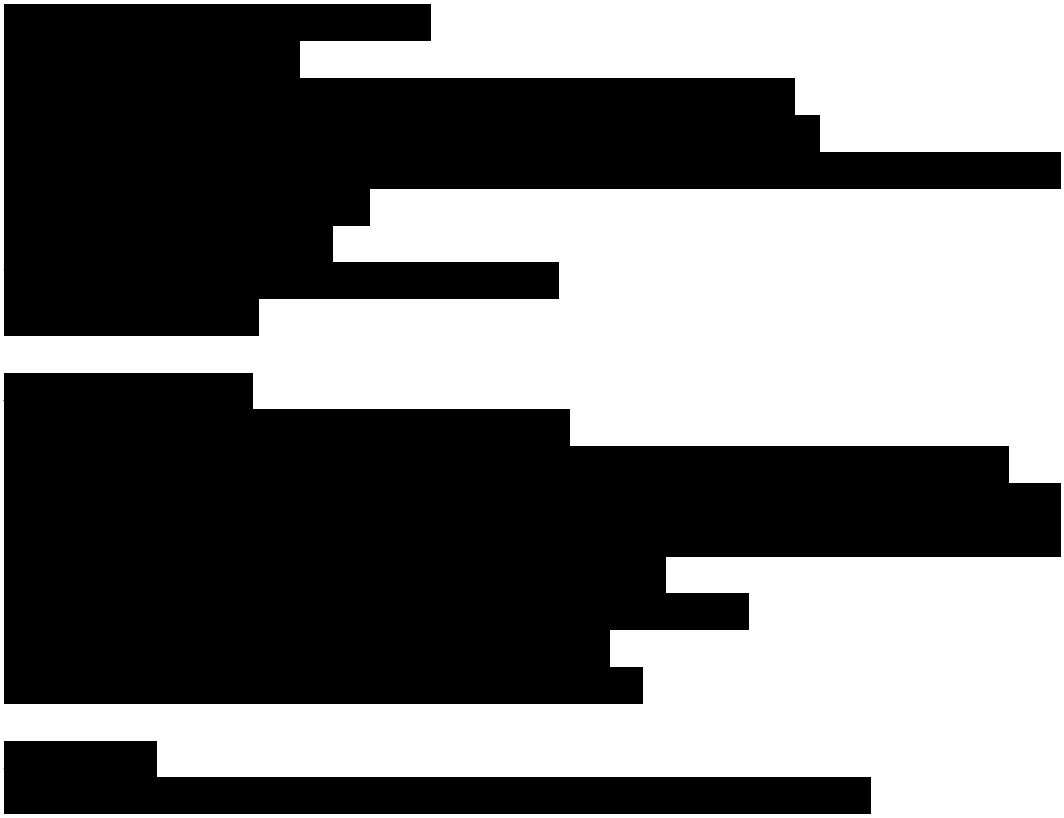
6.2 Delay for Organ Dysfunction

If renal dysfunction (creatinine ≥ 3 X institutional upper limit of normal) or hepatic dysfunction (bilirubin ≥ 2.5 mg/dl or ALT/AST ≥ 3 X institutional upper limit of normal) occurs, therapy may not resume until the abnormalities have resolved to below the limits stated above. If renal or hepatic dysfunction occurs during a treatment course, the next dose of decitabine should be held, and serum chemistries should be checked twice per week. Decitabine can be resumed when creatinine, bilirubin, ALT and AST are below the limits listed above.

For grade 3-4 non-hematologic toxicities, decitabine treatment should be delayed until recovery to baseline or to grade ≤ 1. For toxicities felt to be related to decitabine, decitabine may be reintroduced with a 50% dose reduction, or at the discretion of the investigator.

6.3 Modification in Dose or Schedule

Once patients achieve morphologic complete remission with incomplete blood count recovery (CRi) (morphologic leukemia-free state, defined as less than 5% blasts in aspirate with marrow spicules and a count of ≥ 200 nucleated cells and no blasts with Auer rods, any persistence of extramedullary disease, or persistent pre-treatment morphologic abnormalities, such as pseudo-Pelger-Hüet cells, ringed sideroblasts, or dysplastic megakaryocytes), administration may be decreased to 5 consecutive days to limit myelosuppressive toxicity, per treating physician. After two cycles with bone marrow blast counts < 5% and no evidence of disease by cytogenetics or immunophenotype, patients may further decrease therapy to 3 consecutive days per cycle, per treating physician.



8.0 CORRELATIVE STUDIES SAMPLE COLLECTIONS

Bone marrow samples will be collected at Baseline, on Cycle 1 Day 10 ± 1, and on Day 28 ± 4 of Cycle 1 and of even number cycles for correlative studies. Bone marrow aspirate (6-10 ml) will be collected and divided between two EDTA (lavender top) tubes. The bone marrow samples and peripheral blood samples should be delivered by hand to Sandra McDonald, M.D. PhD, director of the Tissue Procurement Core Facility at the Siteman Cancer Center. At the Tissue Procurement Core Facility, patient identification will be removed from the samples, and the cells will be stored as frozen pellets in DMSO at 10^7 per tube. Subsequently, the samples will be thawed, and cells will be assayed.

Peripheral blood sample (3 mL) will be collected on Cycle 1 Day 4 ± 1. Sample must be obtained 45 minutes ± 15 minutes after beginning the infusion, before the decitabine infusion is complete, and should be obtained from the opposite arm as the infusion. The sample should be collected in a 3 mL K3EDTA tube pre-loaded with 8 mcL of tetrahydrouridine (THU 500 mcg/mL), an inhibitor of cytidine deaminase. The sample should be processed by the Tissue Procurement Core Facility at the Siteman Cancer Center. Serum should be separated and stored per protocols.

9.0 EFFICACY, SAFETY AND CORRELATIVE STUDY MEASURES

A secondary endpoint of this study is to estimate morphologic complete remission rate.

9.1 Response Criteria

Patients will be assessed for response according to the IWG criteria:[1]

Morphologic complete remission (CR) – Defined as morphologic leukemia-free state, defined as less than 5% blasts in aspirate with marrow spicules and a count of ≥200 nucleated cells and no blasts with Auer rods or any persistence of extramedullary disease. When erythroid precursors constitute less than 50% of bone marrow nucleated cells, the percentage of blasts is based on all nucleated cells; when there are 50% or more erythroid cells, the percentage blasts should be based on the nonerythroid cells. The presence of mild megaloblastoid changes may be permitted if they are thought to be consistent with treatment effect. However, persistence of pretreatment abnormalities (eg, pseudo-Pelger-Hüet cells, ringed sideroblasts, dysplastic megakaryocytes) are not consistent with CR. In addition, ANC >1000/µl and platelet count of ≥100,000/µl. Patient must be independent of transfusions. No duration requirement for this designation.

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

Morphologic complete remission with incomplete blood count recovery (CRi) – Defined as morphologic complete recovery with the exception of neutropenia <1000/µl or thrombocytopenia <100,000/µl.

Partial remission (PR) – Requires that the above criteria for complete remission be met, with the following exceptions: decrease of ≥50% in the percentage of blast to 5-25% in the bone marrow aspirate. A value of ≤5% blasts in bone marrow with Auer rods present may also be considered a partial remission.

Treatment failure – Includes all patients who did not achieve a complete or partial remission following 4 cycles of therapy.

Recurrence/morphologic relapse - Relapse following 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. New dysplastic changes are considered a relapse. 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.

9.2 Guidelines for Evaluation of Disease

Bone marrow biopsy should be performed at baseline for both the correlative study and clinical evaluation. Bone marrow biopsy should be performed on Day 28 ± 4 of cycle 1 and on Day 28 ± 4 of each even cycle for clinical disease assessment.

Peripheral blood counts will be followed by weekly CBC with differential and platelets. After the first cycle, this may be changed to every other week or monthly at the discretion of the treating physician.

9.3 Progressive Disease

Progressive disease is defined as a clear increase in bone marrow blast count, typically a greater than 50% increase compared with baseline. Patients with progressive disease will be removed from the study.

Patients may also be removed from the study for progressive disease based on evidence of new extramedullary disease or based on the clinical judgment of the treating physician.

Patients with a > 50% increase in peripheral blast count with an increase of the total peripheral white blood cell count to > 10,000/μl should undergo evaluation by bone marrow biopsy regardless of the cycle number.

9.4 Other Secondary Efficacy Measures

9.4.1 Overall Survival and Event-free Survival

Overall survival is defined as the date of first dose of study drug to the date of death from any cause. Patients still alive at the time of the final analysis will be right-censored.

Event-free survival is defined as the interval from the date of first dose of study drug to date of treatment failure, recurrence, death due to any cause, or loss to follow up.

9.4.2 Duration of Remission and Relapse-free Survival for Patients Having Complete Remission

Duration of complete remission is defined as the interval from the date that complete remission is documented to the date of recurrence, death due to any cause, or loss 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.

9.4.3 Safety

Safety evaluation will include assessments of adverse experiences, medical history, physical examinations, vital signs, and laboratory assessments at baseline and throughout the study period. Local laboratories may be used for this study.

10.0 ADVERSE EVENT REPORTING

10.1 Adverse Events (AEs)

Definition: any unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site: http://ctep.cancer.gov/reporting/ctc.html.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website: (http://www.hhs.gov/ohrp/policy/AdvEvntGuid.htm).

10.2 Unanticipated Problems

Definition:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

10.3 Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

10.4 Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

10.5 Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

Pre-approval of all protocol exceptions must be obtained prior to the event.

10.6 Reporting to the Human Research Protection Office (HRPO) and the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University:

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH or SLCH institution, or that impacts participants or the conduct of the study.
- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within **10 working days** of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification to the PI of the event.

10.7 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. **PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC.** It is the responsibility of the investigator to report any unanticipated problem to the FDA as follows:

- Report any unexpected fatal or life-threatening adverse experiences associated with use of the drug (i.e., there is a reasonable possibility that the experience may have been caused by the drug) by telephone or fax no later than **7 calendar days** after initial receipt of the information. A life-threatening adverse experience is defined as any adverse drug experience that places the subject (in the view of the investigator) at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Report any serious, unexpected adverse experiences, as well as results from animal studies that suggest significant clinical risk within **15 calendar days** after initial receipt of this information. A serious adverse drug experience is defined as any

adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions)
- A congenital anomaly/birth defect
- Any other experience which, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

An unexpected adverse drug experience is defined as any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure (or risk information, if an IB is not required or available).

All MedWatch forms will be sent by the investigator or investigator's team to the FDA at the following address or by fax:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Oncology Drug Products
5901-B Ammendale Rd.
Beltsville, MD 20705-1266
FAX: 1-800-FDA-0178

10.8 Timeframe for Reporting Required Events:

Reportable adverse events will be tracked for 30 days following the last day of study treatment.

Deaths	
Any reportable death while on study or within 30 days of study	Immediately, within 24 hours, to PI and the IRB
Any reportable death while off study	Immediately, within 24 hours, to PI and the IRB
Adverse Events/Unanticipated Problems	
Any reportable adverse events as described in Sections 10.1 and 10.2 (other than death) and 10.7	Immediately, within 24 hours to PI and within 10 working days to the IRB, and within 7 or 15 days to the FDA
All adverse events regardless of grade and attribution should be submitted cumulatively	Include in DSM report
Noncompliance and Serious Noncompliance	
All noncompliance and serious noncompliance as described in Sections 10.3 and 10.4	Immediately, within 24 hours, to PI and within 10 working days to the IRB

11.0 DATA SAFETY MONITORING PLAN

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months after accrual has opened (if at least five patients have been enrolled) or one year after accrual has opened (if fewer than five patients have been enrolled at the six-month mark).

The Principal Investigator will review all patient data at least every six months, and provide a semi-annual report to the QASMC. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual
- Protocol activation date
- Average rate of accrual observed in year 1, year 2, and subsequent years
- Expected accrual end date
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

12.0 STATISTICAL ANALYSIS METHODS AND SAMPLE SIZE

12.1 Analysis Populations

Intent-to-Treat - The intent-to-treat (ITT) population will be defined as all patients who receive at least one dose of decitabine.

Efficacy-Evaluable - The efficacy-evaluable (EE) population will be defined as all patients who receive at least one cycle of treatment.

Safety - The safety population will be defined as all patients with at least one dose of decitabine.

12.2 Statistical Analysis

12.2.1 Descriptive Analyses

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

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.

12.2.2 Primary Endpoint Analysis

The primary end-point of this phase II study is the correlation biomarkers with overall response in AML/MDS patients to decitabine.

We will correlate the presence of patient specific mutations (especially mutations in *DNMT3A, IDH1, IDH2,* and *TET2*) with overall response rates. Mutation rates in these genes and in other patient-specific genes will be correlated with overall response and their respective 95% confidence intervals will be provided.

Statistical considerations. The relationship between AML/MDS-associated mutations and the responsiveness to decitabine will be described by contingency tables and assessed using Chi-square test or Fisher's exact test as appropriate. The differentially expressed genes between responders and non-responders will be determined using the significance analysis of microarray (SAM). Multivariate logistic regression will also be fit to adjust the potential confounding effects of other demographic and clinical characteristics. The resultant critical *P* values will be corrected for multiple testing. Since the Bonferroni correction is known to be too conservative, a permutation analysis (e.g., 10,000 random assignments for

responsive status) will be used to derive an empirical critical *P* value. The new critical p-value is the number of permutation tests which yield a lower p-value than the original test divided by the total number of permutation tests.[61]

A power analysis was performed using Chi-square test for the association between mutation and response to decitabine. The frequency of *DNMT3A, IDH1/2,* and *TET2* mutations in AML and MDS patients are each between 15% and 30% in multiple cohorts.[29-37] **Table 3** shows the minimal detectable differences of response rates between mutant and wild-type cases, assuming an expected overall 45% CR/CRi rate and 80% power. For an average of 20% frequency of a mutated gene, for example, 100 patients will provide 80% power to detect 30% difference at a 2-sided 0.1 alpha error. The purpose of this study is to determine whether any of these mutations result in a clinically significant association that would warrant pre-treatment testing. We believe that a reasonable cut off would be if at least 30% of clinical response could be attributed to the presence of a specific mutation. However, we anticipate that more power could be achieved as we assess patient groups with combined characteristics (e.g. *DNMT3A* mutation or loss of *DNMT3A* expression). In addition, we expect that the majority of the enrolled 125 patients can be used in the analysis (since all of our molecular information will be acquired within 30 days of enrollment, we anticipate a drop-out rate of 20% or less).

Table 3. Detectable mutation associated response rates (80% power, $\alpha = 0.05$)

Total N	% of patients with a given mutation	Detectable response difference	Sensitivity of mutation to predict response at minimum detectable response difference*
100	15%	0.40	80%
	20%	0.35	75%
	30%	0.30	67%
	40%	0.27	61%
	50%	0.26	58%

* Assuming the overall response rate (CR/Cri) of 45%

12.2.3 Secondary Endpoint Analyses

The overall response rate (CR/CRi/PR) and complete response rate (CR/CRi) will be compared with historical controls[2], and the 95% confidence intervals will be provided.

The rate of patient specific mutation clearance after 10, 28, and 56 days of therapy will be determined using an Illumina based platform. We will compare the rate of mutation clearance between the patients who achieve a CR/CRi after 4 cycles and those who do not. We anticipate that we will identify between 6 and 25 exonic SNVs per case (the range obtained with >200 AML samples sequenced to date with whole genome or exome sequencing), and that the expected 95% confidence

interval of the mean variant allele frequency will be ±2.56% and ±1.25%, respectively, if we assume an anticipated standard deviation of 3.2% within the same clone. The change of tumor burden over time and its association with clinical response will be assessed using 2-way ANOVA for repeated measurement data, followed by the ad-hoc multiple comparisons for between-group differences (e.g., responders vs. non-responders) at given time points as well as the between-time differences within each group. The resultant critical *P* values will also be corrected using permutation analysis for multiple testing.

The steady-state serum decitabine concentration on day 4 ± 1 will be measured and correlated with clinical overall response.

The percent decrease in bone marrow methylcytosine will be calculated on day 10±1. We will compare this reduction with previously published data and between the patients who achieve CR/CRi and those who do not. The correlation of peak serum levels of decitabine with response will be assessed using 2-sample t-test or Mann-Whitney non-parametric rank-sum test as appropriate. The change of total bone marrow DNA methylcytosine from baseline and its association with both steady-state serum drug levels and response will be assessed using 2-way ANOVA for repeated measurement data, followed by the ad-hoc multiple comparisons for between-group differences (e.g., responders vs. non-responders) and between-time differences. Similar analyses will be performed for the correlation of expression profiles and SNP analysis with pharmacokinetic data.

Statistical power justification. A power analysis was performed using two-sample t-test for between-group difference and paired t-test for the change over time. For tumor clearance, this will provide > 99% power to detect a 10% difference in the means at two separate time points for clones with 6 or more variants, and > 98% power to detect a 15% difference if there are 3 or more variants per clone with $\alpha = 0.05$ (power will decrease to 91% and 82%, respectively, in cases where the standard deviation is 4.7%, the maximum we have observed thus far). For pharmacologic correlations, we anticipate 85% power to determine a 25 ng/ml difference between the average C_{max} of 45 evaluable responders and 55 evaluable non-responders with an anticipated average C_{max} of 70 ng/ml and a standard deviation of 40 ng/ml within the entire cohort, and with $\alpha = 0.05$.[22] We believe a less significant difference would not be sufficient to warrant application in clinical decision making (i.e. whether to continue therapy for more than 1 cycle). We anticipate 99% power to determine a difference of 20% in the reduction of bone marrow methylcytosine between 45 evaluable responders and 55 evaluable non-responders with an expected standard deviation of 15% and $\alpha = 0.05$.[28, 66-68] The difference in methylcytosine reduction previously observed was 30% between patients achieving CR/PR and those with only hematologic improvement.[28]

Time to relapse and time to death will also be listed for each patient.

12.2.4 Safety Analysis

Extent of Exposure – Duration (number of cycles/infusions) of decitabine doses (cumulative and intensity) will be summarized.

Adverse Events - All adverse events will be coded using NCI CTCAE v. 4.0. 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. In addition, adverse events will be summarized by investigator attribution of relationship to study medication and by grade. Similar summaries will be provided by treatment cycle.

Clinical Laboratory Evaluation – Applicable laboratory parameters will be graded according to NCI CTCAE version 4.0. The incidence of maximum grade (number and percent of patients experiencing the maximum grade during the study) will be summarized for each laboratory parameter. Similar summaries will be provided by treatment cycle. Graphical displays of select hematological parameters will also be provided.

Vital Signs, Physical Findings, and Other Safety Observations – Vital signs, findings from physical examinations, ECOG scores and weights will be presented in listing format.

12.2.5 Description of Planned Subgroup Analyses

The following subgroups (if sufficient numbers of patients) will be explored with respect to the primary and overall survival endpoints.

- Age (60-69 years old vs ≥ 70 years old)
- Cytogenetics
- Patients with AML-associated mutations in *DNMT3A, TET2, IDH1,* or *IDH2* vs those who do not have somatic variants in these genes.

12.3 Sample Size

We anticipate initially enrolling 25 patients at Washington University. Once we establish a routine exome analysis with validation and calculation of the rate of patient-specific mutation clearance, we will enroll an additional 100 patients from at least 2 additional centers.

13.0 DATA SUBMISSION SCHEDULE

Case Report Form	**Submission Schedule**
Original Consent Form	*Prior to registration*
Patient Registration Form *Disease Diagnosis Form* *Inclusion Criteria Worksheet* *Exclusion Criteria Worksheet*	*Prior to starting treatment*
Treatment Record Form	*To be maintained throughout a patient's treatment cycle. The complete record is due at completion of treatment.*
Bone Marrow Sample Collection Form	*To be maintained throughout the course of the study at designated bone marrow sample collections time points (See Appendix 2).*
Pathology: Bone Marrow Biopsy	*To be completed upon the return of pathology report for each bone marrow biopsy.*
Study Calendar Form	*To be completed with the date of each time point upon occurrence.*
Labs Form	*To be completed upon the return of laboratory reports for each study time point (See Appendix 2)*
Dose Modifications	*To be maintained throughout the course of the study if dose modifications are performed.*
Therapy Response Form	*To be filled out upon remission (prior to, and/or on therapy)*
End of Study Form	*Due at the completion of the EOS visit, at the time of death, and/or at the time that the patient is lost to follow-up*
Follow Up Form	• *6 weeks post date of last decitabine dose.* • *Every 2 months for 2 years after the first dose of study drug.*
Record of Adverse Events	*At the time of any AE*

Appendix 1 ECOG Performance Scale

Activity Status	Description
0	Asymptomatic, fully active, and able to carry on all predisease performance without restrictions.
1	Symptomatic, fully ambulatory but restricted in physically strenuous activity and able to carry out performance of a light or sedentary nature, eg, light housework, office work.
2	Symptomatic, ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours: in bed less than 50% of day.
3	Symptomatic, capable of only limited self-care, confined to bed or chair more than 50% of waking hours, but not bedridden.
4	Completely disabled. Cannot carry on any self-care. Totally bedridden.
5	Dead

Appendix 2 Study Flow Chart

Study Flow Chart

Test	Pre-study	Base-line	All Cycles Day 1	All Cycles Cohort A Days 1-10[a, b]	Cycle 1 day 4 ± 1	Cycle 1 Day 10 ± 1 and 28 ± 4	Every week	Every 2 weeks	Even cycles Day 28±4	End of Study
Informed Consent	X									
Medical History		X	X							
Physical Exam		X	X							X
Concomitant Medications		X								
Transfusion Requirements[c]		X[c]								
Bone Marrow Aspirate and Biopsy	X[d,e]					X[d]			X[d]	X[d]
Peripheral blood for correlative studies					X					
Pregnancy test		X								
CBC with differential and platelets		X	X				X[f]			X
Serum Chemistries[g]		X	X					X[f]		X
Decitabine administration[a,b,h]				X						
Adverse Events				Ongoing						
Post-study follow-up										X[i]

[a] Once bone marrow blast count is ≤ 5% (measured by cytomorphology, immunophenotype, and cytogenetics), the number of days per cycle of decitabine may be decreased to 5 per treating physician discretion.
[b] Once bone marrow blast count is ≤ 5% for two or more cycles and the peripheral blood counts have normalized, the number of days per cycle of decitabine may be decreased to 3 per treating physician discretion.
[c] Care should be given to record the frequency of transfusions from 8 weeks prior to first dose of study drug.
[d] Standard bone marrow procurement procedures will be followed for collection of the tissue. Prestudy samples should be collected within 14 days of first dose of study drug.
[e] The bone marrow biopsy for correlative studies may be collected with the prestudy evaluation. If not, it should be done as part of the base-line evaluation
[f] Once stable, these labs may be obtained every other week or once a month at the discretion of the treating physician.
[g] Serum chemistries include bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, and LDH.
[h] Decitabine treatment will be 20 mg/m^2 over a 1-hour IV infusion on consecutive Days 1-10 every 28-day cycle. The dose will be calculated based on actual patient weight at the beginning of each cycle.
[i] Patients will be followed for 6 weeks following the date of the last decitabine dose to monitor for toxicity, survival and response. Thereafter, the investigator or designee will record information on subsequent therapy, disease progression, and death, every 2 months for 2 years after first dose of study drug or until the patient is lost to follow-up.

References:

1. Cheson, B.D., et al., *Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia.* J Clin Oncol, 2003. **21**(24): p. 4642-9.
2. Cashen, A.F., et al., *Multicenter, phase II study of decitabine for the first-line treatment of older patients with acute myeloid leukemia.* J Clin Oncol, 2010. **28**(4): p. 556-61.
3. Blum, W., et al., *Clinical response and miR-29b predictive significance in older AML patients treated with a 10-day schedule of decitabine.* Proc Natl Acad Sci U S A, 2010. **107**(16): p. 7473-8.
4. Schlenk, R.F., et al., *Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia.* N Engl J Med, 2008. **358**(18): p. 1909-18.
5. Erba, H.P., *Has there been progress in the treatment of older patients with acute myeloid leukemia?* Best Pract Res Clin Haematol, 2010. **23**(4): p. 495-501.
6. Heaney, M.L. and D.W. Golde, *Myelodysplasia.* N Engl J Med, 1999. **340**(21): p. 1649-60.
7. Bennett, J.M., et al., *Proposals for the classification of the myelodysplastic syndromes.* Br J Haematol, 1982. **51**(2): p. 189-99.
8. Kantarjian, H., et al., *Results of intensive chemotherapy in 998 patients age 65 years or older with acute myeloid leukemia or high-risk myelodysplastic syndrome: predictive prognostic models for outcome.* Cancer, 2006. **106**(5): p. 1090-8.
9. Martin, M.G. and C.N. Abboud, *Induction therapy for elderly patients with acute myeloid leukemia.* Blood Rev, 2008. **22**(6): p. 311-20.
10. Bow, E.J., et al., *Therapy of untreated acute myeloid leukemia in the elderly: remission-induction using a non-cytarabine-containing regimen of mitoxantrone plus etoposide.* J Clin Oncol, 1996. **14**(4): p. 1345-52.
11. Goldstone, A.H., et al., *Attempts to improve treatment outcomes in acute myeloid leukemia (AML) in older patients: the results of the United Kingdom Medical Research Council AML11 trial.* Blood, 2001. **98**(5): p. 1302-11.
12. Stone, R.M., *The difficult problem of acute myeloid leukemia in the older adult.* CA Cancer J Clin, 2002. **52**(6): p. 363-71.
13. Liu Yin, J.A., et al., *Mitozantrone and cytosine arabinoside as first-line therapy in elderly patients with acute myeloid leukaemia.* Br J Haematol, 1991. **79**(3): p. 415-20.
14. Stone, R.M., et al., *Granulocyte-macrophage colony-stimulating factor after initial chemotherapy for elderly patients with primary acute myelogenous leukemia. Cancer and Leukemia Group B.* N Engl J Med, 1995. **332**(25): p. 1671-7.
15. Kantarjian, H., et al., *Decitabine improves patient outcomes in myelodysplastic syndromes.* Cancer, 2006. **106**(8): p. 1794-1803.

16. Lubbert, M., et al., *A multicenter phase II trial of Decitabine as first-line treatment of older AML patients judged unfit for induction chemotherapy.* Haematologica, 2011. **97**(3): p. 393-401.
17. Issa, J.P., et al., *Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies.* Blood, 2004. **103**(5): p. 1635-40.
18. Petti, M.C., et al., *Pilot study of 5-aza-2'-deoxycytidine (Decitabine) in the treatment of poor prognosis acute myelogenous leukemia patients: preliminary results.* Leukemia, 1993. **7 Suppl 1**: p. 36-41.
19. Wijermans, P.W., et al., *Continuous infusion of low-dose 5-Aza-2'-deoxycytidine in elderly patients with high-risk myelodysplastic syndrome.* Leukemia, 1997. **11**(1): p. 1-5.
20. Kantarjian, H., et al., *Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study.* Cancer, 2006. **106**(8): p. 1794-803.
21. Wijermans, P.W., et al., *An epigenetic approach to the treatment of advanced MDS; the experience with the DNA demethylating agent 5-aza-2'-deoxycytidine (decitabine) in 177 patients.* Ann Hematol, 2005. **84 Suppl 1**: p. 9-17.
22. Cashen, A.F., et al., *Pharmacokinetics of decitabine administered as a 3-h infusion to patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS).* Cancer Chemother Pharmacol, 2008. **61**(5): p. 759-66.
23. Chabot, G.G., J. Bouchard, and R.L. Momparler, *Kinetics of deamination of 5-aza-2'-deoxycytidine and cytosine arabinoside by human liver cytidine deaminase and its inhibition by 3-deazauridine, thymidine or uracil arabinoside.* Biochem Pharmacol, 1983. **32**(7): p. 1327-8.
24. Momparler, R.L., et al., *Cell cycle effects and cellular pharmacology of 5-aza-2'-deoxycytidine.* Cancer Chemother Pharmacol, 1984. **13**(3): p. 191-4.
25. Raza, A., et al., *Biological significance of cell cycle kinetics in 128 standard risk newly diagnosed patients with acute myelocytic leukemia.* Br J Haematol, 1991. **79**(1): p. 33-9.
26. Brons, P.P., et al., *Proliferation patterns in acute myeloid leukemia: leukemic clonogenic growth and in vivo cell cycle kinetics.* Ann Hematol, 1993. **66**(5): p. 225-33.
27. Raza, A., et al., *Direct relationship between remission duration in acute myeloid leukemia and cell cycle kinetics: a leukemia intergroup study.* Blood, 1990. **76**(11): p. 2191-7.
28. Shen, L., et al., *DNA methylation predicts survival and response to therapy in patients with myelodysplastic syndromes.* J Clin Oncol, 2010. **28**(4): p. 605-13.
29. Tefferi, A., et al., *IDH1 and IDH2 mutation studies in 1473 patients with chronic-, fibrotic- or blast-phase essential thrombocythemia, polycythemia vera or myelofibrosis.* Leukemia, 2010. **24**(7): p. 1302-9.
30. Ley, T.J., et al., *DNMT3A mutations in acute myeloid leukemia.* N Engl J Med, 2010. **363**(25): p. 2424-33.
31. Mardis, E.R., et al., *Recurring mutations found by sequencing an acute myeloid leukemia genome.* N Engl J Med, 2009. **361**(11): p. 1058-66.

32. Abdel-Wahab, O., J. Patel, and R.L. Levine, *Clinical Implications of Novel Mutations in Epigenetic Modifiers in AML.* Hematol Oncol Clin North Am, 2011. **25**(6): p. 1119-33.
33. Reuther, G.W., *Recurring mutations in myeloproliferative neoplasms alter epigenetic regulation of gene expression.* Am J Cancer Res, 2011. **1**(6): p. 752-62.
34. Paschka, P., et al., *IDH1 and IDH2 mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3 internal tandem duplication.* J Clin Oncol, 2010. **28**(22): p. 3636-43.
35. Shen, Y., et al., *Gene mutation patterns and their prognostic impact in a cohort of 1185 patients with acute myeloid leukemia.* Blood, 2011. **118**(20): p. 5593-603.
36. Tefferi, A., et al., *Detection of mutant TET2 in myeloid malignancies other than myeloproliferative neoplasms: CMML, MDS, MDS/MPN and AML.* Leukemia, 2009. **23**(7): p. 1343-5.
37. Walter, M.J., et al., *Recurrent DNMT3A mutations in patients with myelodysplastic syndromes.* Leukemia, 2011. **25**(7): p. 1153-8.
38. Lin, J., et al., *Recurrent DNMT3A R882 Mutations in Chinese Patients with Acute Myeloid Leukemia and Myelodysplastic Syndrome.* PLoS One, 2011. **6**(10): p. e26906.
39. Bejar, R., et al., *Clinical effect of point mutations in myelodysplastic syndromes.* N Engl J Med, 2011. **364**(26): p. 2496-506.
40. Walter, M.J., et al., *Clonal architecture of secondary acute myeloid leukemia.* N Engl J Med, 2012. **366**(12): p. 1090-8.
41. Kantarjian, H.M., et al., *Survival advantage with decitabine versus intensive chemotherapy in patients with higher risk myelodysplastic syndrome: comparison with historical experience.* Cancer, 2007. **109**(6): p. 1133-7.
42. Lubbert, M., et al., *Continued Low-Dose Decitabine (DAC) Is an Active First-Line Treatment in All Cytogenetic Subgroups of Older AML Patients: Results of the FR00331 Multicenter Phase II Study.* ASH Annual Meeting Abstracts, 2007. **110**(11): p. 300-.
43. Blum, W., et al., *Phase I Study of Decitabine Alone or in Combination With Valproic Acid in Acute Myeloid Leukemia.* J Clin Oncol, 2007. **25**(25): p. 3884-3891.
44. Issa, J.-P.J., et al., *Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies.* Blood, 2004. **103**(5): p. 1635-1640.
45. Garcia-Manero, G., et al., *Phase 1/2 study of the combination of 5-aza-2'-deoxycytidine with valproic acid in patients with leukemia.* Blood, 2006. **108**(10): p. 3271-3279.
46. Issa, J.P., S.B. Baylin, and J.G. Herman, *DNA methylation changes in hematologic malignancies: biologic and clinical implications.* Leukemia, 1997. **11 Suppl 1**: p. S7-11.
47. Ganetzky, R.D., et al., *Differences Between Normal and Leukemic Stem Cell-Specific Methylome Indicates Aberrantly Silenced Genes Involved in the*

Pathogenesis of Malignant Evolution. ASH Annual Meeting Abstracts, 2008. **112**(11): p. 599-.

48. Christman, J.K., *5-Azacytidine and 5-aza-2'-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy.* Oncogene, 2002. **21**(35): p. 5483-95.
49. Lubbert, M., *DNA methylation inhibitors in the treatment of leukemias, myelodysplastic syndromes and hemoglobinopathies: clinical results and possible mechanisms of action.* Curr Top Microbiol Immunol, 2000. **249**: p. 135-64.
50. Kantarjian, H., et al., *Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia.* Blood, 2007. **109**(1): p. 52-57.
51. Steensma, D.P., et al., *Multicenter Study of Decitabine Administered Daily for 5 Days Every 4 Weeks to Adults With Myelodysplastic Syndromes: The Alternative Dosing for Outpatient Treatment (ADOPT) Trial.* J Clin Oncol, 2009: p. JCO.2008.19.6550.
52. Cashen, A.F., et al., *Preliminary Results of a Multicenter Phase II Trial of 5-Day Decitabine as Front-Line Therapy for Elderly Patients with Acute Myeloid Leukemia (AML).* ASH Annual Meeting Abstracts, 2008. **112**(11): p. 560-.
53. Jabbour, E., et al., *Evolution of decitabine development: accomplishments, ongoing investigations, and future strategies.* Cancer, 2008. **112**(11): p. 2341-51.
54. Kantarjian, H., et al., *Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia.* Blood, 2007. **109**(1): p. 52-7.
55. Blum, W.G., Klisovic, R., Liu, S., Kefauver, C., Grever, M. R., Schaaf, L., Chan, K., Byrd, J. C., Villalona-Calero, M., Marcucci, G. *Efficacy of a novel schedule of decitabine in previously untreated AML, age 60 or older*. in *ASCO Annual Meeting*. 2009.
56. van Groeningen, C.J., et al., *Phase I and pharmacokinetic study of 5-aza-2'-deoxycytidine (NSC 127716) in cancer patients.* Cancer Res, 1986. **46**(9): p. 4831-6.
57. Rivard, G.E., et al., *Phase I study on 5-aza-2'-deoxycytidine in children with acute leukemia.* Leuk Res, 1981. **5**(6): p. 453-62.
58. Lin, K.T., R.L. Momparler, and G.E. Rivard, *Sample preparation for the determination of 5-aza-2'-deoxycytidine in plasma by high-performance liquid chromatography.* J Chromatogr, 1985. **345**(1): p. 162-7.
59. Momparler, R.L., G.E. Rivard, and M. Gyger, *Clinical trial on 5-aza-2'-deoxycytidine in patients with acute leukemia.* Pharmacol Ther, 1985. **30**(3): p. 277-86.
60. Raska, K., Jurovcik, M., Sormova., Z., *On the metabolism of 5-azacytidine and 5-aza-2'-deoxycytidine in mice.* Coll Czech Chem Commun, 1965. **30**: p. 3001-3005.
61. Momparler, R.L., et al., *Pilot phase I-II study on 5-aza-2'-deoxycytidine (Decitabine) in patients with metastatic lung cancer.* Anticancer Drugs, 1997. **8**(4): p. 358-68.

62. Aparicio, A., et al., *Phase I trial of continuous infusion 5-aza-2'-deoxycytidine.* Cancer Chemother Pharmacol, 2003. **51**(3): p. 231-9.
63. Bryan, J., et al., *Pharmacokinetic evaluation of decitabine for the treatment of leukemia.* Expert Opin Drug Metab Toxicol, 2011. **7**(5): p. 661-72.
64. Scandura, J.M., et al., *Phase 1 study of epigenetic priming with decitabine prior to standard induction chemotherapy for patients with AML.* Blood, 2011. **118**(6): p. 1472-80.
65. Westfall, P.H. and S.A. Young, *Resampling-based multiple testing: examples and methods for p-value adjustment*. 1993, New York: John Wiley & Sons, Inc.
66. Samlowski, W.E., et al., *Evaluation of a 7-day continuous intravenous infusion of decitabine: inhibition of promoter-specific and global genomic DNA methylation.* J Clin Oncol, 2005. **23**(17): p. 3897-905.
67. Blum, W., et al., *Phase I study of decitabine alone or in combination with valproic acid in acute myeloid leukemia.* J Clin Oncol, 2007. **25**(25): p. 3884-91.
68. Yang, A.S., et al., *DNA methylation changes after 5-aza-2'-deoxycytidine therapy in patients with leukemia.* Cancer Res, 2006. **66**(10): p. 5495-503.

Genomic Predictors of Decitabine Response in AML/MDS

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Genomic Predictors of Decitabine Response in AML/MDS

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CONFIDENTIAL

Study Synopsis

Title of study	**Genomic Predictors of Decitabine Response in AML**
Primary investigator/study center location	John S. Welch, M.D./PhD Washington University School of Medicine, Division of Oncology St. Louis, MO Timothy J. Ley, MD Washington University School of Medicine, Division of Oncology St. Louis, MO
Phase of development	Phase II
Study objectives	**Primary:** - To determine whether patient-specific mutations correlate with clinical overall response to decitabine in patients with AML or MDS. **Secondary:** - To determine whether 10 consecutive days per cycle of decitabine improves outcomes compared with historical controls treated with 5 consecutive days per cycle of decitabine. - To determine whether other biomarkers correlate with clinical overall response to decitabine in patients with AML or MDS. These will include: velocity and depth of patient-specific mutation clearance, bone marrow expression profile and change in expression profile during decitabine treatment, steady-state serum decitabine concentrations, and decrease in bone marrow methylcytosine content in response to decitabine.
Study design	Non-randomized, open-label, Phase II study of decitabine in patients with AML or MDS.
Study criteria	**Inclusion Criteria** <u>One of the following:</u> 1. Patient must have non-M3 AML and be > 60 years of age OR 2. non-M3 AML with relapsed disease OR 3. Symptomatic MDS with one of the following: o Symptomatic anemia with either hemoglobin <10.0 g/dL or requiring RBC transfusion o Thrombocytopenia with a history of two or more platelet counts < 50,000 / μL or a significant hemorrhage requiring platelet transfusions, or o Neutropenia with two or more absolute neutrophil counts < 1,000 /μL. <u>All of the following:</u> 1. Patient must have an ECOG performance status ≤ 2. 2. Patient must have >10% disease burden measured by cytomorphology, flow cytometry, or cytogenetics. 3. Patient must have peripheral white blood cell count < 50,000/mcl. 4. Patient must have adequate organ function, defined as: a. Total bilirubin < 1.5 x ULN b. AST/ALT < 2.5 x ULN c. Serum creatinine < 2.0 x ULN 5. Patient must have undergone ≤ 2 cycles of prior hypomethylating agent (decitabine or azacitidine).

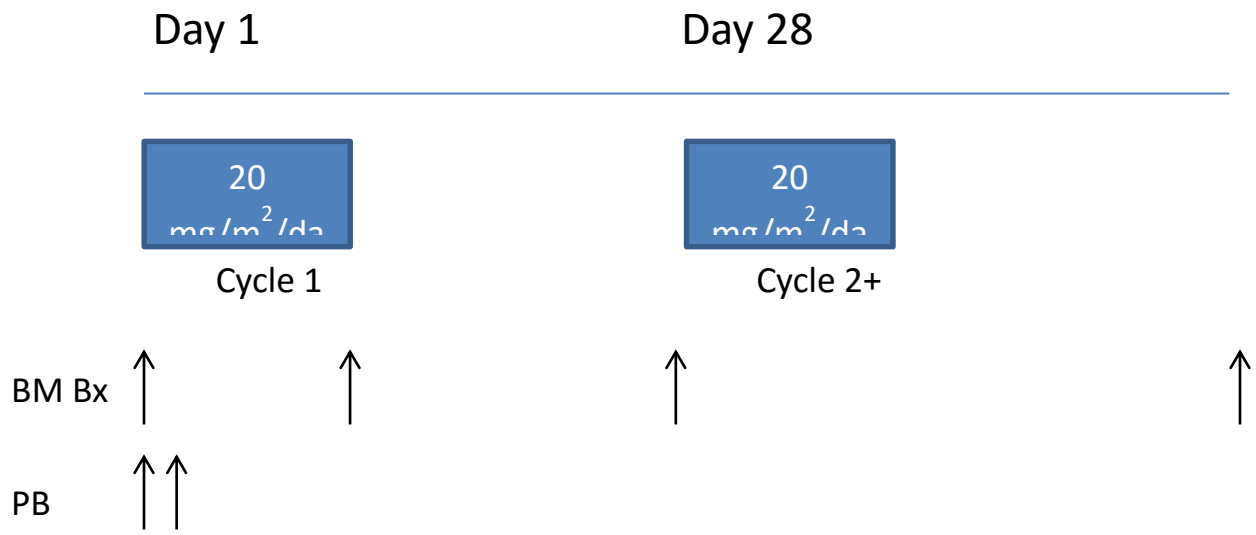
	6. Patient must be able to understand and willing to sign an IRB-approved written informed consent document. 7. Patient must be enrolled in HRPO# 201011766 ("Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Malignancies"). 8. Patient must be ≥ 18 years of age.
	Exclusion Criteria 1. Patient must not be pregnant or nursing. 2. Patient must not have known CNS leukemia. 3. Patient must not have a history of positive HIV serology. 4. Patient must not have a history of positive Hepatitis C serology. 5. Patient must not have any uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, ongoing or active GVHD, congestive heart failure of NYHA class 3 or 4, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situation that would limit compliance with study requirements. 6. Patient must not have had radiation therapy within 14 days of enrollment.
Expected number of patients	Total: 125. Evaluable for all molecular studies: 100. Enrollment time table: 25 patients will be enrolled initially at Washington University. Once the molecular pipeline for routine exome analysis is established, at least 2 additional sites will be added.
Study drug formulation and administration	[redacted]
Duration of treatment	Treatment may continue until one of the following criteria applies: • Death, • Disease progression (as defined in 3.7.1.3), • Intercurrent illness that prevents further administration of treatment, • Unacceptable adverse event(s), • Patient decides to withdraw from the study, or

	• General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the treating physician.
Correlative studies	Bone marrow samples for correlative studies will be collected prestudy, on Cycle 1 day 10 ± 1 and day 28 ± 4, and on day 28 ± 4 of subsequent even numbered cycles. Peripheral blood samples for correlative studies will be collected prestudy, and on Cycle 1 day 4 ± 1. 1. Identify Patient-specific mutations We will perform exome sequencing of each patient's day 0 bone marrow sample and skin sample. We will determine AML/MDS-associated variants, defined as variants found in the bone marrow sample and not in the skin sample. We will correlate the presence of mutations with overall response. 2. Velocity and depth of response. We will assess the rate of tumor clearance by measuring the allele frequency of patient-specific mutations in bone marrow samples during cycles 1 and 2. We will assess mutations in at least 24 recurrently mutated genes (including, but not limited to: *FLT3, DNMT3A, NPM1, TET2, WT1, IDH1, IDH2, RUNX1, NRAS, TP53, U2AF1, KRAS, PHF6, MSTP9, PTPN11, KIT, SMC3, PRUNE2, ETV6, CEBPA, SMC1A, ASXL1, STAG2, RAD21*) as well as all patient-specific mutations discovered during exome sequencing. The leukemic allele frequency will be measured using the Illumnia MySeq or HiSeq platform using DNA capture by gene specific biotinylated-oligonucleotides, or by a comparable technique. Patient bone marrow samples will be assessed on day 0, day 10 ± 1, and day 28 ± 4 of cycle 1, and on day 28 ± 4 of cycle 2 and compared with patient-specific skin samples (normal control). The velocity of response will be calculated as the natural log rate of patient-specific mutation allele frequency clearance. Depth of response will be defined as the lowest patient-specific mutation allele frequency in a bone marrow sample. The velocity and depth of tumor response will be correlated to: • Overall response, • Time to best response, • Mutation status, • Time to disease progression. 3. Expression profile. Expression array profiling will be performed on bone marrow samples from day 0 and day 10 ± 1. We will correlate expression signatures, and changes in expression signatures, with: • Overall response, • Mutational status. 4. Steady-state serum decitabine concentration. We will assess serum decitabine concentration on day 4 ± 1 and correlation with: • Overall response.

	5. Bone marrow DNA hypomethylation. We will assess the percent reduction in bone marrow methylcytosine content using LC-MS. This may serve as a better biomarker of adequate dosing than serum drug concentration. Patients will be evaluated on day 0 and day 10 ± 1 of cycle 1. The change in DNA methylcytosine content will be correlated to: • Overall response, • Change in expression signatures.
Criteria for evaluation	Efficacy- Bone marrow biopsies should be performed at baseline and at day 10 ± 1 and day 28 ± 4 of cycle 1 and at the end of subsequent even numbered cycles. Standard bone marrow procurement procedures will be followed for collection of the tissue. Jeffrey Klco, MD/PhD will act as a central reviewer for all bone marrow biopsies. Peripheral blood counts will be followed by weekly CBC with differential. After cycle 1, the frequency of routine CBC analysis may be decreased by the treating physician as clinically indicated. Patients will be assessed for response according to the IWG criteria:[1] *Morphologic complete remission (CR)*– Defined as morphologic leukemia-free state, defined as less than 5% blasts in aspirate with marrow spicules and a count of ≥ 200 nucleated cells and no blasts with Auer rods or any persistence of extramedullary disease, and no evidence for dysplasia. When erythroid precursors constitute less than 50% of bone marrow nucleated cells, the percentage of blasts is based on all nucleated cells; when there are 50% or more erythroid cells, the percentage blasts should be based on the nonerythroid cells. The presence of mild megaloblastoid changes may be permitted if they are thought to be consistent with treatment effect. However, persistence of pretreatment abnormalities (eg, pseudo-Pelger-Hüet cells, ringed sideroblasts, dysplastic megakaryocytes) are not consistent with CR. In addition, ANC > 1000/µl and platelet count of ≥ 100,000/µl. Patient must be independent of transfusions. No duration requirement for this designation. *Cytogenetic complete remission (CCR)* – 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. Fluorescent in situ hybridization (FISH) may be used as a supplement to follow a specifically defined cytogenetic abnormality. *Morphologic complete remission with incomplete blood count recovery (CRi)* – Defined as morphologic complete recovery with the exception of neutropenia <1000/µl or thrombocytopenia <100,000/µl. *Partial remission (PR)* – Requires that the above criteria for complete remission be met, with the following exceptions: decrease of ≥50% in the percentage of blasts to 5-25% in the bone marrow aspirate. A value of ≤5% blasts in bone marrow with Auer rods present may also be considered a partial remission. *Treatment failure* – Includes all patients who did not achieve a complete or partial remission.

	Recurrence/morphologic relapse - Relapse following 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. New dysplastic changes are considered a relapse. 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. Safety-- All patients receiving at least one dose of decitabine will be evaluated for safety.
Statistical considerations	This trial will be conducted as a phase II biomarker trial. This trial is designed to detect the most clinically meaningful mutational predictors of response. These are mutations that have sufficient positive predictive value to be used as a clinical assay. A power analysis was performed with Fisher's exact test for mutation status and response to decitabine, assuming an expected 45% CR/CRi. With alpha = 0.05, and a total anticipated enrollment of 100 evaluable patients, we anticipate 80% power to detect a 27% and 40% response difference between patients with and without a mutation, if the mutation occurs with a respective frequency of 30% and 15%. Such mutations would provide 80% sensitivity to predict response to decitabine. These are values that would warrant diagnostic clinical testing prior to therapy. This trial is also designed to detect an improvement in clinical response compared to historical controls treated with 5 consecutive days of decitabine (20 mg/m^2/day every 28 days)[2]. A power analysis was performed with Fisher's exact test for response to decitabine compared to a historical rate of 24% observed in 55 patients. With 100 evaluable patients enrolled, we anticipate 90% power with alpha = 0.1 to detect a 21% improvement in response (CR/ PR ≥ 45%). The reported CR/PR rate of the proposed treatment schedule (10 consecutive days of decitabine 20 mg/m^2/day) is 64%[3]; the expected CR/PR rate of 45% is a conservative estimation of expected outcomes.

Study Schema:



Patients with progressive disease after two cycles (defined as a clear increase in bone marrow blast count, typically a > 50% increase in bone marrow blasts over baseline) should be removed from study.

Patients with stable disease may continue on study and should undergo a diagnostic bone marrow biopsy at the end of even numbered cycles.

After 2 cycles, if patients achieve morphologic CRi, administration will be decreased to 5 days to limit myelosuppressive toxicity (20 mg/m^2 over 1 hour on Days 1-5 every 28 ± 4 days). After two cycles with morphologic CRi and no evidence of disease by cytogenetics or immunophenotype, patients may further decrease therapy to 3 days per cycle (20 mg/m^2 over 1 hour on Days 1-3 every 28 ± 4 days).

Sample collection for correlative studies:

Bone marrow biopsies should be performed at baseline and at Day 10 ± 1 and Day 28 ± 4 of Cycle 1 and at the end of even numbered cycles.

Peripheral blood samples should be collected on Day 4± 1 of Cycle 1. Collection should be obtained 45 ± 15 minutes after decitabine infusion is initiated.

Genomic Predictors of Decitabine Response in AML/MDS

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1.0 INTRODUCTION

1.1 Rationale

Personalized therapy for cancer requires that 1) patient-specific factors (e.g. patient-specific mutations, expression profiles, or pharmacogenomics) influence treatment algorithms, and 2) once therapy is initiated, *in vivo* responsiveness is used to guide further therapy. The modern era of genomics has provided significant insights into cancer pathogenesis by describing patterns of germline and somatic variants, as well as defining disease-specific patterns of gene expression. However, the application and integration of this data with therapeutics remains a difficult task.

Decitabine is a commonly used single-agent therapy for patients with acute myeloid leukemia (AML) and/or myelodysplastic syndrome (MDS). Historically, it has yielded clinical complete responses in approximately 25% of patients across diverse clinical trials, it is well tolerated, and it can be given as in the outpatient setting. However, the majority of patients do not respond to decitabine, the molecular etiology of decitabine sensitivity is unknown, and there are no algorithms to predict patient responsiveness *a priori* or even within one month of starting therapy.

AML and MDS are two closely related hematological disorders characterized by ineffective hematopoiesis and malignant expansion of clonal myeloid cells. Both AML and MDS frequently occur in elderly patients and some cases are secondary and/or related to long-term bone marrow damage after radiation or chemotherapy. Current therapy for AML consists of cytotoxic chemotherapy followed by consolidation chemotherapy or stem cell transplantation. Using these approaches, current cure rates may be as high as 60% for patients younger than 60 with good risk cytogenetics (e.g. t(15;17), t(8;21), inv(16)).[4] Outcomes in patients ≥ 60 years old and with relapsed disease remain poor with 2 year survival of only ~25%.[5] Historically, the available treatment for MDS has been symptomatic and has not been shown to be effective in producing sustained improvement in hematopoiesis or in delaying leukemic evolution[6, 7].

Current AML therapy is poorly tolerated in elderly and pre-treated patients due to hematologic and non-hematologic toxicities; following such therapy, patients typically require 3-4 weeks of hospitalization for treatment of neutropenic fevers and for regular transfusions. Even among the relatively healthy patients enrolled in clinical trials, induction mortality in the elderly can be as high as 20-40% following standard induction therapy,[8-11] and moderate to severe non-hematologic toxicity occurs in at least an additional 30% of patients.[9, 12-14] Consequently, many older AML patients are not offered traditional intensive chemotherapy, but receive supportive care alone.[15] More effective and less toxic therapies are needed to provide durable remissions in a significant proportion of elderly AML in populations.

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We and others have recently used whole genome sequencing to identify recurrent, somatic mutations in AML and MDS patients in four genes that are involved in methylcytosine regulation: *DNMT3A, IDH1, IDH2,* and *TET2.*[29-37] Collectively, nearly half of all AML/MDS patients have a mutation in one of these four genes. *DNMT3A* mutations are associated with an adverse outcome in AML patients treated with cytotoxic chemotherapy.[35, 38] However, it is not yet clear whether the presence of mutations in one or more of these genes is associated with response or resistance to decitabine.

In this study, we will treat patients with the current state-of-the-art decitabine schedule.[3] We will perform exome sequencing on all patients and correlate response with mutation status, rate of mutation clearance, expression profile, serum decitabine levels, and DNA

methylcytosine content reduction. This approach will comprehensively assess genomic and pharmacologic parameters that could clinically predict patient response to decitabine.

In this study, we will combine analysis of patients with AML and MDS. Recent genomic analysis has shown that AML and MDS have shared mutations in many of the same genes, shared patterns of mutation acquisition, and shared patterns of mutation evolution.[39, 40] Furthermore, MDS, like AML, presents with clonal hematopoiesis, even when the morphologic blast count is zero.[36]Because this study will identify and track the rate of clearance of patient specific mutations, and because decitabine has been shown to have efficacy in both AML and MDS patients, [2, 3, 16-19, 41-45] combining analysis of AML and MDS patients is reasonable. Asymptomatic MDS patients are excluded because these patients are typically followed with watchful-waiting rather than with chemotherapy.

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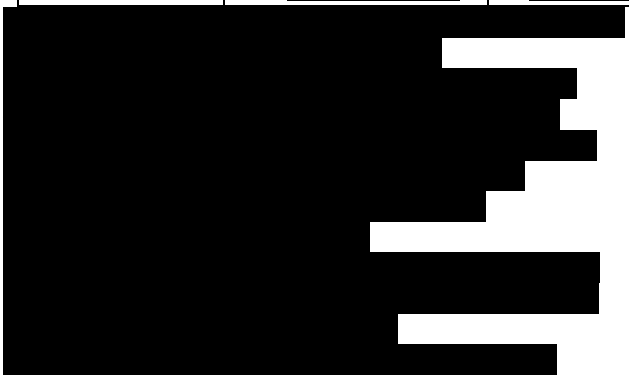

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1.3 Correlative Studies

The goals of the correlative studies include:

1. To correlate patient-specific mutations with response to decitabine.
   We will perform exome sequencing of each patient's Day 0 bone marrow sample and skin sample. We will determine AML/MDS-associated variants found in the bone marrow, but not skin sample. We will correlate the presence of mutations with response (especially mutations in *DNMT3A, IDH1, IDH2,* and *TET2*). This study is powered to identify mutations with >80% sensitivity to predict decitabine response if used as a clinical assay.

2. To correlate the velocity and depth of response with response to decitabine.
   We will assess tumor burden by allele frequency of patient-specific mutations in bone marrow samples collected on day 0, 10±1, 28±4, and 56±4. We will assess mutations in at least 24 recurrently mutated genes (including, but not limited to: *FLT3, DNMT3A, NPM1, TET2, WT1, IDH1, IDH2, RUNX1, NRAS, TP53, U2AF1, KRAS, PHF6, MSTP9, PTPN11, KIT, SMC3, PRUNE2, ETV6, CEBPA, SMC1A, ASXL1, STAG2, RAD21*) as well as all patient-specific mutations discovered during exome sequencing. The leukemic allele frequency will be measured using the Illumnia MySeq or HiSeq platform using DNA capture by gene specific biotinylated-oligonucleotides. Patient bone marrow samples will be assessed on day 0, day 10 ± 1, and day 28 ± 4 of cycle 1, and day 28 ± 4 of cycle 2 and compared with patient specific skin samples (normal control). The velocity of response will be calculated as the natural log rate of patient-specific mutation allele frequency clearance. Depth of response will be defined as the lowest patient-specific mutation allele frequency in a bone marrow sample. The velocity and depth of tumor response will be correlated to:
   - Achievement of clinical CR,
   - Time to best response,

- Mutational status,
- Time to disease progression.

Because all patient-specific exome-discovered mutations will be assessed a second time in the primary bone marrow sample as well as the Day 10 ± 1, 28 ± 4, and 56 ± 4 bone marrow samples, this study will also serve to validate all exome-discovered mutations. This is important, because exome sequencing is associated with a 20-50% false-discovery rate depending on type I error tolerated (mutation-calling algorithms are typically set for greater sensitivity than specificity). Thus, many mutations discovered during exome sequencing are incorrect. This second analysis of these mutations will server to exclude these false positive calls.

3. To correlate the bone marrow expression profile on Day 0 and Day 10 ± 1 with response to decitabine.
   We will correlate expression signatures, and changes in expression signatures, with:
   - Achievement of clinical CR,
   - Mutation status.

4. To correlate steady-state serum decitabine concentration on Day 4 ± 1 with:
   - Achievement of CR.

5. To correlate the extent of bone marrow DNA hypomethylation on Day 10 ± 1 with response to decitabine.
   We will assess the percent reduction in bone marrow methylcytosine content using LC-MS. Patients will be evaluated on Day 0 and Day 10 ± 1 of Cycle 1. The change in DNA methylcytosine content will be correlated to:
   - Achievement of clinical CR,
   - Change in expression signature.

   This will determine whether inadequate blast exposure to decitabine correlates with drug resistance.

2.0 OBJECTIVES

2.1 Primary Objective

To determine whether patient-specific mutations correlate with clinical overall response to decitabine in patients with AML or MDS.

2.2 Secondary Objectives

- To determine whether 10 consecutive days per cycle of decitabine improves outcomes compared with historical controls treated with 5 consecutive days per cycle of decitabine.

- To determine whether other biomarkers correlate with clinical overall response to decitabine in patients with AML or MDS. These will include:

    1. The velocity and depth of patient-specific mutation clearance;
    2. The bone marrow expression profile and change in expression profile during decitabine treatment;
    3. The steady-state serum decitabine concentrations;
    4. The decrease in bone marrow methylcytosine content in response to decitabine.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

One of the following:

- Patient must have non-M3 AML and be ≥ 60 years of age OR
- non-M3 AML with relapsed disease OR
- Symptomatic MDS with one of the following:
  - □ Symptomatic anemia with either hemoglobin < 10.0 g/dL or requiring RBC transfusion
  - □ Thrombocytopenia with a history of two or more platelet counts < 50,000/mcL or a significant hemorrhage requiring platelet transfusions
  - □ Neutropenia with two or more ANC < 1,000/mcL

All of the following:

1. Patient must have an ECOG performance status ≤ 2.
2. Patient must have >10% disease burden measured by cytomorphology, flow cytometry, or cytogenetics.
3. Patient must have peripheral white blood cell count ≤ 50,000/mcl.
4. Patient must have adequate organ function, defined as:
   a. Total bilirubin ≤ 1.5 x ULN
   b. AST/ALT ≤ 2.5 x ULN
   c. Serum creatinine ≤ 2.0 x ULN
5. Patient must have undergone ≤ 2 cycles of prior hypomethylating agent (decitabine or azacitidine).
6. Patient must be enrolled in HRPO# 201011766 (“Tissue Acquisition for Analysis of Genetic Progression Factors in Hematologic Malignancies”).
7. Patient must be ≥ 18 years of age.
8. Patient must be able to understand and willing to sign an IRB-approved written informed consent document.

3.2 Exclusion Criteria

1. Patient must not be pregnant or nursing.
2. Patient must not have known CNS leukemia.
3. Patient must not have a history of positive HIV serology.
4. Patient must not have a history of positive Hepatitis C serology.
5. Patient must not have any uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, ongoing or active GVHD, congestive heart failure of NYHA class 3 or 4, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situation that would limit compliance with study requirements.
6. Patient must not have had radiation therapy within 14 days of enrollment.

3.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4.0 REGISTRATION PROCEDURES

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility
2. Registration of patient in the Siteman Cancer Center database
3. Assignment of unique patient number (UPN)

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility collecting the information listed below

1. The registering MD's name
2. Patient's race, sex, and DOB
3. Three letters (or two letters and a dash) for the patient's initials
4. Copy of signed consent form
5. Completed eligibility checklist, signed and dated by a member of the study team
6. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center Database

All patients must be registered through the Siteman Cancer Center database.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. Patients will also be identified by first, middle, and last initials. If the patient has no middle initial, a dash will be used on the case report forms (CRFs). All data will be recorded with this identification number on the appropriate CRFs.

5.0 INVESTIGATIONAL PLAN

5.1 Summary of Study Design

This is an open-label, single-arm, Phase II, biomarker study of patients with AML or MDS who are treated with 10 consecutive days of decitabine. FAB-M3 AML is excluded from this trial, because other well-tolerated options exist for the treatment of FAB-M3 acute promyelocytic leukemia. AML patients who are < 60 years old and have not received cytotoxic induction chemotherapy are also excluded, because cytotoxic therapy offers a curative possibility for these patients. MDS patients without cytopenias are also excluded because these patients can be followed with watchful-waiting.

All patients will undergo bone marrow biopsies prior to therapy, on Day 10 ± 1, and on Day 28 ± 4 of Cycles 1 and 2 for comprehensive genomic correlative studies. Patients will also provide peripheral blood samples on Day 4 ± 1 for pharmacokinetic studies.

Patients will receive decitabine 20 mg/m^2/day as a 1-hour infusion on consecutive Days 1-10 in 28-day cycles. The dose will be calculated based on actual patient weight at the beginning of each cycle.

After 2 cycles, if patients achieve CRi, the administration of decitabine will be decreased to 5 days. After two cycles with CRi and no evidence of disease by cytogenetics or immunophenotype, patients will further decrease therapy 3 consecutive days. The dose of decitabine will remain 20 mg/m^2/day over 1 hour regardless of decreases in the number of days per cycle of administration.

Patients who achieve stable disease, partial response, or complete response will continue on decitabine therapy (receiving cycles of 3-10 days every 28 days (i.e., Days 1-10, Days 1-5, or Days 1-3), depending on response) until time of progression, unacceptable toxicity, or patient decision to discontinue study drugs.

It will be a logistical goal of this trial consistently to return preliminary exome results to the treating physician by the end of Cycle 1 and validated results by the end of Cycle 2. However, this logistical goal is not a primary or secondary aim of this study. Given the limited data regarding mutation presence and drug response, we do not anticipate that the results will directly influence therapeutic decisions made by the treating physician. However, the development of comprehensive genomic pipelines and integration of this data into a clinical trial setting will be required before personalized medicine can be realized.

5.2 Study Procedures

5.2.1 Pre-study Procedures

Pre-study procedures must take place no more than 14 days prior to the first dose of decitabine.

- Bone marrow morphologic assessment[a]
- Bone marrow or peripheral blood for flow cytometry
- Bone marrow for cytogenetics.

[a] Standard bone marrow procurement procedures will be followed for collection of the tissue.

5.2.2 Baseline Evaluation

Baseline evaluations must take place no more than 7 days prior to the first dose of decitabine and include:

1. Medical history
2. Physical exam including height, weight, vital signs, and ECOG performance score.
3. Concomitant medications
4. Record transfusion requirements for 8 weeks before first dose of study drug
5. CBC with differential and platelets
6. Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH
7. Urine βHCG pregnancy test if appropriate

5.2.3 Day 1 of Each Cycle

1. Record adverse events
2. Physical exam, including weight and ECOG performance score
3. Vital signs, including blood pressure, pulse and temperature
4. CBC with differential and platelets
5. Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH

5.2.4 Cycle 1 Days 1-10

Decitabine administration, 20 mg/m^2 per day given as a 1-hour continuous intravenous infusion. The dose will be calculated based on actual patient weight at the beginning of each cycle. Patients may be premedicated with lorazepam, prochlorperazine, palonosetron, or ondansetron at the treating physician's discretion.

5.2.5 Cycle 1 Day 4 ± 1

Obtain 3 mL peripheral blood for correlative studies. Sample must be obtained 45 minutes ± 15 minutes after beginning the infusion, before the decitabine infusion is complete, and should be obtained from the opposite arm as the infusion. The sample should be collected in a 3 mL K3EDTA tube pre-loaded with 8 mcL of tetrahydrouridine (THU 500 mcg/mL), an inhibitor of cytidine deaminase. The

sample should be processed by the Tissue Procurement Core Facility at the Siteman Cancer Center. Serum should be separated and stored per protocols.

5.2.6 Cycle 1 Day 10 ± 1

Obtain bone marrow biopsy for response and correlative studies. [a]

[a] Standard bone marrow procurement procedures will be followed for collection of the tissue.

5.2.7 Weekly

1. CBC with differential and platelets. After the first cycle, this may be changed to every other week at the discretion of the treating physician.
2. Record adverse events.

5.2.8 Every Other Week

Serum chemistries, including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, LDH. After the first cycle, this may be changed to every month at the discretion of the treating physician.

5.2.9 Cycle 1 and Even Cycles Day 28 ± 4 days

1. Bone marrow biopsy and aspirate collection.[a]
2. Clinical samples for response determination should be obtained at the end of Cycles 1, 2 and 4. Patients who receive > 4 cycles should continue to undergo bone marrow biopsies on even cycles for clinical evaluation (or as clinically indicated).

[a] Standard bone marrow procurement procedures will be followed for collection of the tissue.

5.2.10 End-of-Study Procedures

1. CBC with differential and platelets.
2. Record adverse events.
3. Physical examination including weight, vital signs, and ECOG performance score.
4. Serum chemistries including bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, and LDH.
5. Bone marrow collection, with samples sent for routine pathology.[a]

[a] Standard bone marrow procurement procedures will be followed for collection of the tissue.

5.2.11 Post-study Follow-up

Patients are to be followed according to standard patient care procedures for 6 weeks following the date of the last decitabine dose to monitor for toxicity, survival,

and response. Thereafter, the investigator or designee will record information on subsequent therapy, disease progression, and/or death every 2 months for 2 years after first dose of study drug or until the patient is lost to follow-up or dies.

5.3 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented in the case report forms.

In the absence of treatment delays due to adverse events, treatment may continue indefinitely or until one of the following criteria applies:

- Documented and confirmed disease progression
- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious non-compliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study

Patients who prematurely discontinue treatment for any reason will be followed as indicated in the study calendar.

5.4 Concomitant Therapy and Supportive Care Guidelines

5.4.1 Chemotherapy

Patients cannot receive any chemotherapy within 21 days of study enrollment and must have recovered from any treatment-related toxicities. Patients may have received hydrea prior to enrollment for a WBC > 50,000/μl but must stop hydrea at least one day prior to initiating decitabine. Other than decitabine, patients cannot receive chemotherapy during this trial.

5.4.2 Growth Factors

Erythroid growth factors should not be routinely administered to patients during the study.

Thrombopoietin receptor agonists should not be routinely administered to patients during the study.

Filgrastim (G-CSF) or sargramostim (GM-CSF) may be administered to patients with septic shock or febrile neutropenia at the discretion of the treating physician.

5.4.3 Transfusions

- *Packed red blood cells transfusion*:
  Two units of packed RBCs are to be administered when the hemoglobin drops under 8 g/dL. Transfusion administration when the patient's hemoglobin is 8-10 g/dL may be done according to the treating physician's clinical judgment.

- *Platelet transfusion*:
  One unit of single-donor platelets will be administered when platelets are < 20,000/μL.

5.4.4 Prophylactic Antimicrobial Agents

Antimicrobial agents will be administered at the discretion of the treating physician.

5.4.5 Radiotherapy

Patients cannot receive radiotherapy within 14 days prior to study enrollment or while on study.

6.0 DOSE MODIFICATIONS

6.1 Delay for Cytopenia or Infection

As cytopenias are a defining feature of AML and MDS, their presence alone does not generally warrant dose modifications or delays. Patients will receive four cycles of therapy without adjustment for any cytopenia.

Patients with febrile neutropenia (temperature ≥ 38.5° with ANC < 1,000/μl) or systemic infections should have treatment held until clinical resolution of the infection. A patient will meet criterion for discontinuation of the study if the patient is observed to have a delay of > 8 weeks between any two cycles.

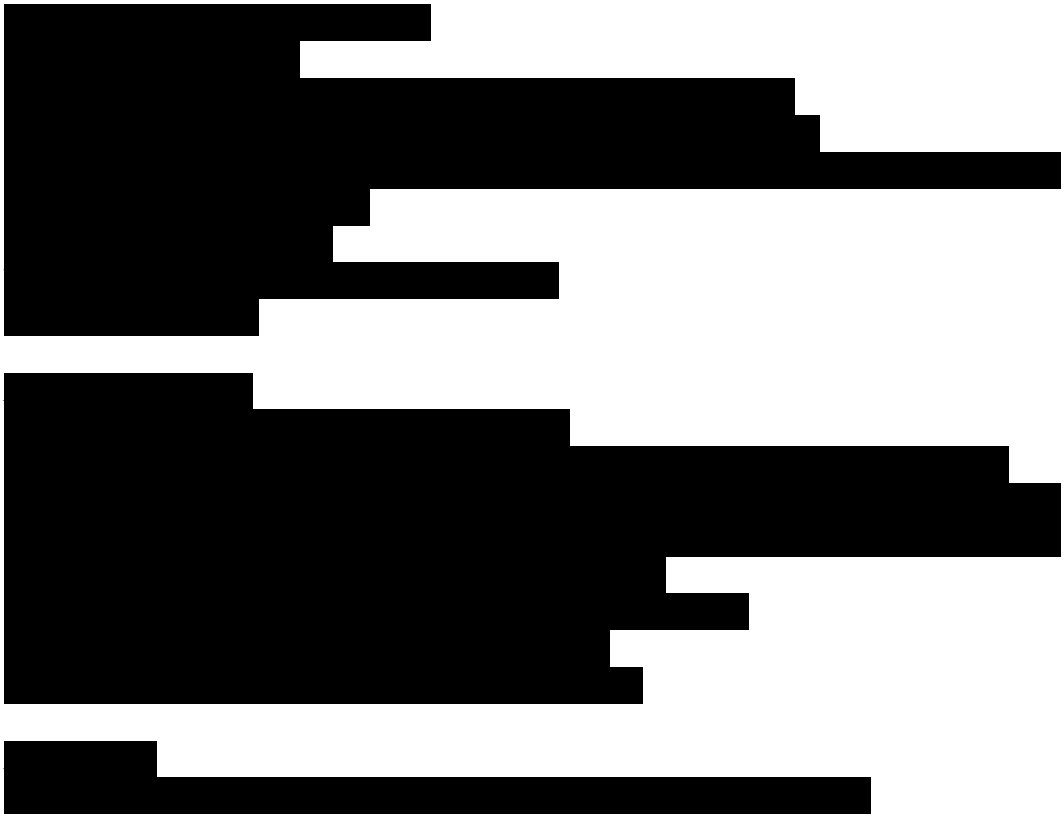
6.2 Delay for Organ Dysfunction

If renal dysfunction (creatinine ≥ 3 X institutional upper limit of normal) or hepatic dysfunction (bilirubin ≥ 2.5 mg/dl or ALT/AST ≥ 3 X institutional upper limit of normal) occurs, therapy may not resume until the abnormalities have resolved to below the limits stated above. If renal or hepatic dysfunction occurs during a treatment course, the next dose of decitabine should be held, and serum chemistries should be checked twice per week. Decitabine can be resumed when creatinine, bilirubin, ALT and AST are below the limits listed above.

For grade 3-4 non-hematologic toxicities, decitabine treatment should be delayed until recovery to baseline or to grade ≤ 1. For toxicities felt to be related to decitabine, decitabine may be reintroduced with a 50% dose reduction, or at the discretion of the investigator.

6.3 Modification in Dose or Schedule

Once patients achieve morphologic complete remission with incomplete blood count recovery (CRi) (morphologic leukemia-free state, defined as less than 5% blasts in aspirate with marrow spicules and a count of ≥ 200 nucleated cells and no blasts with Auer rods, any persistence of extramedullary disease, or persistent pre-treatment morphologic abnormalities, such as pseudo-Pelger-Hüet cells, ringed sideroblasts, or dysplastic megakaryocytes), administration may be decreased to 5 consecutive days to limit myelosuppressive toxicity, per treating physician. After two cycles with bone marrow blast counts < 5% and no evidence of disease by cytogenetics or immunophenotype, patients may further decrease therapy to 3 consecutive days per cycle, per treating physician.



8.0 CORRELATIVE STUDIES SAMPLE COLLECTIONS

Bone marrow samples will be collected at Baseline, on Cycle 1 Day 10 ± 1, and on Day 28 ± 4 of Cycle 1 and of even number cycles for correlative studies. Bone marrow aspirate (6-10 ml) will be collected and divided between two EDTA (lavender top) tubes. The bone marrow samples and peripheral blood samples should be delivered by hand to Sandra McDonald, M.D. PhD, director of the Tissue Procurement Core Facility at the Siteman Cancer Center. At the Tissue Procurement Core Facility, patient identification will be removed from the samples, and the cells will be stored as frozen pellets in DMSO at 10^7 per tube. Subsequently, the samples will be thawed, and cells will be assayed.

Peripheral blood sample (3 mL) will be collected on Cycle 1 Day 4 ± 1. Sample must be obtained 45 minutes ± 15 minutes after beginning the infusion, before the decitabine infusion is complete, and should be obtained from the opposite arm as the infusion. The sample should be collected in a 3 mL K3EDTA tube pre-loaded with 8 mcL of tetrahydrouridine (THU 500 mcg/mL), an inhibitor of cytidine deaminase. The sample should be processed by the Tissue Procurement Core Facility at the Siteman Cancer Center. Serum should be separated and stored per protocols.

9.0 EFFICACY, SAFETY AND CORRELATIVE STUDY MEASURES

A secondary endpoint of this study is to estimate morphologic complete remission rate.

9.1 Response Criteria

Patients will be assessed for response according to the IWG criteria:[1]

Morphologic complete remission (CR) – Defined as morphologic leukemia-free state, defined as less than 5% blasts in aspirate with marrow spicules and a count of ≥200 nucleated cells and no blasts with Auer rods or any persistence of extramedullary disease. When erythroid precursors constitute less than 50% of bone marrow nucleated cells, the percentage of blasts is based on all nucleated cells; when there are 50% or more erythroid cells, the percentage blasts should be based on the nonerythroid cells. The presence of mild megaloblastoid changes may be permitted if they are thought to be consistent with treatment effect. However, persistence of pretreatment abnormalities (eg, pseudo-Pelger-Hüet cells, ringed sideroblasts, dysplastic megakaryocytes) are not consistent with CR. In addition, ANC >1000/µl and platelet count of ≥100,000/µl. Patient must be independent of transfusions. No duration requirement for this designation.

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

Morphologic complete remission with incomplete blood count recovery (CRi) – Defined as morphologic complete recovery with the exception of neutropenia <1000/µl or thrombocytopenia <100,000/µl.

Partial remission (PR) – Requires that the above criteria for complete remission be met, with the following exceptions: decrease of ≥50% in the percentage of blast to 5-25% in the bone marrow aspirate. A value of ≤5% blasts in bone marrow with Auer rods present may also be considered a partial remission.

Treatment failure – Includes all patients who did not achieve a complete or partial remission following 4 cycles of therapy.

Recurrence/morphologic relapse - Relapse following 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. New dysplastic changes are considered a relapse. 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.

9.2 Guidelines for Evaluation of Disease

Bone marrow biopsy should be performed at baseline for both the correlative study and clinical evaluation. Bone marrow biopsy should be performed on Day 28 ± 4 of cycle 1 and on Day 28 ± 4 of each even cycle for clinical disease assessment.

Peripheral blood counts will be followed by weekly CBC with differential and platelets. After the first cycle, this may be changed to every other week or monthly at the discretion of the treating physician.

9.3 Progressive Disease

Progressive disease is defined as a clear increase in bone marrow blast count, typically a greater than 50% increase compared with baseline. Patients with progressive disease will be removed from the study.

Patients may also be removed from the study for progressive disease based on evidence of new extramedullary disease or based on the clinical judgment of the treating physician.

Patients with a > 50% increase in peripheral blast count with an increase of the total peripheral white blood cell count to > 10,000/μl should undergo evaluation by bone marrow biopsy regardless of the cycle number.

9.4 Other Secondary Efficacy Measures

9.4.1 Overall Survival and Event-free Survival

Overall survival is defined as the date of first dose of study drug to the date of death from any cause. Patients still alive at the time of the final analysis will be right-censored.

Event-free survival is defined as the interval from the date of first dose of study drug to date of treatment failure, recurrence, death due to any cause, or loss to follow up.

9.4.2 Duration of Remission and Relapse-free Survival for Patients Having Complete Remission

Duration of complete remission is defined as the interval from the date that complete remission is documented to the date of recurrence, death due to any cause, or loss 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.

9.4.3 Safety

Safety evaluation will include assessments of adverse experiences, medical history, physical examinations, vital signs, and laboratory assessments at baseline and throughout the study period. Local laboratories may be used for this study.

10.0 ADVERSE EVENT REPORTING

10.1 Adverse Events (AEs)

Definition: any unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site: http://ctep.cancer.gov/reporting/ctc.html.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website: (http://www.hhs.gov/ohrp/policy/AdvEvntGuid.htm).

10.2 Unanticipated Problems

Definition:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

10.3 Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

10.4 Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

10.5 Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

Pre-approval of all protocol exceptions must be obtained prior to the event.

10.6 Reporting to the Human Research Protection Office (HRPO) and the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University:

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH or SLCH institution, or that impacts participants or the conduct of the study.
- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within **10 working days** of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification to the PI of the event.

10.7 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. **PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC.** It is the responsibility of the investigator to report any unanticipated problem to the FDA as follows:

- Report any unexpected fatal or life-threatening adverse experiences associated with use of the drug (i.e., there is a reasonable possibility that the experience may have been caused by the drug) by telephone or fax no later than **7 calendar days** after initial receipt of the information. A life-threatening adverse experience is defined as any adverse drug experience that places the subject (in the view of the investigator) at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Report any serious, unexpected adverse experiences, as well as results from animal studies that suggest significant clinical risk within **15 calendar days** after initial receipt of this information. A serious adverse drug experience is defined as any

adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions)
- A congenital anomaly/birth defect
- Any other experience which, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

An unexpected adverse drug experience is defined as any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure (or risk information, if an IB is not required or available).

All MedWatch forms will be sent by the investigator or investigator's team to the FDA at the following address or by fax:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Oncology Drug Products
5901-B Ammendale Rd.
Beltsville, MD 20705-1266
FAX: 1-800-FDA-0178

10.8 Timeframe for Reporting Required Events:

Reportable adverse events will be tracked for 30 days following the last day of study treatment.

Deaths	
Any reportable death while on study or within 30 days of study	Immediately, within 24 hours, to PI and the IRB
Any reportable death while off study	Immediately, within 24 hours, to PI and the IRB
Adverse Events/Unanticipated Problems	
Any reportable adverse events as described in Sections 10.1 and 10.2 (other than death) and 10.7	Immediately, within 24 hours to PI and within 10 working days to the IRB, and within 7 or 15 days to the FDA
All adverse events regardless of grade and attribution should be submitted cumulatively	Include in DSM report
Noncompliance and Serious Noncompliance	
All noncompliance and serious noncompliance as described in Sections 10.3 and 10.4	Immediately, within 24 hours, to PI and within 10 working days to the IRB

11.0 DATA SAFETY MONITORING PLAN

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months after accrual has opened (if at least five patients have been enrolled) or one year after accrual has opened (if fewer than five patients have been enrolled at the six-month mark).

The Principal Investigator will review all patient data at least every six months, and provide a semi-annual report to the QASMC. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual
- Protocol activation date
- Average rate of accrual observed in year 1, year 2, and subsequent years
- Expected accrual end date
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

12.0 STATISTICAL ANALYSIS METHODS AND SAMPLE SIZE

12.1 Analysis Populations

Intent-to-Treat - The intent-to-treat (ITT) population will be defined as all patients who receive at least one dose of decitabine.

Efficacy-Evaluable - The efficacy-evaluable (EE) population will be defined as all patients who receive at least one cycle of treatment.

Safety - The safety population will be defined as all patients with at least one dose of decitabine.

12.2 Statistical Analysis

12.2.1 Descriptive Analyses

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

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.

12.2.2 Primary Endpoint Analysis

The primary end-point of this phase II study is the correlation biomarkers with overall response in AML/MDS patients to decitabine.

We will correlate the presence of patient specific mutations (especially mutations in *DNMT3A, IDH1, IDH2,* and *TET2*) with overall response rates. Mutation rates in these genes and in other patient-specific genes will be correlated with overall response and their respective 95% confidence intervals will be provided.

Statistical considerations. The relationship between AML/MDS-associated mutations and the responsiveness to decitabine will be described by contingency tables and assessed using Chi-square test or Fisher's exact test as appropriate. The differentially expressed genes between responders and non-responders will be determined using the significance analysis of microarray (SAM). Multivariate logistic regression will also be fit to adjust the potential confounding effects of other demographic and clinical characteristics. The resultant critical *P* values will be corrected for multiple testing. Since the Bonferroni correction is known too conservative, a permutation analysis (e.g., 10,000 random assignments for

responsive status) will be used to derive an empirical critical *P* value. The new critical p-value is the number of permutation tests which yield a lower p-value than the original test divided by the total number of permutation tests.[61]

A power analysis was performed using Chi-square test for the association between mutation and response to decitabine. The frequency of *DNMT3A, IDH1/2,* and *TET2* mutations in AML and MDS patients are each between 15% and 30% in multiple cohorts.[29-37] **Table 3** shows the minimal detectable differences of response rates between mutant and wild-type cases, assuming an expected overall 45% CR/CRi rate and 80% power. For an average of 20% frequency of a mutated gene, for example, 100 patients will provide 80% power to detect 30% difference at a 2-sided 0.1 alpha error. The purpose of this study is to determine whether any of these mutations result in a clinically significant association that would warrant pre-treatment testing. We believe that a reasonable cut off would be if at least 30% of clinical response could be attributed to the presence of a specific mutation. However, we anticipate that more power could be achieved as we assess patient groups with combined characteristics (e.g. *DNMT3A* mutation or loss of *DNMT3A* expression). In addition, we expect that the majority of the enrolled 125 patients can be used in the analysis (since all of our molecular information will be acquired within 30 days of enrollment, we anticipate a drop-out rate of 20% or less).

Table 3. Detectable mutation associated response rates (80% power, $\alpha = 0.05$)

Total N	% of patients with a given mutation	Detectable response difference	Sensitivity of mutation to predict response at minimum detectable response difference*
100	15%	0.40	80%
	20%	0.35	75%
	30%	0.30	67%
	40%	0.27	61%
	50%	0.26	58%

* Assuming the overall response rate (CR/Cri) of 45%

12.2.3 Secondary Endpoint Analyses

The overall response rate (CR/CRi/PR) and complete response rate (CR/CRi) will be compared with historical controls[2], and the 95% confidence intervals will be provided.

The rate of patient specific mutation clearance after 10, 30, and 60 days of therapy will be determined using an Illumina based platform. We will compare the rate of mutation clearance between the patients who achieve a CR/CRi after 4 cycles and those who do not. We anticipate that we will identify between 6 and 25 exonic SNVs per case (the range obtained with >200 AML samples sequenced to date with whole genome or exome sequencing), and that the expected 95% confidence

interval of the mean variant allele frequency will be ±2.56% and ±1.25%, respectively, if we assume an anticipated standard deviation of 3.2% within the same clone. The change of tumor burden over time and its association with clinical response will be assessed using 2-way ANOVA for repeated measurement data, followed by the ad-hoc multiple comparisons for between-group differences (e.g., responders vs. non-responders) at given time points as well as the between-time differences within each group. The resultant critical *P* values will also be corrected using permutation analysis for multiple testing.

The steady-state serum decitabine concentration on day 4 ± 1 will be measured and correlated with clinical overall response.

The percent decrease in peripheral blood methylcytosine will be calculated on day 10±1. We will compare this reduction with previously published data and between the patients who achieve CR/CRi and those who do not. The correlation of peak serum levels with response will be assessed using 2-sample t-test or Mann-Whitney non-parametric rank-sum test as appropriate. The change of total bone marrow DNA methylcytosine from baseline and its association with both steady-state serum drug levels and response will be assessed using 2-way ANOVA for repeated measurement data, followed by the ad-hoc multiple comparisons for between-group differences (e.g., responders vs. non-responders) and between-time differences. Similar analyses will be performed for the correlation of expression profiles and SNP analysis with pharmacokinetic data.

Statistical power justification. A power analysis was performed using two-sample t-test for between-group difference and paired t-test for the change over time. For tumor clearance, this will provide > 99% power to detect a 10% difference in the means at two separate time points for clones with 6 or more variants, and > 98% power to detect a 15% difference if there are 3 or more variants per clone with $\alpha = 0.05$ (power will decrease to 91% and 82%, respectively, in cases where the standard deviation is 4.7%, the maximum we have observed thus far). For pharmacologic correlations, we anticipate 85% power to determine a 25 ng/ml difference between the average C_{max} of 45 evaluable responders and 55 evaluable non-responders with an anticipated average C_{max} of 70 ng/ml and a standard deviation of 40 ng/ml within the entire cohort, and with $\alpha = 0.05$.[22] We believe a less significant difference would not be sufficient to warrant application in clinical decision making (i.e. whether to continue therapy for more than 1 cycle). We anticipate 99% power to determine a difference of 20% in the reduction of bone marrow methylcytosine between 45 evaluable responders and 55 evaluable non-responders with an expected standard deviation of 15% and $\alpha = 0.05$.[28, 66-68] The difference in methylcytosine reduction previously observed was 30% between patients achieving CR/PR and those with only hematologic improvement.[28]

Time to relapse and time to death will also be listed for each patient.

12.2.4 Safety Analysis

Extent of Exposure – Duration (number of cycles/infusions) of decitabine doses (cumulative and intensity) will be summarized.

Adverse Events - All adverse events will be coded using NCI CTCAE v. 4.0. 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. In addition, adverse events will be summarized by investigator attribution of relationship to study medication and by grade. Similar summaries will be provided by treatment cycle.

Clinical Laboratory Evaluation – Applicable laboratory parameters will be graded according to NCI CTCAE version 4.0. The incidence of maximum grade (number and percent of patients experiencing the maximum grade during the study) will be summarized for each laboratory parameter. Similar summaries will be provided by treatment cycle. Graphical displays of select hematological parameters will also be provided.

Vital Signs, Physical Findings, and Other Safety Observations – Vital signs, findings from physical examinations, ECOG scores and weights will be presented in listing format.

12.2.5 Description of Planned Subgroup Analyses

The following subgroups (if sufficient numbers of patients) will be explored with respect to the primary and overall survival endpoints.

- Age (60-69 years old vs ≥ 70 years old)
- Cytogenetics
- Patients with AML-associated mutations in *DNMT3A, TET2, IDH1,* or *IDH2* vs those who do not have somatic variants in these genes.

12.3 Sample Size

We anticipate initially enrolling 25 patients at Washington University. Once we establish a routine exome analysis with validation and calculation of the rate of patient-specific mutation clearance, we will enroll an additional 100 patients from at least 2 additional centers.

13.0 DATA SUBMISSION SCHEDULE

Case Report Form	**Submission Schedule**
Original Consent Form	*Prior to registration*
Patient Registration Form *Disease Diagnosis Form* *Inclusion Criteria Worksheet* *Exclusion Criteria Worksheet*	*Prior to starting treatment*
Treatment Record Form	*To be maintained throughout a patient's treatment cycle. The complete record is due at completion of treatment.*
Bone Marrow Sample Collection Form	*To be maintained throughout the course of the study at designated bone marrow sample collections time points (See Appendix 2).*
Pathology: Bone Marrow Biopsy	*To be completed upon the return of pathology report for each bone marrow biopsy.*
Study Calendar Form	*To be completed with the date of each time point upon occurrence.*
Labs Form	*To be completed upon the return of laboratory reports for each study time point (See Appendix 2)*
Dose Modifications	*To be maintained throughout the course of the study if dose modifications are performed.*
Therapy Response Form	*To be filled out upon remission (prior to, and/or on therapy)*
End of Study Form	*Due at the completion of the EOS visit, at the time of death, and/or at the time that the patient is lost to follow-up*
Follow Up Form	• *6 weeks post date of last decitabine dose.* • *Every 2 months for 2 years after the first dose of study drug.*
Record of Adverse Events	*At the time of any AE*

Appendix 1 ECOG Performance Scale

Activity Status	Description
0	Asymptomatic, fully active, and able to carry on all predisease performance without restrictions.
1	Symptomatic, fully ambulatory but restricted in physically strenuous activity and able to carry out performance of a light or sedentary nature, eg, light housework, office work.
2	Symptomatic, ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours: in bed less than 50% of day.
3	Symptomatic, capable of only limited self-care, confined to bed or chair more than 50% of waking hours, but not bedridden.
4	Completely disabled. Cannot carry on any self-care. Totally bedridden.
5	Dead

Appendix 2 Study Flow Chart

Study Flow Chart

Test	Pre-study	Base-line	All Cycles Day 1	All Cycles Cohort A Days 1-10[a, b]	Cycle 1 day 4 ± 1	Cycle 1 Day 10 ± 1 and 28 ± 4	Every week	Every 2 weeks	Even cycles Day 28±4	End of Study
Informed Consent	X									
Medical History		X	X							
Physical Exam		X	X							X
Concomitant Medications		X								
Transfusion Requirements[c]		X[c]								
Bone Marrow Aspirate and Biopsy	X[d,e]					X[d]			X[d]	X[d]
Peripheral blood for correlative studies					X					
Pregnancy test		X								
CBC with differential and platelets		X	X				X[f]			X
Serum Chemistries[g]		X	X					X[f]		X
Decitabine administration[a,b,h]				X						
Adverse Events				Ongoing						
Post-study follow-up										X[i]

[a] Once bone marrow blast count is ≤ 5% (measured by cytomorphology, immunophenotype, and cytogenetics), the number of days per cycle of decitabine may be decreased to 5 per treating physician discretion.
[b] Once bone marrow blast count is ≤ 5% for two or more cycles and the peripheral blood counts have normalized, the number of days per cycle of decitabine may be decreased to 3 per treating physician discretion.
[c] Care should be given to record the frequency of transfusions from 8 weeks prior to first dose of study drug.
[d] Standard bone marrow procurement procedures will be followed for collection of the tissue. Prestudy samples should be collected within 14 days of first dose of study drug.
[e] The bone marrow biopsy for correlative studies may be collected with the prestudy evaluation. If not, it should be done as part of the base-line evaluation
[f] Once stable, these labs may be obtained every other week or once a month at the discretion of the treating physician.
[g] Serum chemistries include bicarbonate, calcium, glucose, total protein, albumin, BUN, potassium, chloride, sodium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, and LDH.
[h] Decitabine treatment will be 20 mg/m^2 over a 1-hour IV infusion on consecutive Days 1-10 every 28-day cycle. The dose will be calculated based on actual patient weight at the beginning of each cycle.
[i] Patients will be followed for 6 weeks following the date of the last decitabine dose to monitor for toxicity, survival and response. Thereafter, the investigator or designee will record information on subsequent therapy, disease progression, and death, every 2 months for 2 years after first dose of study drug or until the patient is lost to follow-up.

References:

1. Cheson, B.D., et al., *Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia.* J Clin Oncol, 2003. **21**(24): p. 4642-9.
2. Cashen, A.F., et al., *Multicenter, phase II study of decitabine for the first-line treatment of older patients with acute myeloid leukemia.* J Clin Oncol, 2010. **28**(4): p. 556-61.
3. Blum, W., et al., *Clinical response and miR-29b predictive significance in older AML patients treated with a 10-day schedule of decitabine.* Proc Natl Acad Sci U S A, 2010. **107**(16): p. 7473-8.
4. Schlenk, R.F., et al., *Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia.* N Engl J Med, 2008. **358**(18): p. 1909-18.
5. Erba, H.P., *Has there been progress in the treatment of older patients with acute myeloid leukemia?* Best Pract Res Clin Haematol, 2010. **23**(4): p. 495-501.
6. Heaney, M.L. and D.W. Golde, *Myelodysplasia.* N Engl J Med, 1999. **340**(21): p. 1649-60.
7. Bennett, J.M., et al., *Proposals for the classification of the myelodysplastic syndromes.* Br J Haematol, 1982. **51**(2): p. 189-99.
8. Kantarjian, H., et al., *Results of intensive chemotherapy in 998 patients age 65 years or older with acute myeloid leukemia or high-risk myelodysplastic syndrome: predictive prognostic models for outcome.* Cancer, 2006. **106**(5): p. 1090-8.
9. Martin, M.G. and C.N. Abboud, *Induction therapy for elderly patients with acute myeloid leukemia.* Blood Rev, 2008. **22**(6): p. 311-20.
10. Bow, E.J., et al., *Therapy of untreated acute myeloid leukemia in the elderly: remission-induction using a non-cytarabine-containing regimen of mitoxantrone plus etoposide.* J Clin Oncol, 1996. **14**(4): p. 1345-52.
11. Goldstone, A.H., et al., *Attempts to improve treatment outcomes in acute myeloid leukemia (AML) in older patients: the results of the United Kingdom Medical Research Council AML11 trial.* Blood, 2001. **98**(5): p. 1302-11.
12. Stone, R.M., *The difficult problem of acute myeloid leukemia in the older adult.* CA Cancer J Clin, 2002. **52**(6): p. 363-71.
13. Liu Yin, J.A., et al., *Mitozantrone and cytosine arabinoside as first-line therapy in elderly patients with acute myeloid leukaemia.* Br J Haematol, 1991. **79**(3): p. 415-20.
14. Stone, R.M., et al., *Granulocyte-macrophage colony-stimulating factor after initial chemotherapy for elderly patients with primary acute myelogenous leukemia. Cancer and Leukemia Group B.* N Engl J Med, 1995. **332**(25): p. 1671-7.
15. Kantarjian, H., et al., *Decitabine improves patient outcomes in myelodysplastic syndromes.* Cancer, 2006. **106**(8): p. 1794-1803.

16. Lubbert, M., et al., *A multicenter phase II trial of Decitabine as first-line treatment of older AML patients judged unfit for induction chemotherapy.* Haematologica, 2011. **97**(3): p. 393-401.
17. Issa, J.P., et al., *Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies.* Blood, 2004. **103**(5): p. 1635-40.
18. Petti, M.C., et al., *Pilot study of 5-aza-2'-deoxycytidine (Decitabine) in the treatment of poor prognosis acute myelogenous leukemia patients: preliminary results.* Leukemia, 1993. **7 Suppl 1**: p. 36-41.
19. Wijermans, P.W., et al., *Continuous infusion of low-dose 5-Aza-2'-deoxycytidine in elderly patients with high-risk myelodysplastic syndrome.* Leukemia, 1997. **11**(1): p. 1-5.
20. Kantarjian, H., et al., *Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study.* Cancer, 2006. **106**(8): p. 1794-803.
21. Wijermans, P.W., et al., *An epigenetic approach to the treatment of advanced MDS; the experience with the DNA demethylating agent 5-aza-2'-deoxycytidine (decitabine) in 177 patients.* Ann Hematol, 2005. **84 Suppl 1**: p. 9-17.
22. Cashen, A.F., et al., *Pharmacokinetics of decitabine administered as a 3-h infusion to patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS).* Cancer Chemother Pharmacol, 2008. **61**(5): p. 759-66.
23. Chabot, G.G., J. Bouchard, and R.L. Momparler, *Kinetics of deamination of 5-aza-2'-deoxycytidine and cytosine arabinoside by human liver cytidine deaminase and its inhibition by 3-deazauridine, thymidine or uracil arabinoside.* Biochem Pharmacol, 1983. **32**(7): p. 1327-8.
24. Momparler, R.L., et al., *Cell cycle effects and cellular pharmacology of 5-aza-2'-deoxycytidine.* Cancer Chemother Pharmacol, 1984. **13**(3): p. 191-4.
25. Raza, A., et al., *Biological significance of cell cycle kinetics in 128 standard risk newly diagnosed patients with acute myelocytic leukemia.* Br J Haematol, 1991. **79**(1): p. 33-9.
26. Brons, P.P., et al., *Proliferation patterns in acute myeloid leukemia: leukemic clonogenic growth and in vivo cell cycle kinetics.* Ann Hematol, 1993. **66**(5): p. 225-33.
27. Raza, A., et al., *Direct relationship between remission duration in acute myeloid leukemia and cell cycle kinetics: a leukemia intergroup study.* Blood, 1990. **76**(11): p. 2191-7.
28. Shen, L., et al., *DNA methylation predicts survival and response to therapy in patients with myelodysplastic syndromes.* J Clin Oncol, 2010. **28**(4): p. 605-13.
29. Tefferi, A., et al., *IDH1 and IDH2 mutation studies in 1473 patients with chronic-, fibrotic- or blast-phase essential thrombocythemia, polycythemia vera or myelofibrosis.* Leukemia, 2010. **24**(7): p. 1302-9.
30. Ley, T.J., et al., *DNMT3A mutations in acute myeloid leukemia.* N Engl J Med, 2010. **363**(25): p. 2424-33.
31. Mardis, E.R., et al., *Recurring mutations found by sequencing an acute myeloid leukemia genome.* N Engl J Med, 2009. **361**(11): p. 1058-66.

32. Abdel-Wahab, O., J. Patel, and R.L. Levine, *Clinical Implications of Novel Mutations in Epigenetic Modifiers in AML.* Hematol Oncol Clin North Am, 2011. **25**(6): p. 1119-33.
33. Reuther, G.W., *Recurring mutations in myeloproliferative neoplasms alter epigenetic regulation of gene expression.* Am J Cancer Res, 2011. **1**(6): p. 752-62.
34. Paschka, P., et al., *IDH1 and IDH2 mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3 internal tandem duplication.* J Clin Oncol, 2010. **28**(22): p. 3636-43.
35. Shen, Y., et al., *Gene mutation patterns and their prognostic impact in a cohort of 1185 patients with acute myeloid leukemia.* Blood, 2011. **118**(20): p. 5593-603.
36. Tefferi, A., et al., *Detection of mutant TET2 in myeloid malignancies other than myeloproliferative neoplasms: CMML, MDS, MDS/MPN and AML.* Leukemia, 2009. **23**(7): p. 1343-5.
37. Walter, M.J., et al., *Recurrent DNMT3A mutations in patients with myelodysplastic syndromes.* Leukemia, 2011. **25**(7): p. 1153-8.
38. Lin, J., et al., *Recurrent DNMT3A R882 Mutations in Chinese Patients with Acute Myeloid Leukemia and Myelodysplastic Syndrome.* PLoS One, 2011. **6**(10): p. e26906.
39. Bejar, R., et al., *Clinical effect of point mutations in myelodysplastic syndromes.* N Engl J Med, 2011. **364**(26): p. 2496-506.
40. Walter, M.J., et al., *Clonal architecture of secondary acute myeloid leukemia.* N Engl J Med, 2012. **366**(12): p. 1090-8.
41. Kantarjian, H.M., et al., *Survival advantage with decitabine versus intensive chemotherapy in patients with higher risk myelodysplastic syndrome: comparison with historical experience.* Cancer, 2007. **109**(6): p. 1133-7.
42. Lubbert, M., et al., *Continued Low-Dose Decitabine (DAC) Is an Active First-Line Treatment in All Cytogenetic Subgroups of Older AML Patients: Results of the FR00331 Multicenter Phase II Study.* ASH Annual Meeting Abstracts, 2007. **110**(11): p. 300-.
43. Blum, W., et al., *Phase I Study of Decitabine Alone or in Combination With Valproic Acid in Acute Myeloid Leukemia.* J Clin Oncol, 2007. **25**(25): p. 3884-3891.
44. Issa, J.-P.J., et al., *Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies.* Blood, 2004. **103**(5): p. 1635-1640.
45. Garcia-Manero, G., et al., *Phase 1/2 study of the combination of 5-aza-2'-deoxycytidine with valproic acid in patients with leukemia.* Blood, 2006. **108**(10): p. 3271-3279.
46. Issa, J.P., S.B. Baylin, and J.G. Herman, *DNA methylation changes in hematologic malignancies: biologic and clinical implications.* Leukemia, 1997. **11 Suppl 1**: p. S7-11.
47. Ganetzky, R.D., et al., *Differences Between Normal and Leukemic Stem Cell-Specific Methylome Indicates Aberrantly Silenced Genes Involved in the*

Pathogenesis of Malignant Evolution. ASH Annual Meeting Abstracts, 2008. **112**(11): p. 599-.

48. Christman, J.K., *5-Azacytidine and 5-aza-2'-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy.* Oncogene, 2002. **21**(35): p. 5483-95.
49. Lubbert, M., *DNA methylation inhibitors in the treatment of leukemias, myelodysplastic syndromes and hemoglobinopathies: clinical results and possible mechanisms of action.* Curr Top Microbiol Immunol, 2000. **249**: p. 135-64.
50. Kantarjian, H., et al., *Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia.* Blood, 2007. **109**(1): p. 52-57.
51. Steensma, D.P., et al., *Multicenter Study of Decitabine Administered Daily for 5 Days Every 4 Weeks to Adults With Myelodysplastic Syndromes: The Alternative Dosing for Outpatient Treatment (ADOPT) Trial.* J Clin Oncol, 2009: p. JCO.2008.19.6550.
52. Cashen, A.F., et al., *Preliminary Results of a Multicenter Phase II Trial of 5-Day Decitabine as Front-Line Therapy for Elderly Patients with Acute Myeloid Leukemia (AML).* ASH Annual Meeting Abstracts, 2008. **112**(11): p. 560-.
53. Jabbour, E., et al., *Evolution of decitabine development: accomplishments, ongoing investigations, and future strategies.* Cancer, 2008. **112**(11): p. 2341-51.
54. Kantarjian, H., et al., *Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia.* Blood, 2007. **109**(1): p. 52-7.
55. Blum, W.G., Klisovic, R., Liu, S., Kefauver, C., Grever, M. R., Schaaf, L., Chan, K., Byrd, J. C., Villalona-Calero, M., Marcucci, G. *Efficacy of a novel schedule of decitabine in previously untreated AML, age 60 or older*. in *ASCO Annual Meeting*. 2009.
56. van Groeningen, C.J., et al., *Phase I and pharmacokinetic study of 5-aza-2'-deoxycytidine (NSC 127716) in cancer patients.* Cancer Res, 1986. **46**(9): p. 4831-6.
57. Rivard, G.E., et al., *Phase I study on 5-aza-2'-deoxycytidine in children with acute leukemia.* Leuk Res, 1981. **5**(6): p. 453-62.
58. Lin, K.T., R.L. Momparler, and G.E. Rivard, *Sample preparation for the determination of 5-aza-2'-deoxycytidine in plasma by high-performance liquid chromatography.* J Chromatogr, 1985. **345**(1): p. 162-7.
59. Momparler, R.L., G.E. Rivard, and M. Gyger, *Clinical trial on 5-aza-2'-deoxycytidine in patients with acute leukemia.* Pharmacol Ther, 1985. **30**(3): p. 277-86.
60. Raska, K., Jurovcik, M., Sormova., Z., *On the metabolism of 5-azacytidine and 5-aza-2'-deoxycytidine in mice.* Coll Czech Chem Commun, 1965. **30**: p. 3001-3005.
61. Momparler, R.L., et al., *Pilot phase I-II study on 5-aza-2'-deoxycytidine (Decitabine) in patients with metastatic lung cancer.* Anticancer Drugs, 1997. **8**(4): p. 358-68.

62. Aparicio, A., et al., *Phase I trial of continuous infusion 5-aza-2'-deoxycytidine.* Cancer Chemother Pharmacol, 2003. **51**(3): p. 231-9.
63. Bryan, J., et al., *Pharmacokinetic evaluation of decitabine for the treatment of leukemia.* Expert Opin Drug Metab Toxicol, 2011. **7**(5): p. 661-72.
64. Scandura, J.M., et al., *Phase 1 study of epigenetic priming with decitabine prior to standard induction chemotherapy for patients with AML.* Blood, 2011. **118**(6): p. 1472-80.
65. Westfall, P.H. and S.A. Young, *Resampling-based multiple testing: examples and methods for p-value adjustment*. 1993, New York: John Wiley & Sons, Inc.
66. Samlowski, W.E., et al., *Evaluation of a 7-day continuous intravenous infusion of decitabine: inhibition of promoter-specific and global genomic DNA methylation.* J Clin Oncol, 2005. **23**(17): p. 3897-905.
67. Blum, W., et al., *Phase I study of decitabine alone or in combination with valproic acid in acute myeloid leukemia.* J Clin Oncol, 2007. **25**(25): p. 3884-91.
68. Yang, A.S., et al., *DNA methylation changes after 5-aza-2'-deoxycytidine therapy in patients with leukemia.* Cancer Res, 2006. **66**(10): p. 5495-503.