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HM-091 A Phase Ib/II Study of the Safety and Activity of Digoxin with Decitabine in Adult AML and MDS

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Schema

Population *up to 100 Patients with newly diagnosed or relapsed AML/MDS who are deemed unfit for standard cytotoxic chemotherapy intervention*

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Treatment ***(28 days per cycle):***

- *Digoxin: 0.5mg PO BID on Day 1 then 0.25mg/d for Days 2-10*
- *Decitabine: 20mg/m^2 IV Days 6-10*

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Disease Assessment

- *Evaluate response after cycle 3 and cycle 6 with Bone Marrow Biopsy*
- *During Cycle 1+2 collect PB samples for correlative studies*

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Continue treatment until:

- *Disease progression*
- *Unacceptable toxicity*
- *Patient preference*
- *Completion of planned 6 cycles of therapy*

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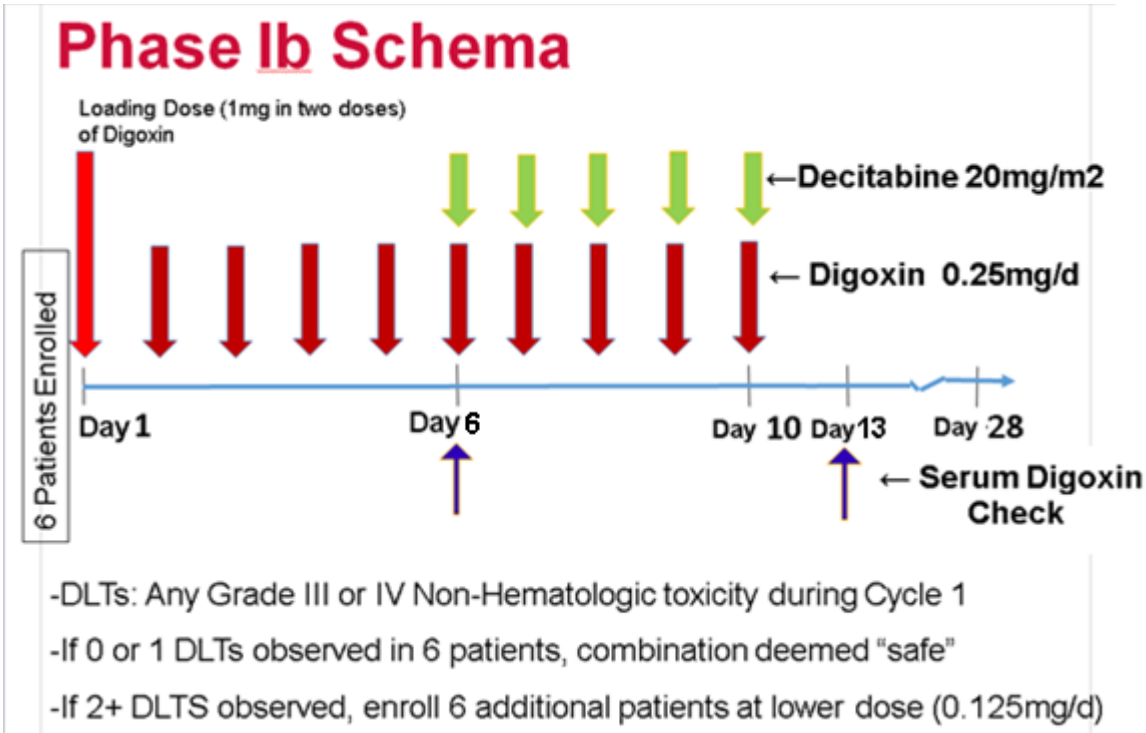
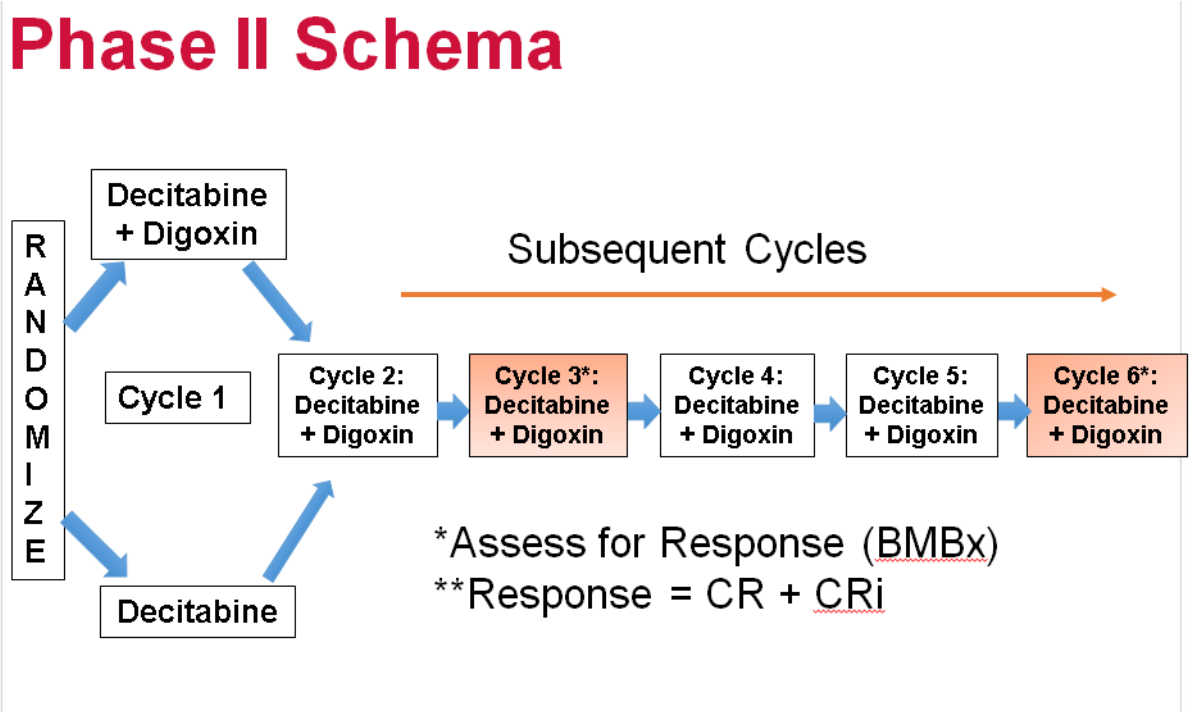
Follow up

Upon treatment discontinuation, patients will be followed up every month for 6 months for disease progression





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1.0 Introduction

1.1. Study Disease

Acute myeloid leukemia (AML)/Myelodysplastic Syndrome (MDS) is a heterogeneous disease group typically treated with chemotherapy and frequently stem cell transplantation. However, a certain percentage of patients are considered "unfit" for intensive induction chemotherapy. Furthermore, despite an excellent response to initial therapy, a significant portion of patients with acute myeloid leukemia [AML] will relapse, either after their initial chemotherapy induction or after stem cell transplantation.[1] In addition, many fail to respond to initial induction therapy and are categorized as "refractory."[4] A retrospective study conducted at the MD Anderson Cancer Center, looking at the outcomes of 1069 who achieved first complete remission [CR] and did not undergo allogeneic stem cell transplantation (SCT), highlighted that the probability of relapse-free survival at 3 years was only 29%.[2] Patients with relapsed or refractory disease are often offered treatment with a hypomethlytaing agent [HMA], such as decitabine or salvage chemotherapy, usually utilizing a cytarabine based regimen.[8,9] Furthermore, for elderly patients stem transplantation is not always a viable option and many are too frail to undergo multiple lines of chemotherapy and they too are offered treatment with HMAs or low dose cytarabine. While these drugs have clinical activity in AML, with reported response rates of 16-60%, neither offers a long term remission or cure.[3,6] This has fueled the search for novel alternative therapeutic agents, ranging from such targeted therapies as FLT3 inhibitors to the novel anti-CD33 monoclonal antibody Gemtuzumab Ozogamicin, new purine analogs such as clofarabine and different iterations of various HMAs.

1.2. Agent under Investigation/intervention

Cardiac glycosides have been shown in vitro to inhibit the differentiation and proliferation of tumor cells via multiple pathways, including promotion of cell cycle arrest and differentiation via epigenetic pathways. Studies using the human leukemic cell line, HL-60, demonstrated that the cardiac glycoside, ouabain, at micromolar concentrations induces leukemic cell apoptosis (as indicated by nuclear fragmentation, reduced nuclear DNA and loss of clonogenic potential).[5] In preliminary studies from our laboratory, the epigenetic activity of digoxin, alone and in combination with decitabine, was investigated utilizing the human cell lines, YB5, HL-60 and K562. In these studies, treatment with digoxin, at 10 uM and 50 uM concentrations, led to increased expression of normally silenced regulatory genes and was associated with increased tumor cell apoptosis.

1.3. Study Rationale

We initially utilized the human colon cancer cell line, YB5, by incubating with a variety of different FDA-approved drugs, obtained from the NCI-Developmental Therapeutics Program, at two dosing schedules (72hr treatment at 10 uM or 24hr at 50uM). The cells were then analyzed for epigenetic changes in tumor suppressor gene expression by flow cytometry to analyze for expression of green fluorescent protein (GFP), which has low baseline expression in YB5 cells. This initial analysis highlighted that digoxin exerts an epigenetic effect on the tumor cells as indicated by the observation of increased GFP expression in cell lines treated with digoxin. Further experiments using specially labeled YB5 cell lines helped to delineate that the epigenetic effect of digoxin, in contrast to that

of HMAs, is modulated by Ca2+ signaling and activation of Calcium/calmodulin-dependent protein kinase (CamK). This highlighted that digoxin exerted its epigenetic effect via a different pathway than traditional epigenetic agents such as HMAs. The pre-clinical studies from our lab suggest that not only does the cardiac glycoside have the ability to alter epigenetic pathways in cancer cells but it also appears to work concurrently with HMAs. Specifically we observed that greater gene expression and cell apoptosis were observed with combinations of digoxin and decitabine than either agent alone.

Our primary hypothesis is that digoxin can be safely added to decitabine and will increase the response rates in medically unfit patients with newly diagnosed AML/MDS or those with relapsed/refractory AML/MDS. Furthermore, we hypothesize that the addition of digoxin to decitabine will result in distinct epigenetic alterations in AML/MDS patients.

The study population will include newly diagnosed and relapsed/refractory AML/MDS patients who are deemed to be unfit for standard induction chemotherapy or further lines of standard therapy. We chose to focus on this group given the ongoing need for new therapeutic options in this group given the low rates of efficacy with current standard treatment options

Our specific endpoints are to assess the safety of digoxin + decitabine in AML/MDS patients and to also assess the epigenetic effects of this combination compared to decitabine alone. In addition, as an exploratory aim, for the three “best” and three “worst” responders, we aim to carry out in-depth epigenetic and gene expression analysis to generate preliminary data on profiles that may predict response to the study regimen. Once differences are identified they will be verified using samples from the other patients enrolled in the trial.

We chose specifically digoxin as our cardiac glycoside for this study because it is readily available for clinical use. Furthermore, there is an extensive cardiology literature on the safe dosing and titration of digoxin in the clinical setting. We chose to utilize the congestive heart failure dosing for this study because it utilizes a fixed dosing schedule and minimizing the risk of having toxic serum levels of digoxin.

With regards to risk and benefits; an HMA is standard of care for patients who fall into our study population and decitabine is commonly utilized in this clinical setting.[7] Patients will be getting the standard therapeutic treatment and in addition will also be getting digoxin, which based on available pharmacological data is not postulated to affect each other’s pharmacokinetics. We will be closely monitoring patient’s serum digoxin levels and EKGs to minimize the risk of patients developing digoxin toxicity while they are on study.

Despite extensive research efforts, there are a limited number of therapeutic options for newly diagnosed and relapsed/refractory AML/MDS patients considered unfit for induction chemotherapy or further lines of chemotherapy. Though stem cell transplantation remains a potential option for some in this group; donor availability and a patient's physical status often limits the accessibility of this option. Therefore, there is an

unmet need to expand the treatment options available in order to provide patients with better disease control and to potentially act as a bridge to a stem cell transplant. Our preliminary in vitro data has already highlighted the epigenetic potential of combining digoxin with decitabine and our goal is to assess the safety and clinical activity of digoxin combined with decitabine. We also hope to generate preliminary data on the epigenetic impacts of this regimen with the future goal of further assessing the epigenetic profiles of a larger cohort of patients treated with this regimen in order to develop a biomarker profile to guide decision making in selecting treatment regimens and improve outcomes for patients with AML. This proposed project strives to increase the number of options available for medically unfit or relapsed/refractory AML patients and additionally will generate additional epigenetic data that can be utilized in the construction of biomarker profiles to guide both treatment choices and insights on clinical outcomes in this difficult to treat group of patients.

1.4. Correlative Testing

1. Genome methylation assessment in AML blasts via LINE-1 methylation status assessment prior to and during therapy.

LINE-1 methylation is an expedient method of assessing global DNA methylation changes. It involves bisulfite treatment of DNA and PCR amplification of repetitive DNA elements (specifically long interspersed nucleotide elements, LINE-1) utilizing LINE-1 specific primers. The results elements are then pyrosequenced. Analysis of these DNA elements, which are normally heavily methylated, serves as a substitute marker for global genomic methylation. [10,11,12]We chose this assay as our method of choice to assess global genomic methylation status based on its relatively quick turn-around time, ease use of the assay and Dr. Issa's lab's expertise in this methodology.

2. Targeted gene methylation and expression assessment of p15, p21, RIL prior to and during therapy.

Decitabine has a clear effect on inducing gene expression of aberrantly silenced genes, which may be boosted by the combinations. To study the effects of the combination (vs. decitabine alone), we will use real-time PCR to measure expression of p15, p21 and RIL, which are known tumor suppressor genes normally hypermethylated in myeloid leukemia. We will also assess the methylation status of p15, p21, RIL genes which are known to be hypermethylated in AML/MDS and are also postulated to have a prognostic impact on outcomes.[8,9] We will utilize GAPDH as a control gene during our analysis. The methylation assessment will be carried out in a manner similar to that described for LINE-1 methylation assessment but will utilize RNA and gene specific primers. The assay methodology chosen is based on that established in the literature for this type of analysis and which Dr. Issa's lab carries out on a regular basis in their pre-clinical studies.

3. For three "best" and three "worst" responders, carry out in-depth epigenetic and gene expression analysis to generate preliminary data on profiles that may predict response to study regimen.

For these patients we will analyse their bone marrow biopsy material collected at pre-study, at the end of cycle 3 and cycle 6 to carry out deep sequencing in order to analyze their DNA methylation profile, transcriptome profile and exome profile. We will determine gene methylation status using the Digital Restriction Enzyme Analysis of Methylation (DREAM) technique, which is a simple, cost effective and quantitative method of determining differences in gene methylation greater than 10-30% with a low false positive rate.[16] This assay was first described in Dr. Issa's lab and its sensitivity, cost-effectiveness and Dr. Issa's expertise makes it an ideal assay for this assessment. Transcriptome profile will be assessed via RNA sequence analysis. This will be carried out based on the standard methodology established at the FCCC Core facility and sample processing will be handled by Dr. Issa's lab. Whole exome profile assessment will be carried out by exome sequencing; which is based on the established literature and will be facilitated by the Otogenetics Company. Finally, once differences in profiles are identified we will attempt to verify our findings by analyzing samples from the other patients enrolled on the trial.

2.0 Objectives

2.1. Primary Objective

1. To assess the maximum tolerated dose (MTD) of digoxin when added to a standard dose of decitabine in patients newly diagnosed AML/MDS or those with relapsed/refractory AML/MDS considered unfit for induction chemotherapy.

2. To assess the safety and efficacy of digoxin when added to a standard dose of decitabine in patients newly diagnosed AML/MDS or those with relapsed/refractory AML/MDS considered unfit for induction chemotherapy.

2.2. Secondary Objectives

1. To determine the in vivo epigenetic effect of digoxin in AML and MDS by assessing overall genome methylation (based on LINE-1 global methylation).

2. To determine gene-specific methylation and expression of tumor suppressor genes normally hypermethylated in myeloid leukemia (p15, p21, RIL).

2.3. Exploratory Objectives

To carry out in-depth epigenetic and gene expression analysis to generate preliminary data on profiles that may predict response to study regimen.

3.0 Study Plan

3.1. Description of Study Design, Population and Duration of Study Therapy

We will conduct a Phase Ib/II clinical trial to determine the safety and clinical activity of digoxin with decitabine. The phase Ib portion of the study will be a safety assessment. All patients will receive a standard dose of intravenous decitabine (20mg/m^2 IV) daily for five total doses and will also receive a dose of digoxin daily, cycled every 4 weeks. Patients will take digoxin for a total of ten days, starting five days prior to their first dose of decitabine and continuing concurrently with each dose of decitabine. The first day of digoxin will consist of a loading dose of digoxin (0.5mg PO BID on day 1) and subsequent doses will be 0.25mg/day PO. Patients will have their digoxin level checked on the first day of decitabine and on day 13 to ensure that serum levels of digoxin are not toxic (>2ng/mL); they will also have an EKG on the first day of digoxin administration and on day 13. During the first cycle the patients will be monitored for dose limiting toxicities (DLTs) which will be defined as any grade III or IV non-hematologic toxicity per the NCI CTCAE v4 guidelines. Initially 3 patients will be enrolled onto the phase Ib study. If 0 or 1 DLTs are observed then an additional 3 patients will be enrolled and monitored for toxicity during their first cycle. If 0 or 1 DLTs are observed in the total 6 patients enrolled, the combination of digoxin plus decitabine will be considered safe and we will proceed to the phase II segment of the study. However, if 2 or more DLTs are observed the dose of digoxin will be decreased to 0.125mg/d (with same loading dose of 0.5mg PO BID on day 1)and the safety study will be repeated with a new group of 6 patients (enrolled as cohorts of 3 patients). If 2 or more toxicities are encountered with the decreased dose then the combination of digoxin and decitabine will be considered too toxic for further study.

Once/if a safe dose is determined we will proceed to the phase II portion of the study. This will utilize a Simon's two stage design for phase II studies. This part of the study will be carried out in parallel with two groups; Group#1 will consist of patients with newly diagnosed AML or MDS. Group#2 will include patients with relapsed/refractory AML or MDS.

For Group#1 our target will be to enroll a total of 37 patients, including the eligible patients originally enrolled in the phase Ib portion (safe dose group) of our study, with the goal of determining the clinical activity of our experimental regimen. In the phase II segment, all new patients who are enrolled will initially be randomized in a 1 to 1 fashion to receive decitabine alone or decitabine plus digoxin for one cycle before receiving decitabine plus digoxin for all subsequent cycles for a total of 6 cycles. Patients will have peripheral blood samples collected (prior to any treatment) on days 1, 6, 13, 20 during the first two cycles and Day 1 of cycle 3 for our biologic correlative and comparative studies. Patients will have a bone marrow biopsy to assess response to therapy at the end of cycle 3 and 6 to determine clinical activity of our proposed regimen, with the marrow after cycle 3 being utilized to determine response rate and the marrow after cycle 6 will be to determine ongoing response to therapy. The study is designed such that patients will receive a total of 6 cycles of the experimental treatment regimen.

For Group#2 our target will be to enroll a total of 60 patients, including the eligible patients enrolled in the phase Ib group. This group will have randomization and treatments similar to Group#1. Samples will be collected for comparative and correlative studies as previously described for Group#1 and response to therapy will be assessed in a similar fashion.

4.0 Patient Selection Inclusion & Exclusion

4.1. Inclusion Criteria

4.1.1 Patients must have a confirmed diagnosis of one of the following:

- Newly diagnosed AML (excluding APL)
- Newly diagnosed intermediate-2 (INT-2) or high-risk MDS
- Relapsed or Refractory AML, or INT-2 or high-risk MDS

4.1.2 For patients with refractory disease they must be at least 4 weeks out from most recent therapeutic intervention.

4.1.3 Age $\geq$ 18 years.

4.1.4 ECOG performance status 0 – 2.

4.1.5 Patients must have normal organ function as defined below:

• Total bilirubin	within normal institutional limits
• AST/ALT (SGOT/SGPT)	$\leq$ 2 times institutional normal limits
• Creatinine	within normal institutional limits
	OR
• Creatinine clearance	$\geq$ 60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal

4.1.6 Ability to understand and willingness to sign a written informed consent and HIPAA consent document.

4.1.7 Agreement on the part of any male participant to use effective contraception during sexual activity throughout the duration of treatment and for 2 months after discontinuation, for protection against the risk of embryofetal toxicity.

4.2. Exclusion Criteria

4.2.1 Patients who have had chemotherapy or radiotherapy within 4 weeks prior to entering the study or those who have not recovered from adverse events (less than or equal to Grade 1 toxicity) due to agents administered more than 4 weeks earlier.

4.2.2 Patients receiving any other investigational agents.

4.2.3 Patients with known brain metastases, active infection, or untreated CNS leukemia.

4.2.4 Patients with prior or current history of digoxin exposure.

4.2.5 Patients requiring treatment with one or more medications known to interact adversely with digoxin, namely thiazide and/or loop diuretics, quinidine, ritonavir, amiodarone, cyclosporine, itraconazole, propafenone, spironolactone, verapamil.

4.2.6 Patients requiring treatment with one or more beta-blockers (metoprolol, atenolol, propranolol) or calcium channel blockers with AV-nodal blocking activity (verapamil, diltiazem). Patients being treated with AV nodal blocker (β-blocker or calcium channel blocker) are allowed if the agent is being used only for correcting hypertension, and if an acceptable alternative is available (for example, transitioning from a drug such as atenolol to Lisinopril or amlodipine) prior to starting treatment on therapy.

4.2.7 Patient with history of prior exposure to decitabine.

4.2.8 Patients eligible for intensive induction chemotherapy and "Medically unfit" based on a TRM score ≥ 13.1*

* TRM Score= A scoring model which predicts early death following intensive induction chemotherapy in newly diagnosed AML.
-Model looks at ECOG PS, Age, Platelet Count, Albumin, 2nd AML, WBC, % Peripheral Blasts, Creatinine
-Score above 13.1 associated with 31%+ chance of death after induction

4.2.9 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

4.2.10 Known HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with **digoxin.** In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy.

4.2.11 Pregnant or breast feeding. Refer to section 4.4 for further detail.

4.3. Inclusion of Women and Minorities

Men and women, regardless of race, ethnic group or sexual orientation are eligible for this study.

4.4. Pregnancy

The effects of digoxin on the developing human fetus at the recommended therapeutic dose are unknown. For this reason and because hypomethylating agents like decitabine used in this trial are known to be teratogenic, women of child-bearing potential (WOCBP) and men must agree to use adequate contraception (hormonal or barrier method of birth

control; abstinence) prior to study entry, for the duration of treatment, and for at least 3 months after the completion of treatment. Should a woman become pregnant or suspect she is pregnant while participating in this study, she must inform her treating physician immediately.

Prior to study enrollment, WOBCP must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy. In additional, men enrolled on this study should understand the risks to any sexual partner of childbearing potential.

All WOCBP must have a negative pregnancy test within *72 hours* prior to receiving the first dose of the investigational agent(s). If the pregnancy test is positive, the patient must not receive protocol treatment and must not be enrolled in the study.

WOCBP is defined as follows: Any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or a bilateral oophorectomy) or is not postmenopausal (defined as amenorrhea $\geq$ 12 consecutive months, or women on hormone replacement therapy (HRT) with documented plasma follicle-stimulating hormone (FSH) level > 35 mIU/ml). Even women who are using oral, implanted, or injectable contraceptive hormones or mechanical products (diaphragm, condoms, spermicides) to prevent pregnancy or practicing abstinence or where partner is sterile (e.g. vasectomy), should be considered to be WOCBP.

4.5. Patient Registration

Participants may be registered from 8:00 am to 4:00 pm EST excluding holidays by emailing the Investigator-Sponsored Research Unit (ISRU) at: **FCCC.MONITOR@fccc.edu.** Eligible participants will be entered on study centrally once the following items have been received by email:

- Completed registration form
- Consent and HIPAA signature pages
- Eligibility checklist

Following registration, participants must begin protocol treatment within 14 calendar days of registration. Issues that would cause treatment delays must be discussed with the Sponsor-Investigator. If a registered participant does not receive protocol therapy following registration, the participant will be recorded as withdrawn from study. The Study Monitor must be notified as soon as possible if a participant does not begin protocol treatment as scheduled. For additional registration questions, please email **FCCC.MONITOR@fccc.edu**

The FCCC ISRU will notify the site by email once registration is confirmed and the sequence number has been assigned. Participants must be registered and have received a sequence number prior to the initiation of treatment.

Exceptions to the current registration policies will not be permitted.

5.0 Treatment Plan

Patients will be stratified by diagnosis at time of study enrollment. Treatment will be administered on an outpatient basis, and will proceed for a planned total of 6 cycles in the absence of any event or delay as detailed below in section 5.3. During the phase II portion of the study, randomization to standard decitabine versus digoxin will dictate treatment only for the first cycle, after which both study arms will receive combined digoxin plus decitabine for the remainder of the cycles as depicted in the phase II schema on page 4.

Treatment will be administered during each respective cycle as described below; with initial loading of digoxin given as two 0.5mg doses on Day 1. Dose delays and modifications will only be done following protocol guidelines described. Missed days will not be made up. If treatment delays are ≥ 4 weeks study therapy will be discontinued.

5.1. Treatment Administration

Regimen description					
Agent	***Premedications, precautions***	***Dose***	***Route***	***Schedule***	***Cycle Length***
Decitabine	Premedicate with Zofran 16mg IV prior to decitabine	20 mg/m^2 in 100 cc NS	IV over 1 hrs	Days 6-10,	4 weeks (28 days)
Digoxin	N/A	1mg Loading Dose *(administered as two 0.5mg doses)* 0.25mg	PO PO	Day 1 Of each cycle of Phase 1b and Phase II Days 2-10	

5.2. Concomitant Medications, Supportive Care, Excluded Therapies and Restrictions

The investigator will be permitted to prescribe supportive treatment(s) at his or her discretion. Appropriate hydration and supportive care (eg, antiemetics and blood and platelet transfusions) may be administered according to study center standards. Aggressive surveillance, prophylaxis, and the treatment of bacterial, fungal, viral, and opportunistic infections are essential to prevent morbidity and mortality. Any supportive treatment or infusion should be recorded in the patient record.

Antibiotics may be utilized to prevent or manage febrile neutropenia based on institutional standard practice. Febrile neutropenia is defined as temperature at least 38.5ºC when the ANC is < 1000 μL. Febrile subjects are to be evaluated by physical examination, CBC

with differential, and blood culture. Subjects with febrile neutropenia or suspected sepsis on the basis of the physical examination are to be hospitalized for appropriate broad spectrum antibiotic coverage, consistent with local pathogen sensitivities.

Prohibited concomitant therapies while on study are: radiation therapy, chemotherapy, immunotherapy, hematopoietic growth factors unless for emergency use or if judged by the investigator to be clinically necessary (erythropoietin can be used after Cycle 1), or any experimental therapy.

In vitro studies in human liver microsomes suggest that decitabine is unlikely to inhibit or induce cytochrome P450 enzymes. However based on standard guidelines the combination of decitabine with clozapine or dipyrone should be avoided due to increased risk for agranulocytosis and pancytopenia.

For Digoxin, standardized precautions should be taken to avoid concomitant administration of digoxin with a variety of drugs without close supervision by the treating physician.

5.3. Duration of Therapy

In the absence of treatment delays due to adverse events, treatment may continue for 6 cycles or until one of the following criteria applies:

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse events
- Patient required > 3 dose reductions of decitabine or >1 dose reductions of digoxin
- Treatment held ≥ 4 Weeks
- Patient becomes pregnant
- Patient decides to withdraw from the study or
- General or specific changes in the patient's condition that render the patient unacceptable for further treatment in the judgment of the investigator

5.4. Duration of Follow up

Patients will be followed every month for 6 months upon discontinuation of study treatment for disease assessment and survival. Patients removed from study for unacceptable adverse events that are related to the study treatment will be followed until resolution or stabilization of the adverse event.

5.5. Criteria for Discontinuation

Patients will be removed from study when any of the criteria listed in Section 5.3 applies. The reason for study removal and the date the patient was removed must be documented in the source documents and case report form.

6.0 Dose Modifications

6.1. General Principles

Dose modifications will be made based only on the guidelines described in Section 6.0. Dose reductions are permanent, there are no dose re-escalations. Patients requiring > 3 dose reductions of decitabine or >1 dose reductions of digoxin must discontinue protocol treatment. Missed doses are not to be made up. Patients requiring treatment to be held > 4 weeks for recovery from toxicity must discontinue protocol treatment.

6.2. Dose Level Adjustment Table [Dose Adjustment for Subsequent Cycle]

During the phase 1b portion of the study:

Dose level	**Digoxin Dose**
-3 dose level	n/a
-2 dose level	n/a
-1 dose level	0.125mg/d
0 (baseline)	0.25mg/day

During the phase II portion of the study:

Dose level	**Decitabine Dose**	**Digoxin Dose**
-3 dose level	5 mg/m2	n/a
-2 dose level	10 mg/m2	n/a
-1 dose level	15 mg/m2	n/a
0 (baseline)	20 mg/m^2	0.25 or 0.125 mg/day

6.3. Specific Toxicities and Modifications

6.3.1. Decitabine

If myelosuppression is present, subsequent treatment cycles of decitabine will be delayed until there is hematologic recovery (ANC ≥ 1,000/μL platelets ≥ 50,000/μL). Once hematologic recovery has been achieved the dose of decitabine will be decreased by one dose level for the subsequent cycle.

During the Phase II portion of the study; if grade III or IV non-hematologic toxicities are encountered, further treatment will be delayed until patients have recovered to grade 0 or 1 before initiation of further treatment. At time of start of next course of treatment, the dose of decitabine will be decreased by 1 dose level.

6.3.2. Digoxin

If 2 or more DLTs are encountered during the phase 1b part of the study, the dose of digoxin will be decreased to 50% of starting dose and the safety study will be repeated with a new group of 6 patients.

For the Phase II portion of the study no digoxin dose modifications will be made for toxicities encountered, all modification will be made with decitabine dose as appropriate.

At time of serum digoxin check, if serum digoxin is at upper limit of normal (normal range: 0.8-2 ng/mL) then subsequent doses for cycle will be held.

6.4. Definition of Dose Limiting Toxicities (DLTs):

Dose limiting toxicities (DLTs) will be defined as any grade III or IV non-hematologic toxicity per the NCI CTCAE v4 guidelines during Phase Ib and cycle 1 of Phase II. Also qualifying as a DLT will be grade IV neutropenia lasting for 28 days or longer from the start of any given cycle of therapy, in the absence of evidence of active AML/MDS and any treatment-related deaths.

7.0 Study Agent Information

7.1. Decitabine Formulation, Product identification, Package and Labeling

7.1.1. Product description:

Antimetabolite, DNA hypomethylating agent
Molecular Formula: $C_8H_{12}N_4O_4$ M.W.: 228.21 g/mol

7.1.2. Availability:

Decitabine is commercially available and FDA approved for MDS. The literature supports its use in AML as well.

7.1.3. Solution preparation:

When reconstituted with 10 mL of sterile water for injection each mL will contain 5 mg of decitabine, 6.8 mg of KH_2PO_4, and approximately 1.1 mg NaOH. The pH of the resulting solution is 6.5 - 7.5. The reconstituted solution can be further diluted to a concentration of 1 mg/mL or 0.1 mg/mL in cold infusion fluids (0.9% Sodium Chloride Injection USP, 5% Dextrose in Water Injection, USP, or Lactated Ringer's Injection USP).

7.1.4. Storage requirements:

The intact vials should be stored under refrigeration (2-8°C; 36-46°F) in the original package.

7.1.5. Stability

The intact vials are stable for at least 1 year at room temperature (22-25°C), 2 years at 2-8°C or 6 months at -40° C. Reconstitution and dilution of the powder for injection (with 10mL of sterile water for injection) results in a rapidly decomposing solution. The concentration of decitabine in the reconstituted and diluted solution decreases about 10 % after 4 hours at 25°C or about 10% after 24 hours at 4°C. Since 10% is the maximum allowable decomposition, and the solution will also decompose during administration (infusion), the solution will be prepared just prior to administration. If this is not possible the solution should be prepared at least twice a day and kept in a refrigerator (2-8°C) until administration. Furthermore, the solution will be prepared only with cold infusion fluids at a temperature of 2-8°C; (36-46°F). This solution can be infused over a maximum period of 3 hours.

7.1.6. Route of administration:

Intravenous

7.2. Digoxin Formulation, Product Identification, Package and Labeling

7.2.1. Product description:

Antiarrhythmic Agent, Miscellaneous; Cardiac Glycoside
Molecular Formula: C14H64O14 M.W.: 780.94 g/mol

7.2.2. Availability:

Commercially available

7.2.3. Storage requirements:

Store at controlled room temperature, 25 degrees C (77 degrees F); range of temperatures where it can be stores is 15 to 30 degrees C (59 to 86 degrees F). Protect from light and from moisture.

7.2.4. Stability:

Stable at above noted temperature range and when protected from light and moisture

7.2.5. Route of administration:

PO

8.0 Correlative /Special Studies

8.1. Study Correlative #1: Assessment of genome wide methylation before and during therapy.

- This will be done at Dr. Issa's lab at Temple University.
- This will be carried out via bisulfite/PCR/pyrosequencing analysis of LINE1 methylation of peripheral blood samples collected (prior to any treatment) on days 1, 6, 13, 20 during the first two cycles and day 1 of cycle 3.

- All bisulfite pyrosequencing analyses will be done in triplicate for each sample and the methylation degree will be computed as the average of the three measurements. To avoid small changes related to batch effects and PCR biases, all samples from each patient will be analyzed in the same run. We will express changes in DNA methylation after treatment as relative change (%), computed by the following formula: 100*([Methylation on a given post-treatment day] – [Baseline methylation])/[Baseline methylation].

8.2. Study Correlative #2: Assess gene-specific methylation and expression of tumor suppressor genes normally hypermethylated in myeloid leukemia (p15, p21, RIL) before and during therapy.

- This will be done at Dr. Issa's lab at Temple University.
- We will utilize real-time PCR to measure expression of a predefined set of genes of interest (*p15, p21, RIL).*
- Will carry out bisulfite/PCR/pyrosequencing analysis to determine methylation status of the above mentioned genes of interest.
- All analysis will be carried out on peripheral blood samples collected (prior to any treatment) on days 1, 6, 13, 20 during the first two cycles and day 1 of cycle 3.

8.3. Study Correlative #3: In-depth epigenetic and gene expression analysis of three "best" and three "worse" responders to generate preliminary data on profiles that may predict response to study regimen.

- For the three best and three worse responders we will analyse their bone marrow biopsy material collected at pre-study, at the end of cycle 3 and cycle 6 to carry out deep sequencing in order to analyze their DNA methylation profile, transcriptome profile and exome profile.
- We will determine gene methylation status using the Digital Restriction Enzyme Analysis of Methylation (DREAM) technique. This assay will be performed at Dr. Issa's lab.
- Transcriptome profile will be assessed via RNA sequence analysis. This will be carried out based on the standard methodology established at the FCCC Core facility and sample processing will be handled by Dr. Issa's lab.
- Whole exome profile assessment will be carried out by exome sequencing; which is based on the established literature and will be facilitated by the Otogenetics Company. Dr. Issa's lab will do sample processing prior to shipping the samples to the company for analysis. Finally, once differences in profiles are identified we will attempt to verify our findings by analyzing samples from the other patients enrolled on the trial.

8.4. Instructions for collection and shipping of blood samples and bone marrow biopsies.

8.4.1. Blood Sample Collection:

For each patient, peripheral blood samples will be collected (prior to any treatment) on days 1, 6, 13, 20 during the first two cycles and day 1 of cycle 3.

- 2 vials of venous blood 12ml EDTA purple top tubes
- 1 vial of venous blood in PAX Gene RNA tube

8.4.2. Bone marrow biopsy

Bone marrow biopsies from the three best and three worse responders

- 1 vial of bone marrow 12ml EDTA purple top tubes
- 1 vial of bone marrow in PAX Gene RNA tube

Blood and biopsy samples will be shipped to Dr. Issa's lab at Temple University. All samples will be anonymized and distributed based on their sequence number.

9.0 Study Calendar

	Pre-Study [A]	Cycle 1 and 2				Cycle 3				Cycle 4 -6				Biopsy Visit	End of Treatment	Safety Follow up	Follow up
Days	-28 to 1	1	6	13	21	1	6	13	21	1	6	13	21	7 days Post cycle 6		30 days post EOT	
Informed Consent	X																
Medical History	X																
Physical exam	X	X	X	X	X	X	X	X	X	X	X	X	X		X		
Vital signs [C]	X	X	X	X	X	X	X	X	X	X	X	X	X		X		
Pregnancy test [D]	X																
CBC	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X[F]
CMP [B]	X	X	X	X	X	X	X	X	X	X	X	X	X		X		
LDH	X																
EKG	X	X		X		X		X		X		X			X		
ECOG	X	X	X	X	X	X	X	X	X	X	X	X	X		X		
Bone marrow biopsy[E]	X					X								X			
Blood draw		X	X	X	X	X											
PO Digoxin [H]		X	X			X	X			X	X						
IV Decitabine [I]			X				X				X						
Concomitant medication		X	X	X	X	X	X	X	X	X	X	X	X		X		
Serum digoxin			X	X			X	X			X	X					
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X		X	X	
Survival status																	X[G]

A. Pre-study H&P, all labs and EKG must be < 28days prior to study drug initiation. Pre-study assessments may be used for C1D1 assessments if completed < 7days of treatment initiation.

B. Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium
C. Vital signs consist of temperature, pulse, respiration, and blood pressure.
D. Serum pregnancy test (women of childbearing potential) must be completed < 72 hours before treatment initiation.
E. Bone marrow biopsy will be performed Pre-study, at the end of cycle 3 and 6. Pre-study bone marrow should be done at least a week in advance of starting trial, Cycle 3 bone marrow should be done 1-2 days prior to start of cycle 4 and cycle 6 bone marrow should be done 1 week after Day 28 of Cycle 6.
F. Upon treatment discontinuation, patients will be assessed every month for 6 months for disease progression.
G. Follow up every month for 6 months for survival.
H. Digoxin given daily Day 1-10; on Day 1 will get 0.5mg PO BID as a loading dose, on Days 2 – 10 will get 0.25 mg QD. This regimen will be followed for all the cycles where Digoxin plus decitabine is given in both Phase 1b and Phase II.
I. Decitabine administered as IV infusion Day 6-10

10.0 Adverse Events

10.1. Definitions

10.1.1. Adverse Events (AE)

Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, treatment, or procedure regardless of whether it is considered related to the medical treatment or procedure (NCI CTEP Guidelines March 28, 2011).

10.1.2. Serious Adverse Event (SAE)

Serious Adverse Event (SAE) is an AE that results in the following outcomes-

- Death
- Life threatening adverse event
- Requires inpatient hospitalization or prolongation of existing hospitalization (for > 24 hours),
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions,
- Congenital anomaly/ birth defect.

A **"life-threatening**" adverse event places the patient at immediate risk of death in the judgment of the investigator or sponsor.

NOTE: Important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent any of the above outcomes.

10.1.3. Severity Rating

The investigator will evaluate the severity of each adverse event. NCI Common Terminology Criteria for Adverse Events (CTCAE v.4.0) or study specific toxicity tables provided in the protocol define severity. If not included in CTCAE v.4.0, severity is expressed in numerical grade using the following definitions:

- Grade 1: Mild-asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate-minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental ADL.
- Grade 3: Severe-severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
- Grade 4: Life-threatening consequences; urgent intervention indicated.

- Grade 5: Death related to AE.

10.1.4. Attribution/Relationship to study drug

- Definite – clearly related
- Probable – likely related
- Possible – may be related
- Unlikely – doubtfully related
- Unrelated – clearly not related

10.1.5. Expectedness

An Expected Adverse Event is one where the specificity or severity is consistent with the current information available from the resources.

An Unexpected Adverse Event is one where the nature, severity, or frequency of the event is related to participation in the research is not consistent with either:

1. The known or foreseeable risk of adverse events associated with the procedures involved in the research that are described in (a) the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document, and (b) other relevant sources of information, such as product labeling and package inserts: or

2. The expected natural progression of any underlying disease, disorder, or condition of the subject (s) experiencing the adverse event and the subjects(s) predisposing risk factor profile for the adverse event.
(OHRP Guidance on reviewing unanticipated problems 2007).

10.2. Recording and Reporting Responsibilities

10.2.1. Investigative Site Recording Responsibilities:

1. Upon identification of an AE or SAE, the site investigator will utilize the above definitions to properly classify the event. Each category listed above must be recorded for each event.

2. All AEs and SAEs will be recorded in the "AE case report forms" (CRF) and in progress reports with details about the grade and attribution of each episode, action taken with respect to the study drug, and the patient's outcome will be recorded in the CRF. All events will be recorded on case report forms for the duration of the study until they resolve.
3. All SAEs will be recorded on the FDA MedWatch form 3500a. The attribution and expectedness must be recorded on the MedWatch form. If this information is not available at the time of initial reporting, a final report must

be documented with attribution and expectedness. It may be necessary to submit follow up reports to the Sponsor should the event require further investigation. All subsequent SAEs must be recorded for up to 30 days after the last treatment.

4. All patient data must be recorded in eCRFs by the investigative site within 7 days of the patient's visit. eCRFs will be available through Oncore electronic data capture system.

10.2.2. Investigative Site Reporting Responsibilities:

1. The investigator/ site is responsible to report all SAEs that occur on or after the first day of study treatment to the sponsor within 24 hours of becoming aware of the event. All subsequent SAEs must be reported for up to 30 days after the last treatment.

2. Each investigator is responsible to report all AEs/SAEs to their local IRB following guidelines set by that IRB. The FCCC OCR reserves the right to request an event be reported to the IRB at their discretion. Copies of events reviewed by the IRB must be sent email to **SAE.FCCC@fccc.edu.**

3. If the investigator or IRB feels the event warrants a revision to the informed consent that was not already initiated by the ISRU, draft revisions will be made in track changes and submitted to the ISRU for consideration. Any consent revisions must receive ISRU approval **prior** to submission to the IRB.

4. Any investigator who is in doubt of whether a particular AE needs to be reported is directed to call the Study Monitor for confirmation with the Sponsor Investigator.

5. If the results of an investigator or ISRU investigation show an adverse event not initially determined to be reportable is so reportable, the investigator will report the event following the above guidelines based on the date the determination is made.

6. Copies of all related correspondence and reporting documents must be submitted to the ISRU and will be maintained in the trial master file.

Participating sites will report events to:

Investigator-Sponsored Research Unit
Office of Clinical Research
Fox Chase Cancer Center
SAE.FCCC@fccc.edu

10.2.3. Sponsor Reporting Responsibilities:

1. Adverse events which meet **all of the following criteria** must be reported to all participating institutions for IRB submission within 2 weeks of notification of the event:

   a. Unexpected (in terms of nature, severity, or frequency) given
      i. (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and
      ii. (b) the characteristics of the subject population being studied;
   b. Possibly related to participation in the research (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
   c. Serious (refer to above definition) or otherwise one that suggests that the research places subjects or others at a greater risk of physical or psychological harm than was previously known or recognized.

2. If the adverse event requires modification of the study protocol and informed consent, these changes will be provided to all participating institutions in the form of an amendment from the ISRU for each site's IRB of record along with the report of the adverse event.

3. All participating sites must be notified of all amendments approved by FCCC IRB; or if the study is suspended such that the participating sites cannot accrue patients in the study until further notification.

4. All participating sites must be notified when the study is closed by IRB.

5. Copies of all related correspondence and reporting documents will be maintained in a centralized regulatory file for this study at ISRU.

6. SAEs that are related, unexpected, fatal, and life-threatening are reportable through the Food and Drug Administration (FDA) MedWatch program by telephone or fax no later than 7 calendar days after initial receipt of the information. Further information on the timing of submissions as directed by FDA guidelines can be found in the following link:

   (https://www.fda.gov/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/InvestigationalNewDrugINDApplication/ucm362555.htm).

   Serious, unexpected events that suggest significant clinical risk will be submitted to within 15 calendar days after initial receipt of this information.

   The FAX number for reporting SAE to FDA can be found in the FDA "Study may proceed" letter.

The SAE report can also be submitted by email to the Regulatory Project Manager and/or the Chief, Project Management Staff described in the "Study May Proceed" letter

10.3. Pregnancy

All WOCBP should be instructed to contact the Investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation.

In the event of a confirmed pregnancy in a patient participating in the study, the Investigator must immediately notify the Fox Chase Cancer Center Study Monitor who will notify Dr. Philip Pancari.

11.0 Measures of Effect

International Working Group (IWG) standardized response criteria for MDS and AML will be utilized to objectively assess response to our experimental therapeutic regimen.[13,14] Assessments will be performed after cycles 3 and 6 of therapy via analysis of peripheral blood counts and bone marrow biopsy. Once protocol treatment has been completed patients will be assessed at the end of the trial and every month for 6 months.

11.1. Definitions

Evaluable for adverse events. All patients will be evaluable for adverse events from the time of their first treatment with Digoxin + Decitabine.

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

11.2. Disease Parameters

Disease status will be based on analysis of patient's bone marrow and patient's peripheral blood counts per IWG criteria.

11.3. Methods for Evaluation of Measurable Disease

Evaluation of disease will be based upon assessment of peripheral blood counts and assessment of patient's bone marrow prior to start of the clinical trial and at predefined points during the course of the trial.

11.4. Response Criteria

11.4.1. PR/CR/CRi/CRp/HI

These variables will be assessed per standard guidelines (refer to Table 1 and Table 2 below).

Table 1: IWG MDS Response Criteria[13]

Complete Response (CR): the following for 4 weeks		
Peripheral:	normal peripheral counts with persistent granulocyte count ≥ 1.0 X 10^9/L, platelet count ≥ 100 X 10^9/L	
Marrow:	normal bone marrow with persistent marrow blasts ≤ 5%; persistent dysplasia will be noted	
Partial Response (PR): the following for 4 weeks		
Peripheral:	normal peripheral counts with granulocyte count ≥ 1.0 X 10^9/L and platelet count ≥ 100 X 10^9/L	
Marrow:	normal bone marrow with marrow blasts > 5% but were reduced by 50% or more	
Marrow Complete Response (mCR): the following for 4 weeks		
Reduction of bone marrow blasts to ≤ 5% without normalization of peripheral counts		
Hematological Improvement (HI): lasts at least 8 weeks*		
Erythroid Response (HI-E):	*Major Response:*	hemoglobin increase ≥ 1.5 g/dL or RBC transfusion independence
Platelet Response (HI-P):	*Major Response:*	absolute increase of platelet count from <20 to > 20 X 10^9/L and by at least 100%, or if more than 20 X 10^9/L, by an absolute increase of at least 30 X 10^9/L
Neutrophil Response (HI-N):	*Major Response:*	granulocyte increase ≥ 100%, and by an absolute increase ≥ 0.5 X 10^9/L

*Abnormal baseline counts were the averages of at least two measurements over at least one week prior to therapy, not influenced by transfusions.

Table 2: IWG AML Response Criteria[14]

Response	Peripheral Blood	Bone Marrow
CR	ANC > 1.0 x 10^9/L, Platelets ≥ 100 x 10^9/L, no blasts, independence from red cell and platelet transfusions over the past week	<5% blasts
CRp	ANC > 1.0 x 10^9/L, Platelets < 100 x 10^9/L, no blasts, independence from red cell transfusions over the past week	<5% blasts
CRi	ANC < 1.0 x 10^9/L, no blasts	<5% blasts
PR	ANC > 1.0 x 10^9/L, Platelets ≥ 100 x 10^9/L, no blasts	Decrease of ≥ 50% in blasts to level of 5% to 25%

ANC = absolute neutrophil count; CR = complete remission; CRp = complete remission with incomplete platelet recovery; CRi = CR with incomplete blood count recovery; PR = partial remission

11.4.2. Progressive Disease (PD)

In AML it is defined as lack of any response after 3 Treatment cycles, or reappearance of blasts post CR. In MDS it is defined as doubling in bone marrow blasts from baseline to at least 10% or progression to AML.

11.4.3. Stable Disease (SD)

Defined as failure to achieve at least a PR but without progression of disease for 8+ weeks.

11.5. Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

12.0 Statistical Considerations

12.1. Study Design/Endpoints

The phase Ib portion of this aim is a safety analysis. As previously described, all patients will be monitored for DLTs, defined as any grade III or IV non-hematologic toxicity per the NCI CTCAE v4 guidelines. If 0 or 1 patients have toxicity at the starting digoxin dose, that dose will be determined "safe" for the phase II portion. However, if a greater number of DLTs are observed then the dose of digoxin will be decreased to 0.125mg/d and the safety study will be repeated with a new cohort of 6 patients. If 2 or more toxicities are encountered with the decreased dose then the combination of digoxin and decitabine will be considered too toxic for further study.

The phase II portion utilizes a Simon Two-Stage Phase II study design. The phase II portion will be carried out with two Groups. Group#1 will consist of patients with newly diagnosed AML or MDS, and Group#2 will consist of patients with relapsed/refractory AML or MDS. Our primary endpoint will be the CR+CRi rate.

For Group#1, our null hypothesis is a CR+CRi rate $\leq 20\%$ (per historical controls) and our alternative hypothesis is a CR+CRi rate $\geq 40\%$, with 90% power and Type I Error of 10%. We will need to enroll a total of 37 patients, including eligible patients from the phase Ib segment. Initially, we will need to enroll a total 17 patients (including those carried over from the phase Ib portion) to assess for response at cycle 3. If ≥ 4 patients have a CR or CRi we will enroll an additional 20 patients and re-assess response after three cycles. If $\geq$ 11 of the 37 patients have a CR or CRi after three cycles of therapy the regimen will be considered a clinically effective regimen which requires further assessment in a larger trial.

For Group #2, our null hypothesis is a CR rate $\leq 5\%$ (based on historical controls) and our alternative hypothesis is a CR rate $\geq 15\%$, with a 90% power and Type I Error of 10%. Based on this design we will need to enroll a total of 60 patients, which includes patients enrolled in the phase Ib segment. For the initial stage of the phase II trial, we will need to enroll a total 39 patients (including those carried over from the phase Ib portion) to assess for response at cycle 3. If ≥ 2 patients have a CR or CRi we will enroll an additional 21 patients and re-assess response after three cycles. If ≥ 6 of the 60 patients have had a CR

or CRi after three cycles of therapy the regimen will be considered a clinically effective regimen which requires further assessment in a larger trial.

The statistical design for the Phase II portion of the study is based on the assumption, as established in the literature, that randomization of the treatment rendered during the first cycle will not impact the results of response assessment conducted at the end of Cycle 3 and Cycle 6.

12.1.1. Statistical Plan for Assessment of DLTs for Phase II portion of the Study:

Group 1:
Patients treated in cycle 1 with both Decitabine and Digoxin will be assessed for DLTs. If 4 of the first 9 experience DLT (these patients are mostly in the initial set of 17 patients), the trial will be terminated. If ever 5 of 18 experience DLT the same decision will be made. Patients randomized to Decitabine alone will not be counted unless they experience DLTs, in which case these will be added to the total DLTs as if they were in the two-regimen group. DLT rate of 15% will be considered acceptable, but 35% too toxic. The chance of early termination when the true DLT rate is 35% is 39%. The overall chance of declaring the treatment too toxic is 82% in this case. The chance of early termination in error is 3.4% and final termination in error is 12.7%.

Group 2:
Similar to Group 1, if 7 of the initial 19 two-regimen patients (mostly in the first patient group of 39 patients) the trial will be terminated. If 9 of all two-regimen patients experience DLT the treatment will be considered too toxic. Again, single regimen patients will be counted only if they experience DLTs. The chance of early termination is 52% when the treatment is too toxic. The chance of early terminating in error is 1.6%. The chance of final decision of toxicity is 79% when the true DLT rate is 35% and 3.4% when it is 15% (in error).

These computations are only approximate since the exact numbers of patients randomized to both regimens is unknown.

12.2. Analysis of Secondary Endpoints

Patients will be classified as responders or non-responders based on analysis of their peripheral counts and their bone marrow biopsies done at various pre-defined points during their treatment course. The statistical objectives of this portion of the study are exploratory in nature. After pre-processing of raw data, variance stabilizing and normalizing transformations will be applied as needed. As and when appropriate, analysis of variance or its non-parametric equivalent will be used to compare various groups. For each patient sample, gene expression measurements will be obtained using the Illumina HiSeq 2500 system on days 1, 6,13, 20 during the first two cycles and day 1 of cycle 3 (prior to any treatment). Gene expression data will be summarized using descriptive statistics at each time point and cycle and trends in gene expression over time will be graphically presented. Unsupervised clustering of gene expression profiles will be performed using methods such as two-way hierarchical clustering, K-means etc. in order

to identify sub-groups of genes that may be associated with responders and non-responders.[17]

Genome-wide methylation studies will be performed on days 1, 6, 13, 20 during the first two cycles and day 1 of cycle 3 (prior to any treatment) and methylation levels will be compared at each time point. Changes in DNA methylation after treatment will be expressed as relative change (%) for each gene. The degree of genome-wide and gene-specific methylation will be compared between arms at the end of cycle 1.The Mann-Whitney-Wilcoxon test will be used to identify methylated genes as well as differentially expressed targeted genes. For our analysis, we will define hypermethylation as a 2-fold increase of the Cy5 leukemia signal over the Cy3 control based on previous validation experiments. In order to increase the accuracy of all measurements and any observed correlations all test will be done in triplicate to reduce the risk of variance secondary to operatory or machine error.

12.3. Analysis of Exploratory Endpoints

Furthermore, the three best and three worst responders to treatment will be identified at the end of cycle 3 and methylation analysis using the DREAM technique as well as exome and transcriptome analyses will be performed on samples obtained from these responders. These analyses will be exploratory in nature and will primarily consist of descriptive summaries.

All statistical tests will be two-sided and the Benjamini-Hochberg method will be used to account for multiple hypotheses.[18] The R statistical language and environment will be used in the computations.[17]

12.4. Sample Size/Accrual Rate

Planned sample size: 100
Accrual Rate: 2 patients per month

12.5. Stratification Factors

Patients will be stratified based upon disease state (newly diagnosed vs relapsed) and diagnosis (AML vs MDS) at the time of enrollment on the trial. However, interim monitoring and efficacy determination will be done for all enrollees for our primary aim. Based upon the distribution of patients there is a possibility of subgroup analysis based upon original diagnosis.

12.6. Reporting and Exclusions

12.6.1. Evaluation of toxicity

All patients will be evaluable for toxicity from the time of their first treatment with Digoxin + Decitabine.

12.6.2. Evaluation of response

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations. Each patient will be assigned one of the following categories: 1) complete remission 2) partial remission 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.]

All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) will be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

13.0 Data and Safety Monitoring Plan

13.1. Monitoring Plan

FCCC ISRU will monitor the medical and study records of each participant accrued throughout the course of the study. In addition, the IRU will collect and report data to the study Sponsor Investigator who will review these data on a regular basis at a rate dependent on subject accrual. All serious adverse events (SAEs) will be reviewed on a real time basis first by the study site PI and subsequently by the ISRU and Sponsor investigator as applicable.

13.2. Data Safety Monitoring Committee

Interim analysis of toxicity, outcome, and ongoing scientific investigations may be performed at least every 3 months by the Fox Chase Cancer Center Data Safety Monitoring Board (FCCCDSMB). In this capacity the FCCCDSMB will serve as an advisory committee to the sponsor-investigator. The FCCCDSMB will review those aspects of this trial that are outlined in the responsibilities section of the Data and Safety Monitoring Plan (DSMP). If the committee decides that changes should be made to this trial, it will make recommendations in writing to the Sponsor Investigator, the Associate Director of Clinical Research, and the Protocol Management Executive Committee, which, in turn, have the authority to approve or disapprove these recommendations. These changes will be discussed with the Sponsor-Investigator before they are implemented. These changes may include early termination of accrual. Other changes might include altering the accrual goals or changing the eligibility criteria for the trial.

14.0 Administrative

This study will be conducted in accordance with local, state and Federal regulations and according to accepted good clinical practice guidelines.

14.1. Data Reporting

The FCCC Study Monitor will request case report forms to be completed within 2 weeks of the protocol visit. Participating sites are responsible to respond to queries prior to the next scheduled monitoring visit.

The ISRU Staff is responsible for compiling and submitting data to the Sponsor Investigator and statistician on an ongoing basis for monitoring as described in the data safety monitoring plan and reporting to the Data and Safety Monitoring Board.

All patient information will be stored in an EDC system accessible only to the study team members for the purpose of entering, reviewing and analyzing data. Any paper records, such as case report files, produced will be stored in a secure location.

The ISRU is responsible for distributing and tracking review of all IND Action Letters, Safety Reports, study specific Serious Adverse Events

14.2. Retention of Records

Time points for the retention of records are described in detail in the contract between the grantor and the OCR and passed on to the participating site. Please refer to the study specific terms for specific time points. In all cases the Study Monitor must be notified of any plans to move records to an offsite location prior to doing so.

14.3. Study Agents

Any study agent supplied through the OCR from the manufacturer or a third party distributor may not be used for any purpose outside the scope of this protocol. The agent may not be transferred to any party not participating in the clinical trial.

14.4. Informed Consent

The IRB approved informed consent documents must be signed by the patient, or the patient's legally authorized representative, before his or her participation in the study. The case history for each patient shall document that informed consent was obtained prior to participation in the study. A copy of the informed consent documents must be provided to the patient or the patient's legally authorized representative. If applicable, they will be provided in a certified translation of the local language.

Original signed consent forms must be filed in each patient's study file or medical record with a copy in the study file.

15.0 References

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