A Phase I/II Study of Decitabine in Combination with Sequential Rapamycin or Ribavirin in High Risk AML Patients

ULEU-13049

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SYNOPSIS:

OBJECTIVES:

1.1 To determine the efficacy of decitabine followed by rapamycin in previously untreated elderly patients not able to receive standard chemotherapy or in patients with relapsed or refractory AML, through measurement of CR, CRp, PR, and event free and overall survival (ArmA).

1.2 To determine the safety of administration of decitabine with escalating doses of ribavirin in elderly leukemia patients or patients with relapsed/refractory disease with M4/M5 subtypes anticipated to express high eIF4E at diagnosis (ArmB).

1.3 To establish effect of these sequential treatments on expression of PI3K/Akt /mTOR pathway proteins and on eIF4E activation through Western blot and phospho-flow methodologies.

1.4 To correlate the clinical response with baseline expression of phospho-p70S6K/pAKT and with the in vitro inhibitory effects of mTOR inhibition with rapamycin or ribavirin on the level of downstream effectors.

1.5 To determine whether a leukemia stem cell phenotype is inhibited by the sequential administration of decitabine/rapamycin or decitabine/ribavirin.

TARGET POPULATION: Elderly (>65 years old) AML patients who are not otherwise candidates for standard induction chemotherapy or who refuse such therapy OR adults with relapsed or refractory AML. AML may have relapsed after any number of remissions or after stem cell transplantation in the absence of clinically significant GVHD. For inclusion/exclusion criteria, see 3.1 and 3.2.

RATIONALE: Decitabine, a demethylating agent, has been found to have activity in elderly patients with AML and in patients with high risk MDS. mTOR inhibitors such as rapamycin have been found to be inhibitory to AML stem cells and to have activity in some patients with AML. By combining agents, which have been shown to have potential for additive or synergistic effects in vitro, the response rate and safety in relapsed/refractory patients will be determined. In our phase I study, it was found that a daily dose of 2 mg Rapamycin resulted in therapeutic levels and was safely administered.

Rapamycin does not always effectively inhibit protein translation in AML due to inability to inhibit 4E-BP-1. There is data that ribavirin, an agent which inhibits methyl 7G cap translation via inhibition of eIF4E can suppress AML growth in those cases with high baseline expression or aberrant nuclear localization of eIF4E. In those M4/M5 AML cases known to express high eIF4E expression, we will therefore examine ribavirin effects in conjunction with decitabine as it is anticipated that rapamycin would be less effective in these cases.

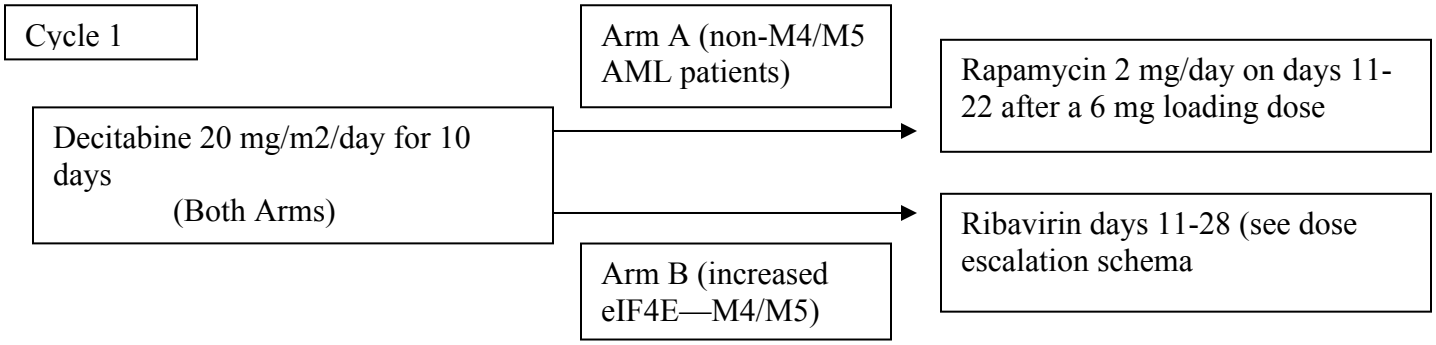
DURATION OF STUDY: Number of cycles will be determined based on response with maximum of 6 cycles to be given. See 4.1.1.

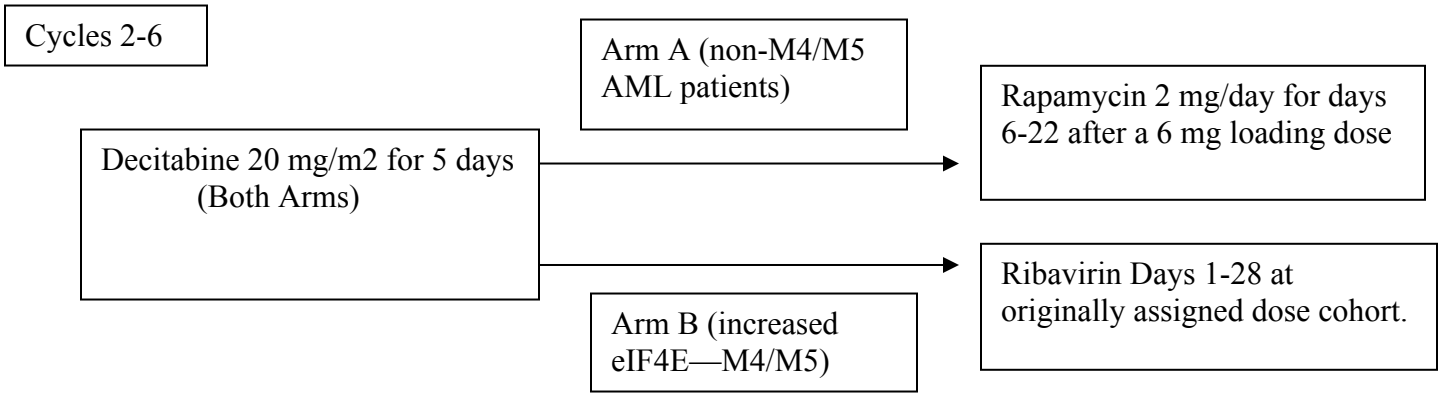
STUDY DESIGN: Open label single arm phase II design in arm A and standard dose escalation design in Arm B. In arm A, Decitabine will be given at 20 mg/m^2 for 10 days in cycle 1 followed by rapamycin orally on days 11 to 22 at 2 mg /day after a 6 mg loading dose on day 11. In subsequent cycles, decitabine will be given for 5 days. This arm of the study will be conducted using a Simon two stage model to maximize efficacy and minimize toxicity.

In arm B, in those with high eIF4E (FAB subtypes M4/M5), after completion of the 10 days of decitabine, ribavirin will be administered at either 1000 mg bid, 1200 mg bid, or 1400 mg bid on days 11 through 28. In cycles beyond the first, ribavirin will be given from days 1 through 28, and decitabine will be given for 5 days.

CORRELATIVE STUDIES: Endpoints of mTOR inhibition and protein translation inhibition will be measured at various time points during the study. We will also assay for inhibition of a leukemia initiating stem cell phenotype and functionality at defined time points during the trial.

STUDY SCHEMA





Outline

1.0 OBJECTIVES

1.1 To determine the efficacy of decitabine followed by rapamycin in previously untreated elderly patients not able to receive standard chemotherapy or in patients with relapsed or refractory AML through measurement of CR, CRp, PR, and event free and overall survival.

1.2 To determine the safety and feasibility of the combination of decitabine given at a fixed dose with sequential rapamycin in previously untreated elderly patients not able to receive standard chemotherapy or in patients with relapsed or refractory acute myeloid leukemia with M4/M5 subtypes known to express phosphorylated eIF4E at diagnosis.

1.3 To establish effect of this sequential treatment on expression of PI3K/Akt /mTOR pathway proteins through Western blot and phospho-flow methodologies. In those receiving ribavirin, effect on levels of phospho-eIF4E expression will be measured as will effects on protein translation.

1.4 To correlate the clinical response to sequential decitabine with rapamycin or ribavirin with baseline expression of phospho-p70S6K/pAKT .

1.5 To determine leukemia stem cell frequency in the setting of AML relapse and in de novo elderly AML patients and to determine if that frequency is decreased after decitabine alone or after decitabine followed by rapamycin or ribavirin.

2.0 BACKGROUND

2.1 Relapsed and refractory AML and AML in the 65 years and older population

Acute myeloid leukemia (AML) is a clonal hematopoietic disorder whereby normal progenitor cells no longer differentiate normally or respond to regulators of proliferation, resulting in the accumulation of leukemic blasts in the blood, bone marrow and other tissues. There are an estimated 14,000 new cases of AML diagnosed in the United States each year with a median age at diagnosis of 69.[1] Standard initial treatment has generally consisted of the combination of an anthracycline, such as daunarubicin or idarubicin, and cytarabine with complete remission rates in patients aged 18-60 years approaching 65% to 75%.[2] In older patients, the complete remission rates are lower, between 40% and 60%, with a probability of survival at 2 years between 19% and 25%.[3] Therefore, there remains a population of patients that will either not achieve a remission after initial induction therapy or will relapse soon thereafter and require salvage therapy. Regardless, complete remission rates and overall survival after relapse tend to be poor.

When possible, relapsed patients are offered high-dose chemotherapy or reduced intensity therapy with hematopoietic stem cell transplant (HSCT), which is the only therapy associated with a reasonable chance of durable remission after relapse. However, the majority of relapsed patients will be ineligible for HSCT either because of a lack of

donor, advanced age, or the presence of other medical co-morbidities that would increase the potential morbidity of the procedure. As a result, therapeutic options available to date remain unsatisfactory for the majority of patients with relapsed AML. Also, relapse after allogeneic stem cell transplantation is usually compatible with a survival of only weeks. Novel therapies should be investigated for the potential to improve remission duration and length of survival while at the same time limiting toxicity in this population of patients.

Because elderly patients often have a lower response rate to standard chemotherapy regimens and have a higher rate of mortality during such treatments, especially when diagnosed at age >70 years, other therapies are now utilized in this patient group such as hypomethylating agents, clofarabine, and other investigational approaches. It is generally held that there is no standard of care for induction therapy in elderly AML patients [2,3].

2.2 Decitabine

2.2.1 Effects in Humans

Decitabine is a unique agent with a dual mechanism of anticancer action. Although the compound was first synthesized over 40 years ago, only recently has the dual mechanism of action been elucidated. High doses of decitabine are cytotoxic while lower doses are associated with DNA hypomethylating activity. This information sparked clinical trials using lower doses since in the original studies higher doses were associated with dose-limiting myelosuppression. Studies using such lower dosing schedules, particularly in patients with hematologic malignancies, have shown significant promise for the future use of demethylating agents. In May of 2006 the United States Food and Drug Administration approved the use of decitabine (DACOGEN for Injection) for the treatment of MDS. The current focus for the clinical use of decitabine is for the treatment of hematologic malignancies, namely MDS as well as AML, as a single agent or in combination with other agents.

2.2.2 Rationale for the Use of Decitabine in Hematologic Malignancies

Reports suggest frequent methylation of the $p15^{INK4B}$ gene promoter in leukemias, and it has been proposed that this methylation could be necessary for leukemic cells to escape inhibitory signals from the bone marrow environment by silencing p15 expression.[4,5] $p15^{INK4B}$ is a regulator of the G1/S phase cell cycle transition which negatively regulates cell-cycle dependent kinases, the activity of which is necessary to activate the Retinoblastoma protein transcription factor.[6] Furthermore, several studies report increased p15 gene methylation in MDS patients, and this methylation pattern appears to become more apparent with MDS disease progression.[7,8] An agent such as decitabine or 5-azacytidine, with documented activity of hypomethylation of DNA would then be expected to affect the natural history of MDS and leukemias and demonstrate clinical activity.

Correlative investigations of Daskalakis *et al*.[9] explored decitabine-induced p15 gene demethylation in bone marrow mononuclear cells collected before and during treatment with decitabine. Samples were collected from 23 intermediate /high risk MDS subjects enrolled in a decitabine Phase 2 study. In these samples, p15 gene hypermethylation (defined as >15% methylation) was observed in 15 of 23 patients at baseline (median, 29%; range, 16-54%). Hypermethylation occurred in all FAB subtypes and thus was not limited to the presence of excess bone marrow blasts. Of the 23 subjects that received one or more courses of low-dose decitabine, 19 were evaluable for changes in p15 gene methylation. Of the 12 subjects with p15 hypermethylation prior to decitabine treatment, 9 responded to treatment with at least a 25% decrease (median, 16%; range, 5 to 34%). One subject had a <25% decrease, and in 2 subjects, p15 methylation increased. Subjects without initial p15 hypermethylation showed no changes in methylation during continued treatment. In 10 subjects, p15 immunohistochemical staining was performed on serial biopsies, and in 4 of 8 cases with p15 gene hypermethylation, cytoplasmic p15 expression was very low or absent prior to treatment while in the other 4 subjects and the 2 subjects without hypermethylation, cytoplasmic p15 levels were comparable to normal bone marrow. Following 1 to 3 courses of decitabine, p15 expression was induced to high levels in all 4 subjects with initially low or absent expression. This was paralleled by hypomethylation. The study concluded that emergence of partially demethylated epigenotypes and re-establishment of normal p15 protein expression following decitabine treatment implicate pharmacologic demethylation as a possible mechanism of response in MDS. Other studies have documented global hypomethylation with decitabine but have not been able to associate responses with increased expression of a given gene. [10]

2.2.3. Clinical Experience with Decitabine in AML and MDS

Treatment of patients with myelodysplastic syndromes (MDS) with decitabine leads to transfusion independence and a longer median time to progression to AML or death as compared to patients treated with supportive care only[11]. In vitro data have shown that decitabine is able to induce terminal differentiation of leukemic blasts, which acquire the morphological and cytologic characteristics of fully differentiated monocytes and macrophages. Additionally, treated cells regained their innate functional ability. Decitabine has also been shown to have activity in MDS patients with intermediate or high risk International Prognostic Scoring System (IPSS) scores. 34% of patients had normalization of bone marrow and peripheral counts. A dosing scheme using 20 mg/m^2 daily intravenously for 5 days has been found to be as efficacious as other more complicated dosing schedules[12]. Subsequent studies have shown activity as a first-line treatment in all cytogenetic subgroups of older AML patients[13]. Furthermore, decitabine has been successfully and safely combined with histone deacetylase inhibitors (HDACs) such as valproic acid, suberoylanilide hydroxamic acid (SAHA) and vorinostat as well as with standard chemotherapy regimens.[14] Recently, decitabine has been utilized in elderly patients with AML

with some success[15]as upfront therapy and response rates up to 40% have been noted with ten day courses in a similar dosing scheme [16].

2.3 mTOR Pathway and Leukemia

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase involved in the regulation of synthesis of proteins involved in cell cycle progression[17]. It appears to be a nutrient sensing protein with dual regulation by amino acid availability as well as mitogen activation by the upstream PI3K/AKT pathways.[17] The eukaryotic initiation factor 4 E (eIF4E), which is downstream from mTOR, is necessary for recruitment of the ribosome to mRNA, the rate limiting step in protein translation. The formation of the eIF4E complex is regulated by 4E-BP1 and in the unphosphorylated form it binds to eIF4E and inhibits its activity and protein translation is halted, while when 4E-BP1 is phosphorylated, there is decreased affinity for eIF4E and translation can occur. A separate downstream target of mTOR, p70S6K, is also involved in protein translation. When activated by mTOR, it is able to phosphorylate the 40S ribosomal protein S6 that then allows translation to occur.[18]

Constitutive activation of PI3K/AKT/mTOR signaling has been shown in leukemic blasts with 50-70% of patients displaying phosphorylated AKT.[19] There are multiple possible mechanisms for this activation which include activating Ras or c-KIT mutations, upregulation of PI3K p110δ, autocrine/paractine VEGF secretion and others.[19] There also appears to be an upregulation of this pathway not only in the bulk AML population, but also in leukemia stem cells transplanted in NOD/SCID mice models. [20] Activation of this pathway results in disrupted control of proliferation resulting in a competitive growth advantage for the leukemic cells. Inhibitors of the mTOR pathway either alone or in combination may block the activated signaling cascade or lead to inhibition of tumor growth and apoptosis.[20]

2.4 Rapamycin

Rapamycin is a macrolide fungicide isolated from the bacteria Streptomyces hygroscopicus with potent antimicrobial, immunosuppressant and antitumor activities and is approved for the prevention of allograft rejection following organ transplantation. Rapamycin binds to the immunophilin FK506 binding protein 12 (FKBP12) and forms a complex which then binds to mTOR and inhibits downstream signaling events.[21] The sensitivity of various AML cell lines to increasing concentrations of rapamycin in clonogenic assays has been measured. There appears to be a variable degree of sensitivity with UT-7EPO and UT-7GM cell lines being insensitive, K562, U937, HEL and HL60 being moderately sensitive and the immature KG1 and KG1a cell lines being strongly inhibited by low concentrations of rapamycin.[22] Nine patients with relapsed, refractory or poor-risk AML were treated with rapamycin as a single agent, with a partial response (> 50% reduction in the absolute number of blasts or at least a 50% reduction in the percentage of marrow blasts) occurring in 4 patients. 4 patients had progressive disease and one had stable disease. [22] As a single agent, rapamycin has had minimal activity in AML. Rapamycin has been combined with cytototoxic chemotherapy in newly

diagnosed patients in early phase trials[23] with no positive effects on CR rates noted. Rapamycin has also been utilized in the therapy of ALL, and it is also used after allogeneic stem cell transplantation for prophylaxis of graft vs. host disease in combination with tacrolimus or methotrexate.

2.5 Ribavirin

One of the reasons postulated for limited impact of mTOR inhibitors in AML is that first generation inhibitors such as rapamycin do not inhibit the mTORC2 component of mTOR and they also fail to inhibit 4E-PB1 phosphoryaltion, thereby allowing translation initiation to occur. Unlike rapamycin, ribavirin is a competitive inhibitor of the cap moiety, thereby inhibiting the mRNA export and translation function of eiF4E. eIF4E activity has been found to be most elevated in M4 and M5 AML (44/44 cases), and there is accumulation of nuclear bodies and less eiF4E in the cytoplasm when overexpression occurs. When used as a single agent in 11 M4 and M5 AML cases, there was 1 CR, 2 PRs, 2 blast responses, 4 stable disease, and 2 progressive disease outcomes [24]

2.6 Rationale for the Combination of Decitabine and an mTOR Inhibitor

Decitabine has shown efficacy in the treatment of patients with myelodysplastic syndrome as well as AML. Rapamycin has also been shown to have activity in patients with AML. The combination of decitabine with rapamycin will allow us to test the potential beneficial effect of targeting two separate pathways that appear aberrant in this disease. Since successful maintenance dose escalation studies with a demethylation agent have been reported after cytotoxic chemotherapy as well as after stem cell transplantation and since rapamycin has effective GVHD activity without adversely impacting donor chimerism, we also wish to test the combination of decitabine and rapamycin in patients who relapse after allografting.[25] It is also anticipated that this combination may be synergistic in that rapamycin by itself may activate pAKT via mTORC2 and IGFR1 mechanisms. [19]

In order to test the rationale for combining a demethylating agent with an mTOR inhibitor preclinically**,** AML cell lines were exposed to 5-azacytidine at 1 to 16 uM, with the IC50 determined to be 4-8 uM. 5-azacytidine increased apoptosis above control whereas rapamycin by itself had minimal effect on apoptosis as assessed in an Annexin V flow cytometric assay. Effects on proliferation at concentrations of Rapamycin up to 100 nM were also minimal. In the U937 cell line, the combination index was 0.465 (10 μM 5-azacytidine and 100 nM rapamycin), indicating synergism. For MV411, the combination index was 0.736, and for KG1a, 0.118, also indicating synergism. For HL60, no synergism was noted. Exposure to the combination of 5-azacytidine and rapamycin resulted in greater suppression of AKT and 4EBP1 phosphorylation than did either agent singly. Four days of AML cell line exposure to decitabine followed by 100 nM Rapamycin also results in inhibition of cell proliferation. This in vitro data (published only in abstract form to date[26]) utilizing a demethylating agent and an mTOR inhibitor lends support to rational combination of this regimen in vitro.

Based on the rationale for combining a hypomethylating agent with an mTOR inhibitor and upon the in vitro data generated, we embarked on a phase I trial to examine the effects of sequential decitabine and rapamycin with the primary endpoint of safety and feasibility: A Phase I Study of Decitabine in Combination with Escalating Doses of Rapamycin in Patients with Relapsed or Refractory Acute Myeloid Leukemia (registered as NCT00861874 at clinical trials.gov). 13 patients were accrued to the study (7 at dose level 1, 3 at dose level 2, and 3 at the third dose level). The median age of the patients was 63 (range 46-78). The mean blast percentage at enrollment was 35% (range 6-93%). Seven patients had complex karyotype. At the end of cycle 1, 5 patients demonstrated disease progression, 5 had stable blast percentage, and 4 demonstrated a decline in blast percentage. There was one CR on the study. Median survival was 4 months (range 1-31+ months). One patient entered on the trial after prolonged neutropenia experienced reversible grade 3 mucositis which was deemed a DLT; hence the additional enrollment on the first doing cohort. No other significant unexpected non-hematologic toxicities have occurred. On the 2 mg cohort, all patients achieved therapeutic rapamycin levels during the first cycle and in 5/7, this occurred within 4 days of beginning therapy. Based on these rapamycin levels, we have chosen 2 mg/day after a loading dose of 6 mg as the dose for the phase II portion of the trial with this combination[27].

2.7 Rationale for combination of decitabine and ribavirin

As noted above, while mTOR inhibitors are not always effective in inhibiting translation in AML, ribavirin has that potential in cases where eIF4E is activated and expressed. In this trial, we will therefore examine the use of ribavirin in dose escalation fashion built on a 10 day decitabine backbone in those M4/M5 subtype patients who express high eIF4E levels.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

3.1.1 Age ≥ 18

3.1.2 Diagnosis of AML according to WHO (World Health Organization) criteria except acute promyelocytic leukemia **AND**

3.1.3 Refractory AML defined as failure to achieve CR after 2 cycles of induction chemotherapy or persistence of > 40% bone marrow blasts after one cycle of chemotherapy induction **OR**

3.1.4 Relapsed AML defined as any evidence of disease recurrence after achieving a documented first or greater CR **OR**

3.1.5 Relapsed AML after stem cell transplantation. 90 days (since stem cell infusion) must have elapsed between transplant and emergence of recurrent AML**OR**

3.1.6 Newly diagnosed AML in a patient >65 years old not considered fit for standard 7+ 3 chemotherapy or who declines such therapy after discussion of therapeutic options available.

3.1.7 ECOG performance status <3 (Appendix 1)

3.2 Exclusion Criteria

3.2.1 Abnormal renal function as evidenced by a calculated creatinine clearance ≤ 30 ml/min (Cockcroft-Gault formula (Appendix 2)
32.2 Abnormal liver function: Bilirubin >2.0 mg/dl, transaminase(s) more than 2.5x the upper limits of normal
3.2.3 Active systemic infection not responding to antibiotics
3.2.4 Known diagnosis of human immunodeficiency virus infection (HIV)
3.2.5 Patients who are post-allogeneic transplantation should not have active GVHD greater than grade 1 of skin at time of enrollment. They may have had DLI but not within 4 weeks of beginning the study.
3.2.6 Pregnant or breast feeding female subjects
3.2.7 Known or suspected CNS leukemia involvement; past involvement is not an exclusion.

3.3 Inclusion of Women and Minorities

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

3.4 Study Duration and Dates

The study will commence in Winter, 2014 and should meet accrual by Autumn , 2015, assuming approximately two accruals per month. These are estimates only. Of note, should a patient be found to exhibit stable or improved disease after completing 6 cycles, a consideration will be given to continuing maintenance treatment of decitabine, but this will be optional and not part of this protocol.

3.5 Prior and concomitant medications

Patients may not have had prior exposure to decitabine, rapamycin, or ribavirin. No other investigational agent may have been received within two weeks, but otherwise there are no exclusions on numbers and types of prior therapies for AML. As discussed below, hydroxyurea and/or leukapheresis may be utilized for control of circulating blast count at the inception of cycle 1 and 10 days into the cycle.

4.0 TREATMENT PLAN

4.1 Agent Administration

Treatment will be administered on an outpatient basis whenever possible. Reported adverse events and potential risks for decitabine, rapamycin, and ribavirin are described in Section 5. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

4.1.1 Study schema

Note: Those patients with morphologic M4/M5 subtypes[28] will be treated on Arm B and all other subtypes on Arm A. At study entry, all patients will have samples procured for testing of eIF4E expression but this will not determine allocation to arm A or B which will be made solely on the basis of FAB subtype. M4 subtype is acute myelomonocytic leukemia and M5 acute monocytic leukemia.

- **Decitabine (Arms A and B)**
- 20mg/m2 will be given as an intravenous infusion daily for 10 consecutive days starting on day 1 of cycle 1.
- In subsequent cycles, decitabine will be given for 5 days (day 1-5)

Rapamycin (Arm A)

- For non-M4/M5 AML cases[28]
- A 6 mg loading dose will be administered on Day 11 in cycle 1 and day 6 in subsequent cycles. Thereafter, 2 mg/day will be administered per schedule below. This may be reduced to 1 mg/day if levels are elevated (>15 ng/mL)..
- Toxicities will be as defined in section 9 using NCI Common Toxicity Criteria for Adverse Events (CTCAE (version 4.0) Appendix 3)
- Rapamycin will be given from day 11 to 22 in cycle 1 and on days 6 to 22 in subsequent cycles.

Dose Level	Rapamycin	Decitabine
Level -1	1 mg	20 mg/m2
Level 1	2 mg	20 mg/m2

The rapamycin dose for phase II of the study was chosen based on results from the phase I study (ULEU08049).

Ribavirin (Arm B)

For M4/M5 cases[28]—These patient are anticipated to have high eIF4E expression as expressed by Western blotting[24].

In cycle 1, ribavirin will be dosed from Day 11 through Day 28.

In subsequent cycles, ribavirin will be started on Day 1 on a continuous basis until study completion. These doses were chosen based on those found effective and tolerated in previous studies[24].

Dose level cohorts are listed in the table below. Subject enrollment will start at **Dose Level 1** (1000 mg). Enrollment in subsequent dose levels (escalation to Dose level 2 and 3, or de-escalation to Level -1 and -2) will follow the dose escalation and dose de-escalation plans as outlined below.

Dose Level	Ribavirin	Decitabine	Description
Level -2	600 mg bid	20mg/m2	Dose de-escalation cohort
Level -1	800 mg bid	20mg/m2	Dose de-escalation cohort
Level 1	1000 mg bid	20mg/m2	Initial cohort
Level 2	1200 mg bid	20 mg/m2	Dose escalation cohort
Level 3	1400 mg bid	20mg/m2	Dose escalation cohort

Dose Escalation Plans for Ribavirin (Arm B)

Number of patients with DLT at a given dose level	Escalation Decision Rule
0 out of 3	Enter 3 patients at the next dose level
≥ 2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Three (3) additional patients will be entered at the next lowest level if only 3 patients were treated previously at that dose.
1 out of 3	Enter at least 3 more patients at this dose level. – If 0 of these 3 patients experience DLT, proceed to the next dose level. -If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
≤ 1 out of 6 at highest dose level below the maximally administered dose	This is generally the recommended phase 2 dose. 6 patients must be entered at the recommended phase 2 dose before going to Phase 2.

An ad hoc confidential person will ensure that no unexpected adverse events have occurred at each dose level before enrollment on the next dose level commences. This will be handled through the DSMB of the James P Wilmot Cancer Center. THIS WILL REQUIRE THAT THE LAST PERSON ENROLLED AT THE PRIOR COHORT HAS BEEN FOLLOWED FOR 30 DAYS FROM THE START OF THERAPY.

Dose De-escalation: If 1000 mg bid proves to be the MTD, a cohort at 800 mg bid will be treated, and if that is too toxic, one at 600 mg bid will be enrolled (level -2). No further dose de-escalation would be planned. Safety assessment at each cohort would be as indicated above.

4.1.1.1

Cycle Duration

Cycles will begin on an every 28 day basis which may be extended to 30 days to account for weekends, holidays, logistics of marrow aspirate and biopsy scheduling.

Routine Clinical Monitoring.

Routine CBC with differential and platelets as well as a comprehensive metabolic profile including renal and hepatic function will be evaluated on a twice weekly basis and more frequently as cytopenias or metabolic abnormalities indicate. For those patients receiving rapamycin, a trough Rapamycin level will be performed at day 14 and 21 of each cycle.

Marrow Status Monitoring.

Marrow aspirate only will be obtained after the first ten days (on days 10 or 11) of decitabine in the first cycle. At the end of the first cycle and third cycle or at the time of full peripheral blood count recovery, whichever comes first, a bone marrow aspirate and biopsy will also be obtained. Marrow may be obtained between days 22 and 26 of the 1st and 3rd cycles, and a marrow at time of withdrawal from study is recommended but is optional.

Number of Cycles.

If there is no overt progression of disease (defined as doubling of the peripheral blood blast count) after the first cycle, the patient will proceed on study and receive two more cycles. If after the third cycle there is evidence of a PR/CR or stable disease, the patient will remain on study for 3 more cycles. The maximum number of cycles that each patient may receive is six. As noted above, physicians may use maintenance decitabine, but this will be off protocol.

Long Term Follow Up

Patients will be followed for survival status every 6 months for up to 2 years after last date of treatment.

4.1.2 Informed Consent

Prior to any study procedures being performed, written informed consent must be obtained for each patient. Each patient will sign the IRB approved informed consent, and the patient will be given a copy of the signed and dated consent. The original signed version will be retained in study files in the Clinical Trials Office of the University of Rochester Cancer Center. Information on all patients screened for the trial will be recorded with indication of reasons for non-participation or non-eligibility. All information will be stored in a secure place.

4.1.3 Registration

To register a patient, the eligibility checklist and consent form will be sent to the research nurse or data manager who will then register the patient. The Clinical Trials Office of the University of Rochester Cancer Center will register all patients by phoning 585-275-9485. The PI will sign each registration form which will include inclusion and exclusion criteria.

4.2 Supportive Care Guidelines

4.2.1 Blood Transfusion: Transfusion Parameters:
Hb ≤ 7 mg/dl or HCT <21 % in the absence of cardiopulmonary disease inpatient and <24% outpatient.
HCT <27 % in presence of cardiopulmonary disease.

4.2.2 Platelet Transfusion: Transfusion Parameters:
Active Bleeding OR
Platelets <10,000/μL inpatient or <15,000/μL outpatient. OR
Platelets < 20,000/μL in the presence of infection or fever.

4.2.3. Infectious Disease Prophylaxis

4.2.3.1 Antibiotics: Ciproflaxcin 500mg po BID or moxifloxacin 400 mg/day may be used during times of neutropenia at the discretion of the treating physician.

4.2.3.2 Antivirals: Acyclovir, Valacyclovir, or Famciclovir are permitted.

4.2.3.3 Antifungals: Fluconazole 200mg QD prophylaxis if patient is neutropenic until ANC >500/uL. Dosage should be adjusted according to liver function. Voriconazole and posaconazole may result in elevated sirolimus levels and should be utilized with caution and only in the case of documented fungal infections. Their use is permitted for those on Arm B.

4.2.3.4 Anti-Emetics: Use of anti-emetics will be at the discretion of the treating physician.

4.2.4 Tumor-lysis prophylaxis

4.2.4.1 Allopurinol 300 mg/day po on day -1 or 0 until day 14 or WBC < 500/ul. Rasburicase may be utilized for extreme uric acid elevations per institutional standards of care.

4.2.5 Graft vs. Host Disease Management—See also 4.3.4. For those patients who are S/P allografting and who have a flare of GVHD during therapy, this can be treated at the investigators' discretion with steroids, mycophenylate mofetil, or photopheresis. If a

calcineurin inhibitor is required, a patient on Arm A would need to be removed from trial based on potential interaction with Rapamycin.

Other supportive care will be per institutional standards of care. This will include treatment of neutropenic fever, management of bleeding, nausea, mucositis, or other toxicities. Neupogen will not be utilized routinely but may be used in settings of infection or fever at the treating physicians' discretion.

4.3 Concomitant Therapy

4.3.1 In general, concomitant medications and therapies deemed necessary for the supportive care and safety of the patient are allowed provided their use is documented in the patient record and on the appropriate case report form. The administration of any other systemic anticancer agents including chemotherapy, radiation therapy or biologic agents including donor leukocyte infusions is **NOT** permitted. Similarly, concurrent use of other investigational agents or their use in the 28 days prior to enrollment is not allowed.

For any patient with a WBC greater than 50,000, consideration will be given for pretreatment with an appropriate dose of hydroxyurea to cytoreduce prior to the first dose of decitabine. Decitabine will not be started until the absolute blast count is less than 15,000/uL. Hydroxyurea may be continued into the first cycle until appropriate cytoreduction has occurred and should then be discontinued. In extreme cases of WBC elevation or in the presence of hyperleukostasis symptoms, leukapheresis may be employed for cytoreduction along with hydroxyurea.

For those patients who relapse after allogeneic transplant, tacrolimus/cyclosporine or steroids should be tapered off if no GVHD occurs. If GVHD occurs/progresses during therapy, it should be treated initially with steroids. Calcineurin inhibitors should be avoided. Mycophenylate mofetil may be utilized. See also 4.2.5.

4.4 Dose Calculation

Dose of decitabine will be based on body surface area calculated on actual body weight. No correction for obesity will be made. The dose of rapamycin or ribavirin is a fixed, non-calculated dose.

4.5 Dose Modification

4.5.1 Rapamycin

4.5.1.1 Dose modifications for non-hematological toxicity

Grade 2 (in accordance with the NCI CTCAE version 4.0)

If the patient experiences a Grade 2 non-hematologic toxicity, study drug must be withheld until the toxicity has resolved to less than or equal to Grade 1. Rapamycin may then be resumed at the 2 mg daily dose. If the grade 2 toxicity recurs, Rapamycin must be withheld until the toxicity has resolved to less than or equal to Grade 1. In those patients receiving 2 mg/day, dosing will be restarted on a 1 mg/day basis. If this recurs at the 1 mg dosing, rapamycin can then be dosed at 1 mg/ qod, but this is the lowest dose allowed.

Grade 3 or 4

If a patient experiences a Grade 3 or 4 toxicity, study drug must be withheld until the toxicity has resolved to less than or equal to grade 1 and the daily dose must be reduced to 1 mg/day from 2 mg per day, If the Grade 3 or 4 toxicity recurs, Rapamycin must be withheld until the toxicity has resolved to less than or equal to Grade 1, and the daily dose must be reduced to 1 mg qod. If the same toxicity occurs again, the drug must be stopped.

4.5.1.2 Dose modifications for hematologic toxicity

Grade 2

No dose interruptions will be performed for grade 1 or 2 hematologic toxicity because it is anticipated that all patients may become pancyopenic as a result of treatment or from the AML itself.

Grade 3 or 4

No dose reductions will be performed for grade 3 or 4 anemia, neutropenia and thrombocytopenia. Patients will receive supportive care as specified in section 4.2. Cycles may be delayed up to a week in the event of stable disease if severe neutropenia and/or active infection are present until the infection is controlled and neutropenia stabilized or improving.

Dose modifications for other reasons

If vomiting occurs following rapamycin administration, an extra dose will not be given that day and antiemetics will be prescribed to enable resumption of the next day's dose.

Grade 3 infections, fatigue, weight loss, and electrolyte imbalances are anticipated in relapsed AML patients and will be appropriately treated but are not deemed study related toxicities and will not result in dose modifications.

4.5.1.3 Dose modification for toxic range rapamycin level

If the rapamycin level at the time of scheduled measurement is >15 ng/mL, the dose will be held for 5 days, and a repeat level will be drawn on the 5th day. If the level is still >15, the drug will be held for 5 more days and the level repeated. Once the repeat level is no longer above the normal range, the daily dose will be reduced to 1 mg and a rapamycin level will be drawn 5 days after the dose adjustment has been made. If the level is again $\geq$ 15 ng/dL, drug will be held until levels return to the therapeutic range and then started again at 1 mg every other day.

4.5.2 Decitabine

No specific dose modification for hematologic toxicities.
Schedule modifications will be based on disease status and count recovery from the previous cycle as follows:

Subjects with >5% blasts in marrow will proceed to a 5 day cycle.
Those with no morphological evidence of leukemia (<5% blasts)will receive a 5 day course of decitabine
Those with no evidence of AML but who have grade 4 neutropenia (<500/uL) of at least 14 days duration will receive 5 days with the next cycle. If this happens again in the subsequent cycle, 3 days will be administrated.
Subjects should receive 3 cycles of treatment as discussed above before treatment failure is determined in the absence of obviously resistant, progressive disease.

4.5.3 Ribavirin

4.5.3.1 Dose Modifications for Hematologic Toxicity

No dose modification for hematologic toxicity will occur. Patients will receive supportive care as specified in section 4.2. Cycles may be delayed up to a week in the event of stable disease if severe neutropenia and/or active infection are present until the infection is controlled and neutropenia stabilizes or is improving.

4.5.3.2 Dose Modifications for non-hematologic toxicity

For non-hematologic toxicities thought due to ribavirin, the drug will be held until toxicity resolves to less than or equal to grade 1. If the resumption of ribavirin results in the same toxicity, dose will be lowered by one cohort, and for those on 1000 bid, dose may be reduced to 800 mg bid (dose level -1). If toxicity occurs at that level, ribavirin will be redosed at 600 mg bid (dose level -2), and if toxicity occurs at that dose, ribavirin will be stopped.

Grade 3 infections, fatigue, weight loss, and electrolyte imbalances are anticipated in relapsed AML patients and will be appropriately treated but are not deemed study related toxicities and will not result in dose modifications

4.5.4 Other dose modifications according to standard clinical practices are to the discretion of the treating physician. The study coordinator and primary investigator (s) should be notified of such modifications. As examples, treatment can be delayed at the discretion of the investigator for disease-related complications such as febrile neutropenia, infection, mucositis, hemorrhage, or extreme fatigue and debility.

4.6 Treatment Compliance

All study drugs will be administered to eligible subjects under the supervision of the investigators. The pharmacist will maintain records of preparation and dispensing, including the applicable lot numbers, patients' height, weight, and body surface, and total drug administered. Any discrepancy between the calculated dose and dose administered and the reason for the discrepancy must be recorded on the data capture records and in the source documents. Patients will also be given a log/diary on which to record rapamycin or ribavirin ingestion.

5.0 AGENT FORMULATION AND PROCUREMENT

5.1 Decitabine

Decitabine will be purchased from commercial sources.

5.1.1 Formulation Information

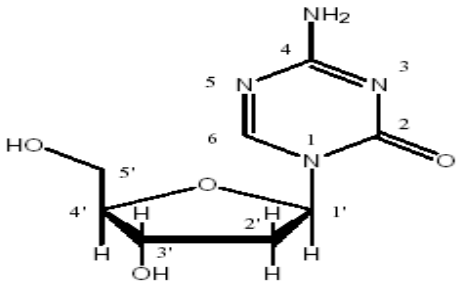
5.1.1.1 Product Identification

The generic name is decitabine or 5-aza-2'-deoxycytidine. The trade name in the United States is DACOGEN™ for Injection.

5.1.1.2 Physical and Chemical Characteristics

Decitabine is a white to almost white finely crystalline powder, odorless substance, with a molecular formula of $C_8H_{12}N_4O_4$ and a molecular weight of 228.21 Daltons. The chemical structure is shown in Figure 1.

Figure 1: Chemical Structure of Decitabine



Decitabine is sparingly soluble in water (8 to12 mg/mL), slightly soluble in ethanol and virtually insoluble in chloroform. Decitabine is an analog of 2'-deoxycytidine in which the #5-carbon atom of the pyrimidine ring has been replaced by a nitrogen atom. Decitabine appears in 2 configurations. The ß-D-anomer is the pharmacologically active form; the α-D-anomer is almost inactive.[29] The drug is most stable at pH 7 and when stored at low temperatures.[30-33]

Dacogen™ for Injection (decitabine) is supplied as a lyophilized preparation. Each 20-mL vial containing 50 mg decitabine should be stored at controlled room temperature per USP and is stable for at least 3 years if unopened.

Decitabine is a hypomethylating drug at lower doses and demonstrates direct cytotoxic properties at higher doses, therefore as with other potentially toxic compounds, caution should be exercised when handling and preparing decitabine. Decitabine should be aseptically reconstituted with 10 mL of Sterile Water for Injection (WFI); upon reconstitution, each mL contains approximately 5.0 mg of decitabine at pH 6.7 to 7.3. Immediately after reconstitution, the solution should be further diluted per recommended procedure with infusion fluids, such as 0.9% Sodium Chloride Injection, 5% Dextrose Injection, or Lactated Ringer's Injection to a final drug concentration of 0.1 to 1.0 mg/mL. Unless used within 15 minutes of reconstitution, the diluted solution must be prepared using cold infusion fluids (2°C to 8°C) and stored at 2°C to 8°C for a maximum of 7 hours prior to administration.

5.1.1.3 Other Pertinent Information

In stability studies, [33] Dacogen for Injection maintains at least 95% of its initial potency for up to 60 minutes when a 50 mg vial is reconstituted

with 10 mL of either cold (2°C to 8°C) or room temperature water for injection (WFI). To assess potency, the decitabine was assayed by high-performance liquid chromatography (HPLC).

Reconstituted Dacogen for Injection (5mg/mL in WFI) was mixed with each of the approved diluents (0.9% sodium chloride injection, 5% dextrose injection or lactated Ringer's solution) and the stability was measured as above. The decitabine potency at diluted concentrations of 0.1 mg/mL and 1.0 mg/mL at either room temperature for 3 hours or refrigerated (2°C to 8°C) for 7 hours followed by 2 hours at room temperature remained ≥ 90%. All admixtures of decitabine remained clear and colorless during storage at room temperature. Additionally, the pH of the solutions remained constant throughout the study.

Handling and Disposal

Procedures for proper handling and disposal of anticancer drugs should be applied. Several guidances on this subject have been published. There is no general agreement that all of the procedures recommended in the guidelines are necessary or appropriate.

5.1.1.4 The dosage of decitabine will be 20 mg/m2 given as an infusion on Days 1-10 of the first cycle and 1-5 of subsequent cycles.. Decitabine must be infused over 1 hour.

5.1.1.5 Adverse reactions

Table 1. Common Adverse Events Reported in a Phase III Trial Comparing decitabine to Supportive Care[33]

	DACOGEN (n=83)	Supportive Care (n=81)
Neutropenia	75 (90%)	58 (72%)
Thrombocytopenia	74 (89%)	64 (79%)
Anemia NOS	68 (82%)	60 (74%)
Febrile neutropenia	24 (29%)	5 (6%)
Nausea	35 (42%)	13 (16%)
Constipation	29 (35%)	11 (14%)
Diarrhea NOS	28 (34%)	13 (16%)
Pyrexia	44 (53%)	23 (28%)
Hyperglycemia NOS	27 (33%)	16 (20%)
Cough	33 (40%)	25 (31%)
Petechiae	32 (39%)	13 (16%)

5.2 Rapamycin (from Rapamycin package insert)

Rapamycin can be obtained as Rapamune® from Wyeth-Ayerst Laboratories. It is approved for graft rejection prevention in kidney transplantation. It will be purchased from commercial sources for this protocol.

The oral solution must be protected from light and stored under refrigeration, 2 degrees Celsius to 8 degrees Celsius and it is stable for 24 months under these conditions. A slight haze may develop in refrigerated solutions, but the quality of the product is not affected. After opening, the solution should be used in 1 month. If necessary, it may be stored at temperatures up to 25 degrees Celsius for several days after opening (not longer than 30 days). Product may be stored in an amber syringe for a maximum of 24 hours (at room temperature or refrigerated). Discard syringe after use. Solution should be used immediately following dilution. The tablet should be stored at room temperature (20 degrees Celsius to 25 degrees Celsius) and protected from light.

Absorption: Rapid
Volume of distribution: 12L/Kg (+/- 7.52L/kg)
Protein binding: 92%, primarily to albumin
Metabolism: Extensively hepatic via CYP3A3/4 and P-glycoprotien
Bioavailability: 14%
Half-life elimination: Mean: 62 hours
Time to peak: 1 – 3 hours
Excretion: Feces (91%); Urine (2.2%)

5.2.1. Adverse reactions

5.2.1.1. Systemic >10%:

Central nervous system: Insomnia, nervousness
Gastrointestinal: Increased appetite, indigestion, esophagitis
Dermatologic: Hirsutism
Endocrine/metabolic: Diabetes mellitus
Neuromuscular & Skeletal: Arthralgia
Ocular: Cataracts
Respiratory: Epistaxis
Metabolic: Increased triglycerides or cholesterol

<1% of case reports:

Seizures, mood swings, headache, delirium, hallucinations, euphoria, skin atrophy, bruising, hyperpigmentation, acne, amenorrhea, sodium and water retention, Cushing's syndrome, hyperglycemia, bone growth suppression, abdominal distention, ulcerative esophagitis, pancreatitis, muscle wasting, hypersensitivity reactions, pneumonitis in renal transplant patients, microangiopathic anemia in GVHD prophylaxis regimens only.

5.2.2. Drug Interactions

Cyclosporine may increase rapamycin concentrations due to inhibition of CYP3A4. In this protocol, active GVHD is an exclusion (see inclusion/exclusion section), so it is unlikely any patient would be on concurrent cyclosporine.

It is recommended that strong inducers and strong inhibitors of CYP3A4 and P-gp be avoided with rapamycin. Such agents include but are not limited to Rifampin, Rirabutin, Ketoconazole, Voriconazole, Itraconazole, Erythromycin, Telithromycin, and Clarithromycin.

Note: None of these are commonly used in AML patients except voriconazole, and it is recommended to avoid this. (See supportive care section of protocol, section 4.2). If any of these agents or other weaker inducers of CYP3A4 require usage, these can be utilized with caution especially since rapamycin levels will be monitored frequently in this protocol.

Grapefruit juice is to be avoided with rapamycin use.

5.3 Ribavirin (from Ribavirin package insert)

5.3.1 Description-- Ribavirin, (COEPEGUS) is a nucleoside analogue with antiviral activity. The chemical name of ribavirin is 1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide. It is available as a light pink to pink colored, flat, oval-shaped, film-coated tablet for oral administration. Each tablet contains 200 mg of ribavirin and the following inactive ingredients: pregelatinized starch, microcrystalline cellulose, sodium starch glycolate, cornstarch, and magnesium stearate. The coating of the tablet contains Chromatone-P® or Opadry® Pink (made by using hydroxypropyl methyl cellulose, talc, titanium dioxide, synthetic yellow iron oxide, and synthetic red iron oxide), ethyl cellulose (ECD-30), and triacetin.

5.3.2 CLINICAL PHARMACOLOGY

Mechanism of Action

- Ribavirin is an antiviral drug

Pharmacokinetics

- Multiple dose ribavirin pharmacokinetic data are available for HCV patients who received ribavirin in combination with peginterferon alfa-2a. Following administration of 1200 mg/day with food for 12 weeks mean±SD (n=39; body weight greater than 75 kg) AUC0-12hr was 25,361±7110 ng·hr/mL and Cmax was 2748±818 ng/mL. The average time to reach Cmax was 2 hours.
- The terminal half-life of ribavirin following administration of a single oral dose of is about 120 to 170 hours. The total apparent clearance following administration of a single oral dose of is about 26 L/h. There is extensive accumulation of

ribavirin after multiple dosing (twice daily) such that the Cmax at steady state was four-fold higher than that of a single dose.

Effect of Food on Absorption of Ribavirin

- Bioavailability of a single oral dose of ribavirin was increased by co-administration with a high-fat meal.
- Elimination and Metabolism
- The contribution of renal and hepatic pathways to ribavirin elimination after administration of is not known. In vitro studies indicate that ribavirin is not a substrate of CYP450 enzymes.
- Renal Impairment
- A clinical trial evaluated 50 subjects with either moderate (creatinine clearance 30 to 50 mL/min) or severe (creatinine clearance less than 30 mL/min) renal impairment or end stage renal disease (ESRD) requiring chronic hemodialysis (HD). The apparent clearance of ribavirin was reduced in subjects with creatinine clearance less than or equal to 50 mL/min, including subjects with ESRD on HD, exhibiting approximately 30% of the value found in subjects with normal renal function. Pharmacokinetic modeling and simulation indicates that a dose of 200 mg daily in patients with severe renal impairment and a dose of 200 mg daily alternating with 400 mg the following day in patients with moderate renal impairment will provide plasma ribavirin exposures similar to that observed in patients with normal renal function receiving the standard 1000/1200 mg daily dose. These doses have not been studied in patients.

5.3.3 Adverse Effects

6.3.3.1 Ribavirin therapy should not be started unless a report of a negative pregnancy test has been obtained immediately prior to planned initiation of therapy. Extreme care must be taken to avoid pregnancy in female patients and in female partners of male patients. Patients should be instructed to use at least two forms of effective contraception during treatment and for 6 months after treatment has been stopped. Pregnancy testing should occur monthly during therapy and for 6 months after therapy has stopped.

5.3.3.2 Anemia

The primary toxicity of ribavirin is hemolytic anemia, which was observed in approximately 13% of all ribavirin/interferon-treated subjects in clinical trials. Anemia occurs within 1 to 2 weeks of initiation of therapy. Because the initial drop in hemoglobin may be significant, it is advised that hemoglobin or hematocrit be obtained pretreatment and at week 2 and week 4 of therapy or more frequently if clinically indicated. This has not been a factor in AML trials thus far. [24]

5.3.3.3 **Hepatic Failure –this has been noted only in hepatitis C patients.**

5.3.3.4 Other reported toxicities:

Pruritis

Severe acute hypersensitivity reactions .

Low calcium levels

Low magnesium levels

Serious skin reactions including vesiculobullous eruptions, reactions in the spectrum of Stevens-Johnson Syndrome (erythema multiforme major) with varying degrees of skin and mucosal involvement and exfoliative dermatitis (erythroderma) have been reported in patients receiving PEGASYS with and without ribavirin. Patients developing signs or symptoms of severe skin reactions must discontinue therapy.

Pulmonary Disorders

Dyspnea, pulmonary infiltrates, pneumonitis, pulmonary hypertension, and pneumonia have been reported during therapy with ribavirin and interferon.

Bone Marrow Suppression

Pancytopenia (marked decreases in RBCs, neutrophils and platelets) and bone marrow suppression have been reported in the literature to occur within 3 to 7 weeks after the concomitant administration of pegylated interferon/ribavirin and azathioprine. This has been reversible and is of less concern in patients with hematologic malignancies.

Pancreatitis

Ribavirin therapy should be suspended in patients with signs and symptoms of pancreatitis, and discontinued in patients with confirmed pancreatitis.

5.3.4 HOW SUPPLIED/STORAGE AND HANDLING

Ribavirin is available as tablets for oral administration. Each tablet contains 200 mg of ribavirin and is light pink to pink colored, flat, oval-shaped, film-coated, and engraved with RIB 200 on one side and ROCHE on the other side. They are packaged as bottle of 168 tablets (NDC 0004-0086-94).

5.3.5 Storage and Handling

The bottle is stored at 25ºC (77ºF); excursions are permitted between 15ºC and 30ºC (59ºF and 86ºF) [see USP Controlled Room Temperature]. Keep bottle tightly closed.

6.0 STUDY CALENDAR (Appendix 5)

Baseline evaluations are to be conducted within 1 week prior to the start of the protocol unless otherwise specified.

Before beginning the treatment protocol, each patient will have the following performed:

- Complete history and physical exam including: Demographics, past medical and surgical history, medications, allergies, vital signs, height, weight, systemic exam including neurological exam
- EKG and Chest x-ray
- Bone marrow aspirate/biopsy, cytogenetics, molecular diagnostic studies to assess for the presence of FLT-3 or NPM-1 mutations and flow cytometry within 2 weeks of treatment. If these studies were performed elsewhere, an aspirate only will be performed to obtain material to perform the baseline correlative studies that are part of the protocol. (See section 8).
- CBC with differential, platelet count
- Chemistry including complete liver function tests, BUN/Cr, LDH and other electrolytes
- Uric acid level
- Urine analysis and urine pregnancy test for female patients of childbearing potential
- DIC panel
- Peripheral blood for correlative studies (See section 8 and Appendix 5)

After the beginning of each cycle, each patient will have the following tests performed at the noted time points:

- CBC with differential and platelets twice per week
- Chemistry including liver function tests, BUN/Cr and other electrolytes twice weekly
- Triglycerides and cholesterol on Day 1 of each cycle for those on Arm A (rapamycin) only
- Abbreviated physical examination and assessment of performance status
- A bone marrow aspirate only will be performed on Day 10 or 11 of therapy after completion of the decitabine and before initiation of rapamycin or ribavirin in CYCLE 1 ONLY for correlative studies including the phosphorylation status of mTOR effectors and stem cell frequency.
- A trough rapamycin level (arm A only) will be drawn on day 14 and 21 of each cycle. A level will also be drawn on Day 1 of Cycle 2 to assure a subtherapeutic level when decitabine is started in subsequent cycles only if the level on Day 21 was elevated.
- A bone marrow aspirate and biopsy will be performed after the completion of cylces 1, 3, and at the end of the study. This may occur on Days 22 through 26 of the cycle.
- A marrow aspirate and biopsy will also be performed at time of suspected disease progression and at study completion or at any other time the treating physician deems important for disease status determination

7.0 MEASUREMENT OF EFFECT Responses will be assessed at the end of cycle 1 (for progression only) and cycle 3 and at study conclusion or patient withdrawal from study (See 8.2 below).

7.1 Definitions

7.1.1 Complete remission: Normocellular marrow (>20%) with < 5% blasts and no extramedullary leukemia. Absence of dysplasia. Normal peripheral blood counts – ANC>1000 and platelets > 100,000. Patients with these criteria and persistently abnormal cytogenetics will be judged as complete remission, but these aberrations will be noted.

7.1.2 Partial remission: Normocellular marrow (>20%) with 5-20% blasts and no extramedullary leukemia.

7.1.3 Treatment failure: failure to achieve CR or PR.

7.1.4 Stable disease: No change in percentage of marrow blasts.

7.1.5 Relapse: The reappearance of circulating blast cells of the malignant phenotype; > 5% blasts in the bone marrow not attributable to another cause; and/or, the development of extramedullary leukemia.

7.1.6 Hematologic recovery: ANC $\geq$ 1500 for 3 consecutive days; non-transfused platelets $\geq$ 100; no leukemic blasts in the peripheral blood.

7.1.7 Duration of remission/response: defined as the time from documented remission/response to the time of progression.

7.2 Confirmatory Measurement/Duration of Response

7.2.1 A bone marrow biopsy and aspirate will be obtained after cycle 3 and after each cycle and at the end of study at which time determination of a PR or CR will be made.

7.2.2 Duration of overall response

The duration of overall response is measured from the time measurement criteria are met for CR or PR until the first date that recurrent or progressive disease is objectively documented.

The duration of CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented. Note will be made of use of maintenance decitabine.

7.2.3 Progression-Free Survival
PFS is defined as the duration of time from start of treatment to time of progression.

7.2.4 Response Review

All bone marrow biopsies and aspirates will be reviewed by a hematopathologist.

8.0 CORRELATIVE STUDIES

Assessment of eIF4E Expression—This will be done on all patients on pre-study marrow aspirate. Light density marrow cells will be processed, and western blotting performed to determine if the AML is eIF4E positive (increased 2 to 3 Xs above normal controls). Immunohistochemistry will also be carried out with eIF4E and DAPI to document initial nuclear localization by confocal microscopy. Having this information at study entry will allow determination of ribavirin effects on eIF4E localization at subsequent sampling points and will also help us determine if some non-M4/M5 cases express this and whether this might affect rapamycin response.

Documentation of mTOR inhibition

Assays will be performed to determine the feasibility of measuring whether the biologic endpoint of mTOR inhibition has been obtained in those receiving rapmycin and whether eIF4E has been inhibited in those receiving ribavirin.

To assess eIF4E, p70S6 kinase, and 4E-BP1 phosphorylation, leukemia blasts will be isolated via Ficoll-Hypaque density gradient centrifugation, lysed with buffer containing 50 nM Tris, pH 8.0, 120 mM Na CL, 0.5% Nonidet P-40, and protease inhibitor cocktail (Roche). The protein concentration of the lysate will be determined by using the Bradford method (Bio-rad, Hercules, CA). Equal amounts of protein will be separated on a 4%-20% Tris-Glycine gel (Invitrogen) and transferred to a BioTrace PVDF membrane. Blots will be probed with primary antibody to p70S6 kinase and to 4E-BP1 (Cell Signaling, Inc). After washing and incubation with secondary antibody, immunoreactive proteins will be visualized by using the ECL Plus detection system (Amersham Biosciences). Blasts will be sampled pre-study and after day 10-11 of the first cycle and at days 22-26 of the first and 3rd cycle. It is anticipated that the number of blasts may be diminished by the latter two samplings to the point where cell numbers may become limiting in the assay, but we will attempt to determine that this endpoint has been met in the whole light density marrow population. In those cases where aspirate is a dry tap, Western analysis will be performed on peripheral blood. Antibodies for these assays will be purchased from Cell Signaling.

If cell numbers and amounts of protein lysate allow, to assess AKT phosphorylation, leukemic blasts isolated and lysed as described above will be blotted and probed with primary antibody to Akt or phospho-AKT (Cell Signaling). After washing and incubating with secondary antibody, immunoreactive proteins were visualized by using the ECL Plus detection system (Amersham Biosciences). If cell numbers are limiting, Akt and Phospho-Akt as well as p70S6K can be detected with a flow cytometric assay. [23, 34]

Other Correlative Studies to Assess apoptosis, cell cycle, and Leukemia Stem Cell Phenotype

At days 0, 11, and 22-26 of the first cycle, at day 22-26 of the 3rd cycle, and at end of study, light density cells will be analyzed for cell cycle status, apoptosis, and expression of an AML progenitor/blast phenotype (CD34+, CD38-, CD123+) by FACS analysis. CFU-L assays will also be plated. Cells will also be injected into NSG mice to determine presence of an engraftable leukemia stem cell. In brief, approximately 10(6) light density marrow cells will be injected in sublethally irradiated NSG mice via tail vein in a final volume of 0.2mL of PBS with 0.5% FBS. After 6 to 8 weeks, animals will be sacrificed and bone marrow will be analyzed for the presence of human cells by evaluation of human CD45 and human CD33 by flow cytometry [35, 36]. Any remaining cells will be frozen for future molecular analysis of response and resistance markers.

Timing of Correlative Studies

Pre-study marrow

a. Cells for in vitro testing (MTT or Alamar Blue assays, apoptosis, cell cycle, viability, and snap and viable freeze for future molecular analysis.)
b. Western blots for p70S6K, 4EBP-1, Akt, and eIF4E expression (10^7 cells) and eIF4E localization by confocal microscopy (both arms); Gli-1 expression by Western or PCR to determine if this might predict for resistance to ribavirin (Arm B only).
c. CFU-L assay—10(6) cells
d. NSG reconstituting assay –10(7) cells

Day 11 aspirate (cycle 1)

a. Light density cells for apoptosis, cell cycle, and viability
b. Western blots for p70S6K, 4EBP-1, Akt, and eIF4E and expression of downstream mediators; eIF4E localization by confocal microscopy
c. CFU-L assay
d. NSG reconstituting assay

Day 28 cycle 1 (same studies)
End of cycle 3 (same studies)
End of cycle 6 (same studies)
End of study marrow (recommended but optional) (same studies)

9.0 REGULATORY AND REPORTING REQUIREMENTS

Each patient will be monitored for side effects associated with treatment. The NCI Common Toxicity Criteria will be used to assess toxicity (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf). All supportive measures consistent with optimal patient care will be given.

9.1 Adverse Event Definitions

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An adverse event is any untoward medical occurrence in a patient or clinical investigation subject administered an investigational product regardless of causality assessment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product,

whether or not related to the investigational product. An adverse event is unexpected when the specificity or severity is not consistent with the current Package Insert for commercial drug.

A serious adverse event (experience) or reaction is any untoward medical occurrence that at any dose: results in death, is a life-threatening adverse event, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/ incapacity, or a congenital abnormality. The definition of serious adverse event (experience) also includes important medical event. Medical and scientific judgment will be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These will also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse

9.2 Adverse Event Reporting

All adverse events occurring after the patient is assigned to a treatment group are to be recorded in the study records. Medical conditions/diseases present before starting study drug are only considered adverse events if they worsen after treatment assignment.

9.3 Serious Adverse Event Recording

Fatal or life –threatening adverse events will be phoned to the Principal Investigator, the Cancer Center Data Safety Monitoring Committee Chairperson within 24 hours of notification.

Follow-up information may include hospital admission records, discharge summaries and autopsy reports, where applicable.
The Principal Investigator (PI) shall also make an accurate and adequate report to the reviewing Institutional Review Board (IRB) on any serious and unexpected AE.

9.4 Definition of Dose Limiting Toxicity and Determination of MTD

In arm B of the study, a dose limiting toxicity will be a grade 3 or 4 non-hematologic toxicity which is deemed probably or definitely due to study drug (ribavirin or combination of decitabine and ribavirin). As noted above, in this population of AML patients, Grade 3 infections, fatigue, weight loss, and electrolyte imbalances will be appropriately treated but are not deemed study related toxicities and will not result in dose modifications.

9.5 Data and Safety Monitoring

Study Investigators will conduct continuous review of data and patient safety. The Investigator will submit quarterly progress reports of these data to the Clinical Trials Monitoring Committee for review. The review will include for each treatment arm/dose level: the number of patients enrolled, withdrawals, significant toxicities as described in the protocol, serious adverse events both expected and unexpected, dose adjustments, and responses observed. The PI

maintains a database of all adverse events with toxicity grade and information regarding treatment required complications, or sequelae. The Investigator will submit a copy of the AE spreadsheet along with the Progress Report to the Clinical Trials Monitoring Committee for review.

- Any serious adverse event that is serious, related AND unexpected must be reported within 10 calendar days to both the DSMC and the RSRB (see RSRB guidelines).
- Serious adverse events that are related AND expected or unrelated AND unexpected will be reported to the Committee for review at the quarterly meeting. SAE reports are expected to include sufficient detail so that the DSMC can determine the severity, toxicity grade, expectedness, treatment required, and a follow up report documenting resolution or if there are sequelae. Unless otherwise specified in the protocol, serious adverse events that require detailed reports (but not necessarily expedited) are expected, related, non-hematologic toxicities of grades 3, 4 or 5.

The Data Safety Monitoring Committee provides oversight of study progress and safety by review of accrual and events at regularly scheduled meetings. The frequency of review is determined by the size, risk and complexity of the trial, and is assigned by the Protocol Review Committee at the time of their initial review and approval. The Data and Safety Monitoring Committee will monitor all adverse event rates utilizing a cumulative spreadsheet listing of all events submitted along with progress reports by the PI. All serious adverse events that have occurred in the prior 3 months will be reviewed at the regular quarterly meeting of the DSMC in order to confirm toxicity grade, expectedness, relatedness, sequelae, follow up required, and risk to current or future subjects.

Events that are serious, unexpected and related will require expedited review within 10 calendar days to the Safety Coordinator. The DSMC Chair will determine whether further action is required, and when patient safety is of concern, an interim meeting may be called.

11.0 STATISTICAL CONSIDERATIONS

Based on the data of Cashen[15], where response rate to single agent decitabine was about 25 %, our null hypothesis would be that the true response rate r to the combination therapy is less than 25% (i.e. H_0:$r \leq 0.25$). We will use Simon's two-stage design to test this null hypothesis against its one-sided alternative (H_1:$r > 0.25$). Assume that the true response rate in fact exceeds 50%. A maximum of 24 evaluable patients would give 80% power to reject the null hypothesis at a significance level of .05. In the first stage of the phase II study, 9 patients will be initially enrolled to assess the response rate to the combination therapy. If there are 2 or fewer of the 9 patients have responded, the phase II study is stopped for accrual and the combination therapy is determined not active. The probability of this early termination is 60%. If at least 3 out of the first 9 evaluable patients respond in the first stage, 15 additional evaluable patients will be accrued for a total of 24 evaluable patients. The null hypothesis is rejected if 10 or more responses are observed in 24 patients.

The ribavirin dose escalation arm of this study will be a phase I dose escalation plan, so only tabular descriptions of toxicity and outcomes will be generated. The design of the phase I portion of the study is a standard 3+3 up and down design (See Section 7 for details). A minimum of 3 and a maximum of 6 patients will be entered at each dose level. The starting dose level as well as each subsequent dose increment (decrement) will be pre-specified. The first 3 patients will be treated at the starting dose level. If none of the 3 patients experiences dose-limiting toxicity, the dose will be escalated to the next higher level, and another 3 patients will be treated at the escalated level. However, if 1 of the first 3 patients experiences a dose-limiting toxicity, three more patients will be treated at the same dose level. The dose escalation continues until at least two patients among 3 or 6 patients experience toxicities, or until the highest prespecified dose level is reached. The MTD is defined as the highest dose level for which at least 5 of 6 subjects did not experience a DLT. Once the MTD has been established, the study will subsequently expand to a phase II study at the MTD level to obtain further safety and efficacy data. The Data Safety and Monitoring Board of the JP Wilmot Cancer Center will be utilized to review the data between cohorts and prior to escalating from phase I to a phase II portion of the study.

As noted in section 8, the correlative studies will be primarily exploratory. We will utilize a Chi square method to determine a correlation between clinical response and mTOR mediator inhibition as assessed by p70S6 kinase expression or 4EBP1 expression. Both response (yes/no) or decreased expression (yes/no) will be treated as binary variables, and decreased expression will be defined as at least a 25% decrease by densitometry or flow cytometric measurement. We will also determine the mean decrease in expression in the responding and non-responding groups for further descriptive correlations. In the treatment arm with ribavirin, similar analysis will be performed for eIF4E expression.

12.0 RETENTION OF RECORDS

12.1 A Case Report Form will be provided by the Clinical Trials Office and submitted once completed. Periodic monitoring of this CRF will be done to ascertain compliance with source documentation requirements.

U.S. FDA regulations (21 CFR 312.62]) require that records and documents pertaining to the conduct of this study including CRFs, consent forms, laboratory test results, and medication inventory records, must be retained by the Principal Investigator as required by the University of Rochester.

13.0 PATIENT CONSENT AND PEER JUDGEMENT

All institutional, NCI, FDA, State, and Federal regulations concerning informed consent and peer judgment will be fulfilled.

14.0 CHANGES IN PROTOCOL

Any change or addition to this protocol requires a written protocol amendment that must be approved by the investigator before implementation. Amendments significantly affecting the safety of subjects, the scope of the investigation or the scientific quality of the study, require additional approval by the RSRB of the University of Rochester.

Amendments affecting only administrative aspects of the study do not require formal protocol amendments or IRB approval but the IRB of each center must be kept informed of such administrative changes

15.0 REASONS FOR WITHDRAWAL

A patient may withdraw from the study at any time for any reason, and patients are under no obligation to explain reason for withdrawal although this will be queried. Any patient may be discontinued from study in the event of progressive disease, unacceptable drug toxicity, patient refusal to continue on study, or if in the physician's judgment discontinuation is in the best interest of the patient.

16.0 REFERENCES

1. National Cancer Institute. Acute myeloid leukemia. http://www.seer.cancer.gov/stat facts
2. Estey E, Dohner H. Acute myeloid leukemia. Lancet 2006; 368: 1894-907.
3. Estey E. Acute Myeloid Leukemia and Myelodysplastic syndromes in older patients. J Clin Oncol 2007;25:1908-15.
4. Preisler HD, Li B, Chen H, et al. P15INK4B gene methyation and expression in normal, myelodysplastic, and acute myelogenous leukemia cells and in the marrow cells of cured lymphoma patients. Leukemia. 2001;15:1589-1595.
5. Christiansen DH, Andersen MK, Pedersen-Bjergaard J. Methylation of p15INK4B is common, is associated with deletion of genes on chromosome arm 7q and predicts a poor

prognosis in therapy-related myelodysplastic and acute myeloid leukemia. Leukemia. 2003;17:1813-1819.
6. Kim WY, Sharpless NE. The Regulation of INK4/ARF in Cancer and Aging. Cell. 2006;127:265-275.
7. Chen H, Wu S. Hypermethylation of the p15(INK4B) gene in acute leukemia and myelodysplastic syndromes. Chin Med J. 2002;115:987-990.
8. Teofili L, Martini M, Luongo M et al. Hypermethylation of GpG islands in the promoter region of p15 (INK4b) in acute promyelocytic leukemia represses p15 (INK4b) expression and correlates with poor prognosis. Leukemia. 2003;17:919-924.
9. Daskalakis M, Nguyen TT, Nguyen C, et al. Demethylation of a hypermethylated P15/INK4B gene in patients with myelodysplastic syndrome by 5-Aza-2'-deoxycytidine (decitabine) treatment. Blood 2002;100:2957-2964.
10. Kico JM, Spencer DH, Lamprecht Tl, et al. Genomic impact of transient low-dose decitabine treatment on primary AML cells. Blood 2013 121:1633-43.
11. Kantarjian H, Issa JP, Rosenfeld CB et al. Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study. Cancer 2006; 108:1794-80.
12. Kantarjian H, Oki Y, Garcia-Manero G, Huang X, O'Brien S, Cortes J, Faderl S, Busso-Ramos C, Ravandl F, Estrov Z, Ferrajoli A, Wierda,W, Shan J, Davis J, Giles F, Saba HI, Issa J-P. Results of randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia. Blood 2007; 109:53-57.
13. Lubbert M, Ruter B, Claus R, 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. Blood 2007; 110: 95a (abstract).
14. Blum W, Klisovic RB, Hackanson B, Liu Z, et al. Phase I study of decitabine alone or in combination with valproic acid in acute myeloid leukemia. J Clin Oncol 25:3884-91, 2007.
15. Cashen AF, Schiller GJ, O'Donnell MR, DiPersio JF. Multicenter, phase II study of decitabine for the first-line treatment of older patients with acute myeloid leukemia. J Clin Oncol 2010 28:556-61
16. Blum W, Garzon R, Kilsovic RB, Schwind S, Walker A, Geyuer S, Liu S, Havelange V, Becker H, Schaaf L, Mickle J, Deveine H, Kefauver C, Devine SM, Chan KK, Heerema NA, bloomfield CD, Grever MR byrd JC, Villalona-Calero M, Croce cM, and Marcucci G. Clinical response and miR-29b predictive significance in older AML patients treated with a 10-day schedule of decitabine. PNAS 2010 107:7473-7478.
17. Vignot S, Faivre S, Aguirre D et al. MTOR-targeted therapy of cancer with rapamycin derivatives. 2005; 16: 525-537.
18. Panwalker A, Verstovsek S, Giles F. Mammalian target of rapamycin inhibition as therapy for hematologic malignancies. Cancer 2004; 100: 657-666.
19. Martelli AM, Tazzari PL, Evangelisti C et al. Targeting the phosphatidylinositol 3-kinase/AKT/Mammalian target of rapamycin module for acute myelogenous leukemia therapy: From bench to bedside. Current Medicinal Chemistry 2007; 14: 2009-2023.
20. 20Yilmaz OH, Valdez R, Theisen BK, Guo W, Ferguson DO, Wu H, Morrison SJ. Pten dependence distinguishes haematopoeitic stem cells from leukaemia-initiating cells. Nature 2006;44:475-482.
21. 21Hidalgo M, Rowinsky E. The rapamycin-sensitive signal transduction pathway as a target for cancer therapy. Oncogene 2000; 19: 6680-6686.

22. 22Recher C, Beyne-Rauzy O, Demur C. Antileukemic activity of rapamycin in acute myeloid leukemia. Blood 2005; 205: 2527-2534
23. Perl AE, Kasner MT, Tsai DE, Vogl DT. Loren LW, Schuster SJ, Porter DL, Stadtmaurer EA, Goldstein SC, Frey NV, Nasta SD, Hexner EO, Dierov JK, Swider CR, Bagg A, Gewirtz AM, Carroll M, and Luger SM. A phase I study of the mammalian target of rapamycin inhibitor sirolimus and MEC chemotherapy in relapsed and refractory acute myelogenous leukemia. Clin Can Res 2009; 15:6732-9.
24. Assouline S, Culjkovic B Cocalakis e, et al. Molecular targeting of the oncogene eIF4E in acute myeloid leukemia (AML): a proof-of-principle clinical trial with ribavirin Blood 2009 114:257-60.
25. de Lima M, Ravandi F, Anderson B, et al. Long-term follow up of a phase I study of high-dose decitabine, busulfan, and cyclophosphamide plus allogeneic transplantation for the treatment of patients with leukemias. Cancer 2003: 94: 1242-7.
26. Liesveld JL, Rosell K, Lu C, Mulford D, Walker A. The mTOR inhibitor Rapamycin demonstrates activity against AML in combination with imatinib mesylate and with 5-azacytidine. Blood 110:147B (abstract).
27. Liesveld JL, O'Dwyer K, Becker MW, Mulford D, Walker A, Chen R, Bechelli J, Rosell K, Jordan CT, Minhajuddin M, and Phillips GLII. Phase I Study of Decitabine and Rapamycin in Relapsed/Refractory Acute Myelogenous Leukemia Blood 2012 120 Abstract 2549
28. Bennett JM, Catovsky D, Daniel MT et al. Proposed revised criteria for the classification of acute myeloid leukemia. A report of the French-American-British Cooperative Group. Ann Intern Med 1985 103:620-5.
29. Vesley J, Piskala A. Effects of the a-D-anomer of 5-aza-2'Deoxycytidine on L1210 mouse leukemic cells in vitro and in vivo. Cancer Res. 1984; 44:5165-5168.
30. Link T, Momparler RL, Rivard GE. High-performance liquid chromatographic analysis of chemical stability of 5-aza-2'deoxyxytidine. J Pharm Sci. 1981; 70: 1228-1232.
31. Tomankova H, Zyka J. A study of the dependence of the stability of 5-aza-2'deoxycytidine in alkaline media independence on time. MicroChem J. 1980; 25:281-288.
32. Liu Z, Marcucci G, et al. Characterization of decomposition products and preclinical and low dose clinical pharmacokinetics of decitabine (5-aza-2'deoxycytidine) by a new liquid chromatography/tandem mass spectrometry quantification method. Rapid Communications in Mass Spectrometry. 2006; 20:1117-1126.
33. MGI Internal report FD-PSP-001-0600, Dacogen® (decitabine) for injection clinical package insert reconstitution and preparation stability 2006.
34. Chow S, Patel H, Hedley. Measurement of MAP kinase activation by flow cytometry using popspho-specific antibodies to MEK and ERK: potential for pharmacodynamic monitoring of signal transduction inhibitors. Cytometry 46:72-78, 2001.
35. Guzman ML, Rossi RM, Neelakantan S, Li X, Corbett CA, Hassane DC, Becker MW, Bennett JM, Sullivan E, Lachowicz JL, Vaughan A, Sweeney CJ, Matthew W, Carroll M, Liesveld JL, Crooks PA, and Jordan CT. An orally bioavailable parthenolide analog selectively eradicates acute myelogenous leukemia stem and progenitor cells. Blood 2007 110; 4427-4435.
36. Liesveld JL, Rosell KE, Lu C, Bechelli J, Phillips G, Lancet JE, et al. Acute myelogenous leukemia—microenvironment interactions: role of endothelial cells and proteasome inhibition. Hematology 2005:10:483-94.

APPENDIX 1

Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX 2

Cockcroft-Gault Equation: (140-age)*wt/(Cr*72)

APPENDIX 3

Common Terminology Criteria for Adverse Events (CTCAE), version 4.0
(http://ctep.cancer.gov)
Publish Date: June 14, 2010

Pdf: http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf

APPENDIX 4

Declaration of Helsinki

World Medical Association Declaration of Helsinki:

Ethical Principles for Medical Research Involving Human Subjects
Adopted by the 18th WMA General Assembly Helsinki, Finland, June 1964 and amended by the 29th WMA General Assembly, Tokyo, Japan, October 1975 35th WMA General Assembly, Venice, Italy, October 1983 41st WMA General Assembly, Hong Kong, September 1989 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996 and the 52nd WMA General Assembly, Edinburgh, Scotland.

A. INTRODUCTION

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.
2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.
3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."
4. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.
5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.
6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the etiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.
7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.
8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.

9. Research Investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

1. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.
2. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.
3. Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.
4. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.
5. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.
6. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.
7. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.
8. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.
9. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.

10. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.
11. The subjects must be volunteers and informed participants in the research project.
12. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.
13. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.
14. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.
15. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.
16. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.
17. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.
18. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

1. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.
2. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists.
3. At the conclusion of the study, every patient entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study.
4. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.
5. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

APPENDIX 5 (ARM A) Decitabine and Rapamycin

Tests	Pre	Days																						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20-22	22-26	28	EOS
Decitabine		X	X	X	X	X	X[10]	X[10]	X[10]	X[10]	X[10]													
Rapamycin							X[11]	X[11]	X[11]	X[11]	X[11]	X	X	X	X	X	X	X	X	X	X			
History & Physical	X	x																						
CBC w/diff & plts	X	X						X[3]																
Chemistries	X	X[8]						X[3]																
LDH	X					X		X[3]																
Urate	x	x						X[4]																
Bone marrow	X										X[1]												X[1]	X[1]
Flow Cytometry	X																					X		
Cytogenetic Studies	X																					X		
DIC Panel	x																							
Molecular Studies[14]	X																							
Bone marrow for Correlative Studies	X[5]40cc										X[5]40cc											X[5]40cc		
Blood for correlative studies	X[7]10cc										X[7]10cc											X[7] 10cc		
EKG/CXR	X																							
U/A, PT, PTT, Pregnancy	X																							
Chimerism	X[2]																						X[2]	
Rapamycin Levels		X[12]													X[9]						X[9]			
Evaluation of Response																							X[6]	X[6]
Long Term Follow Up																								X[13]

1. Aspirate only in cycle 1on day 10-11 and biopsy and aspirate at days 22-26 of cycles 1 and 3 for disease assessment, and at end of study. Marrows may also be performed when clinically indicated at other times for disease assessment. Substitute peripheral blood if aspirate/marrow cannot be obtained.
2. In those patients who have had an allogeneic transplant by chromosome (sex mismatched transplants) or by PCR assay. This can be done on either blood or marrow at physicians' choice.
3. Twice per week or as clinically indicated
4. As indicated clinically
5. 40 cc aspirate in green top (heparinized) tubes; if marrow is a dry tap, substitute 40 cc blood total
6. Cycles 1 and 3 only and at end of study or study discontinuation.
7. 10cc in green top tube.
8. Include cholesterol and triglycerides on day 1 of each cycle only.
9. Rapamycin levels will be drawn (trough) on days 14 and 21. Patients will be asked to hold the daily dose until the level is drawn.
10. Decitabine is given on days 1-10 during cycle 1. In subsequent cycles, decitabine is given days 1-5.
11. Rapamycin starts on Day 6 starting with Cycle 2
12. Cycle 2 Day 1 only
13. Every 6 months for up to 2 years after date of last treatment
14. Molecular studies completed previously are acceptable if results are in the medical record.

All time points refer to all cycles except where noted. The window for protocol visits and assessments is +/- 3 days.

Correlative studies are described in Section 8 above. Blood and marrow for correlative studies will be sent to the BMT laboratory (275-3041). The study coordinator will contact,Jane Liesveld or her laboratory technician for pick up. All are available through the paging system (275-2222 or web paging).

APPENDIX 5 (ARM B) Decitabine and Ribavirin

Tests	Pre	Days																						EOS
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20-22	22-26	28	
Decitabine		X	X	X	X	X	• X[9]	X[9]	X[9]	X[9]	X[9]													
Ribavirin[8]		X[10]	X[10]	X[10]	X[10]	X[10]	X[10]	X[10]	X[10]	X[10]	X[10]	X	X	X	X	X	X	X	X	X	X			
History & Physical	X	X																						
CBC w/diff & plts	X	X						X[3]																
Chemistries	X	X[8]						X[3]																
LDH	X					X		X[3]																
Urate	X	X						X[4]																
Bone marrow	X										X[1]												X[1]	X[1]
Flow Cytometry	X																					X		
Cytogenetic Studies	X																					X		
DIC Panel	X																							
Molecular Studies[12]	X																							
Bone marrow for Correlative Studies	X[5]40cc										X[5]40cc											X[5] 40cc		
Blood for correlative studies	X[7]10cc										X[7]10cc											X[7] 10cc		
EKG/CXR	X																							
U/A, PT, PTT, Pregnancy	X																							
Chimerism	X[2]																						X[2]	
Evaluation of Response																							X[6]	X[6]
Long Term Follow Up																								X[11]

1. Aspirate only in cycle 1on day 10-11and biopsy and aspirate at days 22-26 of cycles 1 and 3 for disease assessment and at end of study. Marrows may also be performed when clinically indicated at other times for disease assessment. Substitution of peripheral blood may be acceptable if aspirate/marrow cannot be obtained.

2. In those patients who have had an allogeneic transplant by chromosome (sex mismatched transplants) or by PCR assay. This can be done on either blood or marrow at physicians' choice.
3. Twice per week or as clinically indicated
4. As indicated clinically
5. 40 cc aspirate in green top (heparinized) tubes; if marrow is a dry tap, substitute 40 cc blood total
6. Cycles 1 and 3 only and at end of study or study discontinuation.
7. 10cc in green top tube.
8. After cycle 1, ribavirin will begin on day 1 and will be given continuously without interruption unless for toxicity.
9. Decitabine is given days 1-10 during cycle 1. In subsequent cycles, decitabine is given days 1-5.
10. Ribavirin is given continuously starting on day 1 of Cycle 2.
11. Every 6 months for up to 2 years after date of last treatment
12. Molecular studies completed previously are acceptable if results are in the medical record.

All time points refer to all cycles except where noted. The window for protocol visits and assessments is +/- 3 days.

Correlative studies are described in Section 8 above. Blood and marrow for correlative studies will be sent to the BMT laboratory (275-3041). The study coordinator will contact Jane Liesveld or her laboratory technician for pick up. All are available through the paging system (275-2222 or web paging).