A Phase II Study of Azacitidine and Sirolimus for the Treatment of High Risk Myelodysplastic Syndrome or Acute Myeloid Leukemia Refractory to or Not Eligible for Intensive Chemotherapy

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Table of Contents

STUDY SUMMARY 1

1 INTRODUCTION 2

1.1 BACKGROUND 2
1.2 INVESTIGATIONAL AGENT 3
1.3 PRECLINICAL DATA 4
1.4 CLINICAL DATA TO DATE 5
1.5 FLOW CYTOMETRIC ANALYSIS OF SIGNAL TRANSDUCTION IN LEUKEMIC POPULATIONS 6
1.6 DOSE RATIONALE AND RISK/BENEFITS 7

2 STUDY OBJECTIVES 8

2.1 PRIMARY OBJECTIVES 8
2.2 SECONDARY OBJECTIVES 8

3 STUDY DESIGN 8

3.1 GENERAL DESIGN 8
3.2 STUDY ENDPOINTS 8

4 SUBJECT SELECTION AND WITHDRAWAL 12

4.1 INCLUSION CRITERIA 12
4.2 EXCLUSION CRITERIA 12
4.3 SUBJECT RECRUITMENT AND SCREENING 13
4.4 EARLY WITHDRAWAL OF SUBJECTS 14

5 STUDY DRUG 14

5.1 SIROLIMUS 14
5.2 AZACITIDINE 15
5.3 TREATMENT REGIMEN 16
5.4 PREPARATION AND ADMINISTRATION OF STUDY DRUG 18
5.5 SUBJECT COMPLIANCE MONITORING 18
5.6 PRIOR AND CONCOMITANT THERAPY 18
5.7 PACKAGING 18
5.8 RECEIVING, STORAGE, DISPENSING AND RETURN 18

6 STUDY PROCEDURES 18

6.1 CORRELATIVE STUDIES 19

7 STATISTICAL PLAN 20

7.1 SAMPLE SIZE AND STUDY DURATION 20
7.2 STATISTICAL ANALYSIS 20

8 SAFETY AND ADVERSE EVENTS 21

8.1 DEFINITIONS 21
8.2 RECORDING OF ADVERSE EVENTS 22
8.3 SAFETY REPORTING 23
8.4 STOPPING RULES 26

9 DATA HANDLING AND RECORD KEEPING 26

9.1 CONFIDENTIALITY 26
9.2 SOURCE DOCUMENTS 26
9.3 CASE REPORT FORMS 26
9.4 RECORDS RETENTION 27

10 CLINICAL SITE MONITORING AND AUDITING 27

11 ETHICAL CONSIDERATIONS 27

12 STUDY FINANCES 27

12.1 FUNDING SOURCE 27
THE DEPARTMENT OF MEDICAL ONCOLOGY WILL BE PAYING FOR THIS STUDY 27
12.2 CONFLICT OF INTEREST 27

13 PUBLICATION PLAN 27

14 REFERENCES 28

15 ATTACHMENTS 30

List of Abbreviations

ALL	Acute Lymphoblastic Leukemia
ANC	absolute neutrophil count
AML	Acute Myeloid Leukemia
AZA	Azacitidine
BUN	blood urea nitrogen
CALGB	Cancer and Leukemia Group B
CBC	complete blood count
CML	Chronic Myeloid Leukemia
CNS	central nervous system
CR	complete response
CRF	case report form
CRp	Complete response in absence of total platelet recovery
CTC	common toxicity criteria
D5W	5% dextrose in water
DLT	dose-limiting toxicity
G-CSF	filgrastim (granulocyte-colony stimulating factor)
GM-CSF	Sargramostim
Hyper-CVAD	Hyperfractionated Cyclophosphamide, Vincristine, Doxorubicin, Dexamethasone
IEC	Independent Ethics Committee
IRB	Institutional Review Board
LFS	Leukemia Free Survival
MDS	Myleodysplastic Syndrome
MEC	Mitoxantrone + Etoposide + Cytarabine
MTI	mTOR inhibitor
NCI	National Cancer Institute
OPRR	US Dept of Health & Human Serv./Office for Protection from Research Risk
PD	progressive disease
PLT	Platelets
PR	partial response
PS	performance status
RR	response rate
RT	Radiotherapy
SAE	Serious Adverse Event
SD	stable disease
SGOT	serum glutamate-oxaloacetate transaminase
SGPT	serum glutamate-pyruvate transaminase
ULN	upper limit of normal
US	United States

Study Summary

Title	**A Phase II Study of Azacitidine and Sirolimus for the Treatment of High-risk MDS and Patients with Acute Myeloid Leukemia Refractory To or Not Eligible for Intensive Chemotherapy.**
Short Title	Sirolimus/Azacitidine
Phase	II
Methodology	Open label
Accrual Duration	83 months
Study Center(s)	1. Jefferson Health Network (Center City Hospital, Abington Hospital, Aria Hospital, Methodist Hospital and NJ Division (Kennedy) Hospital
Objectives	This is a study to determine estimate of efficacy of sirolimus when administered with azacitidine. Secondary objectives are to determine the pharmacodynamic effect of rapamycin on inhibition of mTOR and the safety of the combination of the regimen.
Number of Subjects	74;
Diagnosis and Main Inclusion Criteria	• Adults with high-risk MDS or relapsed or refractory AML or those unable or unwilling to tolerate high dose chemotherapy. • Age ≥18
Study Product, Dose, Route, Regimen	Sirolimus loading dose of 12mg on day 1 followed by a single daily dose of 4 mg/ day on days 2 through 10 or 12 Azacitidine 75 mg/m^2/d for 5 consecutive days, two days rest, then two consecutive days every 28 days or: Azacitidine 75 mg/m^2/d for 7 consecutive days. After 2 cycles, may increase dose to 100 mg/m^2
Duration of administration	Until disease progression or toxicity requiring discontinuation of the drug
Reference therapy	Fenaux et al. J ClinOncol. 2010 Feb 1;28(4):562-9.
Statistical Methodology	This study is a phase II trial. The study will be stratified to arms A, B, and C. Arm A (high risk MDS) will need 40 patients total for an optimal Simon 2-stage design (with alpha=0.1 and 80% power) with 23% standard response rate vs. an expected 40% response rate. Arm B (AML) We will need 43 patients total for an optimal Simon 2-stage design (with alpha=0.05 and 80% power) with 20% standard response rate vs. an expected 40% response rate. Arm C (either MDS or AML with prior Azacitadine therapy) will need 29 patients total for an optimal Simon 2 stage design (with alpha = 0.05 and 80% power) with a 5% response rate versus a 20% response rate.

1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

1.1 Background

Treating high risk myelodysplastic syndrome (MDS), refractory acute myelogenous leukemia (AML), and AML in patients unable to tolerate conventional, high-dose chemotherapy remains, despite advances in understanding the basic science of these diseases, a therapeutic challenge.

MDS is characterized as a malignant, clonal disorder of hematopoetic stem cells which results in cytopenias in the peripheral blood and hypercellular bone marrow with or without trilineage dyspoeisis.[1] Several scoring systems have been created to determine subtype of disease and help clinicians estimate prognosis.[1,2] The most clinically relevant of these today include the World Health Organization's (WHO) histological classification of disease and International Prognosis Scoring system (IPSS) prognostic criteria. The WHO separates disease into eight different categories: refractory anemia (RA), refractory anemia with ringed sideroblasts (RARS), refractory anemia with excess blasts-I (RAER-I), refractory anemia with excess blasts-II (RAER-II), refractory anemia with mutlilineage dysplasia (RCMD), and refractory anemia with multilineage dysplasia and ringed sideroblasts (RCMD/RS). The IPSS system assigns a risk score based on percentage of bone marrow blasts, karyotype, and number of cytopenias and classifies patients into low risk, intermediate risk-1, intermediate risk-2 (IR-2), and high-risk disease. Because of similar survival and transformation to AML outcomes, IR-2 and HR have been classified together as high-risk disease.

The hypomethylating agents, azacitidine and decitabine, are considered first line for the treatment of higher-risk MDS, especially those older and less likely able to tolerate intensive chemotherapy (ICT) or allogenic stem cell transplants (alloSCT).[3] While ICT and alloSCT can potentially induce long-responses, the toxicity of these treatment modalities moderates their clinical usefulness, especially in MDS, where average age of diagnosis is70.[3,4] Several large scale trials have demonstrated the effectiveness of the hypomethylating agents in MDS.[5, 6] The phase III CALGB-9221 trial randomized 191 MDS patients to either receive AZA 75 mg/m2/d for 7 consecutive days every 28 days or a best supportive care, with 60% of patient responding to treatment in the AZA versus 5% in the best supportive care arm. AZA-001 was larger trial conducted in the high risk MDS group and compared azacitidine to a predetermined conventional care regiment consisting of either best supportive care, low-dose cytarabine or intensive chemotherapy. 358 patients were enrolled and followed for 12 months or death, whichever occurred first. Median survival for AZA group was 21.1 months versus 11.5 in the conventional care group.

Despite the advances of hypomethylating agents, the prognosis of high risk MDS remains unsatisfactory, with a median time to leukemic transformation or death that approaches 21 months .[5] For patients whose disease has progressed through hypomethylating agents, survival is poor. In one series, in patients who progress through decitabine, median survival was less than 5 months.[7]

Recent smaller studies have explored the effectiveness of the hypomethylating agents alone and in combination with other drugs in AML.[8-10] Fenaux et al compared azacitidine to predetermined (i.e. before randomization) conventional care regiments. All patients in this retrospective analysis were previously described under the older French-American-British (FAB) classification to have refractory anemia with excess blasts in transformation (RAEB-T) corresponding to a bone marrow biopsy containing 20-30% blasts. Subgroup analysis demonstrated a significant improvement in median survival of the azacitidine group at 24.5 months (95% CI 14.6 – NR) vs conventional care at 14 months (95% CI 11.5-17.5) and an overall improvement in survival at 2 years of 50% vs 16% respectively. Cashen et al studied low-dose (20mg/m^2) decitabine's effectiveness in treatment naïve AML patients over 60 and unable to tolerate conventional high-dose chemotherapy. Their single arm, multicenter, phase II trial showed of the 54 patients studied an overall response rate of 25%, with median survival rate 5.7 months (95% CI, 5.7 to 11.6 months). Major toxicities attributed to treatment included myelosupression, febrile neutropenia, and

fatigue. Recently, Maurillo et al retrospectively evaluated 83 patients with WHO-designated AML treated with azacitidine in a compassionate use fashion.[11] Patients who received at least one cycle of azacitidine were evaluated, and overall, 26 of 82 examined demonstrated at least partial response to treatment, including 12 CRs (15%), 4 CRi (5%), 10 PR (12%). Those with previously treated disease, as well as those with secondary disease did significantly more poorly. Median overall survival was impacted by pretreatment status as well, with those untreated with a median OS of 9 months verses 7 months in the pretreatment group. Response to azacitidine, as in previous reports, was demonstrated between the 4-6 cycle.

In vitro activity of the combination of azacitidine and sirolimus has been preliminarily studied by one group from China. Sun et al tested the combination of drugs against single agent in two gastric cancer lines. MTT survival assays demonstrated additive effect on reduction of cell viability. Western blots demonstrated a marked increase in the upregulation of PTEN and p27 in cell lines treated with both drugs.[12] Zhang et al demonstrated a similar additive effectiveness both in vitro colorectal cancer lines treated and in vivo with mouse models treated with the combination of azacitidine and sirolimus. [13]

Recently, the preliminary results of a combination of azacitidine and MTOR inhibitors in AML have been presented at an international meeting. Wei et al studied the effect of the combination of azacitidine and everolimus on 37 relapsed and refractory AML patients.[14] 32% had a clinical response with a median OS of 211 days and PFS of 178 days, demonstrating the potential for significant activity in this notably difficult population to treat.

We seek new strategies to lengthen survival in patients with high risk MDS, AML refractory to treatment or unable to tolerate ICT. We propose to add a novel agent, an mTOR inhibitor (MTI), sirolimus, to act synergistically with azacitidine to improve response rates. In addition, we note that the benefit of azacitidine is limited by acquired resistance and the addition of sirolimus may rescue or augment the response to those previously treated with azacitidine. This protocol details a phase II study to determine the efficacy of the combination of the two drugs in these settings.

1.2 Investigational Agent

Rapamycin, or sirolimus (Rapamune®, Wyeth), is a naturally occurring compound originally isolated from a soil saprophyte (*Streptomyces hygroscopicus)* found uniquely on Easter Island (Rapa Nui). In addition to its immunosuppressive properties (on which the clinical development of the drug has focused), sirolimus has antifungal, antiviral and antineoplastic properties. It is FDA approved for immunosuppression following solid organ transplant. Rapamycin analogs such as RAD001 have shown anticancer activity in clinical trials of solid and hematologic malignancies and are undergoing FDA review for registration. It is felt that the antitumor mechanism of these derivatives does not differ from that of rapamycin.

Although structurally similar to calcineurin inhibitors, rapamycin binds uniquely to FK binding protein 12 (FKBP12) and then complexes with mTOR (mammalian Target of Rapamycin). Rapamycin does not interact with calcineurin or its downstream effectors. The rapamycin-FKBP12-mTOR complex inhibits several distinct biochemical pathways, resulting in a reduction in DNA transcription, DNA translation, protein synthesis and cell cycling. Ultimately this leads to the inhibition of the induction of activation and proliferation of mature T and B cells.[15, 16]

Upstream pathways that interact with mTOR include the PTEN/PI3 kinase/Akt pathway which regulates cell growth, protein synthesis, and progression through the cell cycle. By inhibiting mTOR, rapamycin mimics growth-factor withdrawal.[17] This is a novel site of blockade not currently targeted by conventional cytotoxic agents.

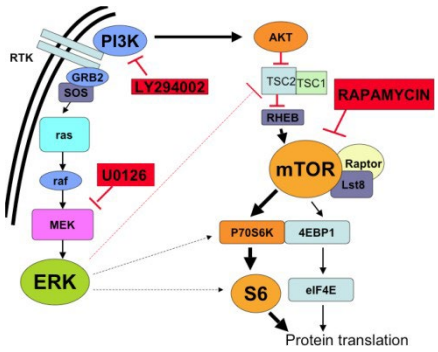


FIGURE 1: *Schematic of PI3K/AKT/mTOR (right) and ras/MAPK (left) signaling in AML. Pharmacologic inhibitors shown in red. Black arrows show stimulation, red bars inhibition. In AML, mutations or autocrine stimulation of transmembrane receptor tyrosine kinases (RTK) such as FLT3or c-KIT initiates signaling through downstream pathways, and activate translational machinery through S6 ribosomal protein (S6). Although the primary input to S6 is mTOR, note that ERK can fine tune the function of S6 cross-talk with PI3/K/mTOR at several levels (dotted lines).*

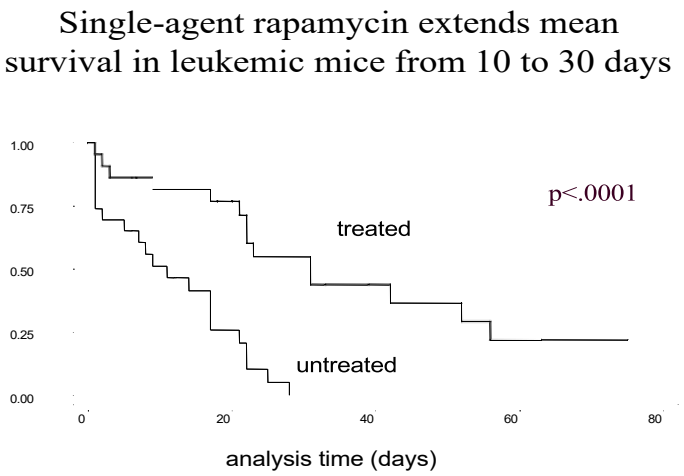
The convention accepted by the medical community has been to call the compound used for clinical therapy Sirolimus (trade name Rapamune) and the compound used in laboratory testing is evolution Rapamycin.

1.3 Preclinical Data

There is extensive evidence that MTI (mTOR inhibitors) inhibit the growth of and/or induce apoptosis in a wide variety of tumor types.[18-21] Additionally there is evidence of preclinical activity of MTI in leukemia as well as data supporting the potentiation of the activity of cytotoxic agents by MTI. Most importantly the inhibitory properties of rapamycin in mature lymphocytes and mature B-cell lymphomas in addition to the following work done at our institution suggest that these diseases may provide particularly exciting targets for MTI.

Researchers at the University of Pennsylvania campus have pioneered the study of mTOR inhibitors in the therapy of hematologic malignancies. The Wasik laboratory in the Department of Pathology originally demonstrated that the mTOR inhibitor, RAD001, or everolimus, was efficacious in a xenotransplantation model of post-transplant lymphoproliferative disease.[20] RAD increased the apoptotic rate in such cells and the drug had a profound inhibitory effect on the growth of PTLD-like Epstein-Barr virus+ B cells xenotransplanted into SCID (severe combined immunodeficient) mice.

Dr. Carroll's laboratory, also at Penn, has been studying the mTOR signaling pathway in primary cells from patients with acute myeloid leukemia. Dr. Carroll's group has shown that the signaling pathway is activated in the great majority (at least 80%) of patient's leukemic cells.[22] Furthermore, incubation of the cells with rapamycin or RAD leads to a modest decrease in survival of the leukemic cells. Other groups have published data on the ability of MTI to induce differentiation of human myeloid leukemia cells.[23] More importantly, combining RAD with chemotherapy leads to a dramatic enhancement in the efficacy of the chemotherapy. Studies suggest that prolonged administration of RAD inhibits AKT. This novel effect of mTOR inhibitors may prove critical for synergy with chemotherapy.[24-27] Based on these studies, a Phase I trial of Sirolimus with MEC chemotherapy for patients with refractory or relapsed AML at Penn was performed at HUP and a Phase II trial has recently finished enrollment.[27] See section 1.4 for clinical data details.



Dr. Grupp's laboratory at the Children's Hospital of Philadelphia (CHOP) has also studied the effect of rapamycin on lymphoid malignancy in an animal model of acute lymphocytic leukemia (ALL).[17] Work at CHOP using sirolimus in precursor B cell malignancies and both human and murine pre-B ALL cell lines have shown both inhibition of growth in culture as well as induction of apoptosis in these cells. The active drug concentration in these studies was well below the achievable serum level in patients. Furthermore, sirolimus is active as a single agent in a murine model of B-precursor leukemia/ lymphoma. Em-Ret transgenic mice are a model of precursor B cell malignancy. These mice develop B-precursor malignancies between 4 and 7 months of life as a result of the activated tyrosine kinase expressed in the B lineage. In these experiments, leukemic mice with significant disease burdens were treated with sirolimus. When compared to untreated littermates, sirolimus-treated mice survived almost 3-fold longer (see Kaplan-Meier analysis of survival in treated and untreated mice in the figure above). In addition to extending survival, sirolimus also normalized the significantly elevated peripheral white blood cell counts in treated mice. There was no statistically significant difference between treated and untreated mice with respect to the hemoglobin or platelet counts. In addition to growth inhibition, there is evidence from experiments performed in the Grupp lab that MTI induce apoptosis in ALL cells.[28,30]

Finally, the Grupp lab has developed 2 assays using primary lymphoblasts obtained from ALL patients: bone marrow stromal layers and NOD/SCID ALL xenografts. Using these models it was shown that MTI treatment resulted in significant decrease in absolute blast count in 4 separate patients as compared to controls. The latest experiments preformed in the Grupp lab demonstrate the ability of rapamycin to enhance the activity of the chemotherapeutic agent methotrexate. Given that this is likely through cell cycle arrest it is reasonable to infer that this is not agent specific and will be applicable to other chemotherapeutic regimens.

1.4 Clinical Data to Date

Several trials using rapamycin as an antitumor agent have been published. A Phase II study of CCI-779 (a second generation MTI in IV formulation that is metabolized to rapamycin) in patients with advanced refractory renal cell carcinoma showed the MTI to have antitumor activity and it was generally well tolerated.[2626] A review by Vignot in 2005 describes responses in lung, renal cell, breast carcinoma and neuroendocrine tumors in the Phase I and Phase II setting.[29] A Phase I trial of CCI-779 in combination with 5-FU and leucovorin showed rash as a prominent toxicity at all dose levels and the dose limiting toxicity (DLT) was stomatitis which resulted in 2 treatment-related deaths from bowel perforation.[3019] Interim Phase II data on a second generation MTI in sarcoma patients demonstrated sustained anti-tumor activity with mild to moderate adverse events including mucositis, rash, fatigue, nausea and hyper-triglyceridemia. A phase II trial of Temsirolimus (CCI-779) for relapsed mantle cell lymphoma showed a 38% response rate with the most frequent toxicities being hematologic.[31]

Larger scale trials of mTOR inhibitors in breast cancer (BOLERO-2 and TAMRAD) have demonstrated that the addition of an mTOR inhibitor can rescue the response to previously effective anti-hormone therapies. The authors concluded that the strategy of adding an oral agent to previously tolerated chemotherapy is an attractive option to regain clinical benefit. [42, 43]

We, along with the University of Pennsylvania have completed a phase I trial and 2 follow up trials of sirolimus with mitoxantrone, etoposide, and cytarabine in relapsed, refractory, or secondary AML. The maximum tolerated dose (MTD) was found to be a 12mg loading dose and 4mg daily dose. 27 patients were treated at this dose. Grade 3 or higher toxicities seen in patients treated at or below the MTD were neutropenic fevers in all patients, documented infections in 15 subjects, transient liver function abnormalities in three subjects, non-infectious diarrhea in two subjects, mucositis in two subjects, and transient metabolic abnormalities (hyperglycemia, hypocalcemia, hyperkalemia, hypernatremia), transient cerebellar ataxia, or thrombosis all in one subject each. We recently reported data from a phase I/II trial of the combination of sirolimus and MEC in relapsed/refractory AML that correlated response to combination therapy with baseline mTOR activation. Patients whose leukemic blasts at baseline had constitutive S6 phosphorylation and who experienced a greater that 50% reduction in the number of blast with pS6 had an impressive 67% response rate, while only 33% of those non-phosphorylated at baseline or rapmycin non-responders has a clinical response.

Our group completed a pilot trial in ALL patients combining sirolimus at the same doses with hyperCVAD and no safety issues were encountered. We have completed enrollment on a phase II trial of sirolimus MEC at Jefferson and the University of Pennsylvania and again, no safety concerns were seen therefore we believe combining this dose of sirolimus with azacitidine will not lead to unexpected toxicities.

1.5 Flow cytometric analysis of signal transduction in leukemic populations

Flow cytometry is a powerful technique for discriminating cells in suspension based upon their immunophenotype.[32,33] It is extraordinarily well suited to both clinical/diagnostic and research fields in leukemia, but has seen little use as a pharmacodynamic analyzer for clinical trials. Recently, advanced techniques have allowed for permeabilization of fixed cells such that the intracellular contents can be examined by this methodology.[34-36] While phospho-specific antibodies have demonstrated the feasibility of extensive intracellular flow cytometric studies on cell lines, marrow and blood samples from patients present a substantial challenge to the methodology due to the admixture of malignant and non-malignant populations. The harsh fixative and detergents or alcohols used to permeabilize cells traditionally create significant artifact, making interpretation challenging.[37] Careful protocols must be therefore be developed that allow for preservation of cell surface epitopes throughout the various fixation, permeabilization, and red cell lysis steps. This has been a major obstacle to widespread use of this technology.

Recently, a whole blood technique for fixation and subsequent permeabilization has been developed and optimized by Chow and colleagues.[38] This methodology has been explored thoroughly in clinical leukemia samples including clinical trials of signal transduction inhibitors by David Hedley's group in Toronto.[39-41] The method is simple and rapid and allows for examination of cytokine stimulation as well as signaling inhibition by various drugs. Importantly, the technique efficiently fixes cell processes, lyses red blood cells, and permeabilizes cells, yet preserves light scatter and cell surface epitopes. This allows for examination of signaling within cells based upon their immunophenotype and light scatter properties. Because simple dot-plots of orthogonal light scatter and hematopoietic markers such as CD45 allow for segregation of hematopoietic cells into lymphocyte, monocyte, neutrophils, granulocytic progenitors, and blast populations,[31] each can be gated and individually explored for intracellular signaling.

In our lab, after optimizing our approach for clinical samples, we explored its potential using peripheral blood samples collected from five subjects from the UPCC 02407 pilot study of Rapamycin with Mitoxantrone, Etoposide and Cytarabine (MEC) in relapsed or refractory AML. Of note, 3 of these 5 subjects had pancytopenia with too few circulating blasts to perform standard Western blot with confidence. All five subjects' baseline samples showed subset S6 positivity in leukemic blasts but not lymphocytes or granulocytes. Four of the five subjects showed definite inhibition of S6 on the sample acquired after 72 hours of sirolimus therapy. Interestingly, in none of these samples obtained at trough

sampling was ex vivo treatment of their post-Rapamycin sample by 1000 nMrapamcyin able to further inhibit S6 phosphorylation.

This suggests that in vivo mTOR inhibition is maximal and that the optimal biologic dose has been achieved with our dose and schedule.

Thus, we demonstrated the feasibility of whole blood processing and flow cytometric data acquisition to provide leukemia specific signaling information devoid of contamination from non-malignant populations. We also demonstrated that ex vivo stimulation and pharmacologic inhibition is necessary both to define positive and negative gates to compare response. It also provides useful data at the pharmacokinetic/pharmacodynamic interface to clarify dosing of mTOR inhibitors. Overall, intracellular flow cytometry is a powerful technique for the application of interpreting the results of clinical trials of signal transduction inhibitors targeting PI3K/AKT/mTOR pathway in AML and, we anticipate in MDS as well.

In a pilot study of sequential sirolimus and MEC, we found flow cytometry yielded consistent and interpretable data in marginal samples with so few blasts that they were not amenable to Western blot analysis at all. Furthermore, even using these samples, we were still able to look specifically at signaling *within* blasts. This yielded leukemia-specific data with confidence and allowed for improved paired comparisons from baseline to post-treatment samples. Western blot can be quite robust among clinical samples where blasts are the predominant cell type among mononuclear cells and the percentage of malignant to non-malignant cells does not vary significantly from collection time point to time point.

1.6 Dose Rationale and Risk/Benefits

Sirolimus will be given as follows: A loading dose of 12mg by mouth will be given on Day 1, and then 4mg by mouth every 24 hours for 9 doses, from Days 2 to 10 inclusive every 28 days if patient is getting consecutively dosed azacitidine, and 11 daily doses Days 2-12 if receiving split doses.

Duration of administration is until disease progression or toxicity requiring discontinuation of the drug.

Azacitidine will be given as follows: 75 mg/m^2/d for 5 consecutive days with two day break and then two additional consecutive days of treatment, i.e. from Day 4-8, 11 and 12 inclusive at least every 28 days. and no longer than 42 days Alternately, azacitidine will be given for 7 consecutive days, day 4-10 inclusive, at least every 28 days and no longer than 42 days.

After 2 cycles (per the current package insert), may increase dose to 100 mg/m^2 if no beneficial effect is seen and no toxicity other than nausea and vomiting has occurred.

EXPERIMENTAL DESIGN SCHEMA*:*

DAY:	1*	2	3	4	5	6	7	8	9	10	11	12
Sirolimus:	12mg	4mg	4mg	4mg	4mg	4mg	4mg	4mg	4mg	4mg	4mg	4mg
Azacitidine:				X	X	X	X	X			X	X
Azacitidine-Alt:				X	X	X	X	X	X	X		
PD:	X[1]			X[2]								
PK:				X[3]								

PD[1]-Sample drawn before sirolimus dosing.
PD[2]- Sample drawn before azacitidine administration
PK[3]- Sample drawn before sirolimus and azacitidine administration

*The dose of azacitidine used in this trial is the FDA approved dose, stemming from multiple phase II-III trials.

This dose of sirolimus was discovered to be the MTD in the Phase 1 trial evaluating sirolimus in addition to MEC chemotherapy for relapsed/refractory AML. It was also utilized in a pilot trial for AML patients, a pilot trial in ALL patients, and a larger phase II AML trial conducted at our institution and/or the University

of Pennsylvania.

Prospective subjects will be informed of all anticipated and possible unanticipated adverse effects of drug treatments. All of the drugs studied in this proposal have been used widely in humans at the doses described. Anticipated treatment-related adverse events include myelosuppression, febrile neutropenia and fatigue. Alternative therapies, including standard chemotherapy, investigational agents, and supportive care-only, will be discussed with potential subjects prior to informed consent. Prospective subjects will also be advised of issues related to confidentiality in accordance with HIPAA guidelines. Because sirolimus is an immunosuppressant, the infectious toxicity of this regimen may be increased compared to azacitidine alone.

A total of 6 infectious deaths have occurred in the more than 80 subjects treated on both sirolimus-MEC studies (7.5%) including 4/62 (6%) at the doses planned for this trial. This rate of toxic deaths does not differ from published data, but this important safety signal will be monitored closely. A stopping rule with respect to safety is included in our design and consideration for early termination will be made in consultation with the medical monitor and biostatistician if excess toxicity is suspected.
Should the combination of azacitidine and sirolimus improve complete response rate, participation would provide substantial benefit for study participants. An additional and more certain benefit is the scientific knowledge gained from this clinical trial's correlative studies. This may improve understanding of chemotherapy response in AML and other myeloid malignancies. Although study participants cannot be guaranteed benefit, the information gained may benefit cancer patients in the future.

2 Study Objectives

2.1 Primary Objectives

- To characterize the rate of response to azacitidine and sirolimus in adults with high-risk MDS, or relapsed or refractory AML or those unable or unwilling to tolerate high dose chemotherapy**.**

2.2 Secondary Objectives

- To determine the pharmacodynamic effect of sirolimus on inhibition of mTOR signaling in adults with high-risk MDS, or relapsed or refractory AML or those unable or unwilling to tolerate high dose chemotherapy.

- To determine the safety and tolerability of sirolimus and azacitidine in adults with high-risk MDS, or relapsed or refractory AML or those unable or unwilling to tolerate high dose chemotherapy.

- To determine the progression free survival and overall survival in adults with high-risk MDS, or relapsed or refractory AML or those unable or unwilling to tolerate high dose chemotherapy
- To determine if the quality of life of patients is improved with the combination of azacitidine and sirolimus when compared to historical controls of azacitidine alone.

3 Study Design

3.1 General Design

3.1.1 Therapy timeline

Refer to section 5.3 for the detailed treatment regimen.

3.2 Study Endpoints

3.2.1 Primary Endpoint

This study's primary endpoint is to determine the rate of response with azacitidine and sirolimus in adults high-risk MDS, or relapsed or refractory AML or those unable or unwilling to tolerate high dose chemotherapy.

3.2.1.1 Response Criteria

3.2.1.1.1 Response Criteria – MDS

- **Complete Response:**
- Bone marrow:
  - <5% myeloblasts with normal maturation of all cell lines
  - Persistent dysplasia will be noted
- Peripheral blood
  - Hgb >11 g/dL
  - Platelets 100x10/L
  - Neutrophils 1.0x10/L
  - Blasts 0%

- **Marrow Complete Response**
- Bone marrow:
- 5% myeloblasts and decrease by _ 50% over pretreatment†
- Peripheral blood: if HI responses, they will be noted in addition to marrow CR

- **Partial remission**
- All CR criteria if abnormal before treatment except:
- Bone marrow blasts decreased by > 50% over pretreatment but still < 5%
- Cellularity and morphology not relevant

- **Cytogenetic response**
- **Complete**
  - Disappearance of the chromosomal abnormality without appearance of
  - new ones
- **Partial**
  - At least 50% reduction of the chromosomal abnormality

- **Hematologic Improvement** - Response criteria responses must last at least 8 weeks and are categorized by cell line impairment at time of diagnosis:
  - **Erythroid response** requires that pretreatment hemoglobin (Hgb) must be Less than 11 g/dL to qualify for response and post-treatment, either:

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

  - **Platelet response** requires pretreatment must be < 100 x 10^9/L to qualifyfor response and post-treatment, either:
    - Absolute increase of ≥ 30 x 10^9/L for patients starting with > 20x 10^9/L platelets

    - Increase from < 20 x 10^9/L to > 20 x 10^9/L and by at least 100%

  - **Neutrophil response** requires pretreatment must be < 1.0 x 10^9/L to qualify for response and at least 100% increase and an absolute increase > 0.5 x 10^9/L.

- **Stable disease**
- Failure to achieve at least PR, but no evidence of progression for 8 wks

- **Disease progression**
- For patients with:

- Less than 5% blasts: 50% increase in blasts to 5% blasts
- 5%-10% blasts: 50% increase to 10% blasts
- 10%-20% blasts: 50% increase to 20% blasts
- 20%-30% blasts: 50% increase to 30% blasts

- Any of the following:
- At least 50% decrement from maximum remission/response in
  - granulocytes or platelets
  - Reduction in Hgb by 2 g/dL
  - Transfusion dependence

  - **Failure**
  - Death during treatment or disease progression characterized by worsening of cytopenias, increase in percentage of bone marrow blasts, or progression to more advanced MDS FAB subtype than pretreatment

  - **Relapse after CR or PR**
  - At least 1 of the following:
- Return to pretreatment bone marrow blast percentage
- Decrement of 50% from maximum remission/response levels in
- granulocytes or platelets
- Reduction in Hgb concentration by 1.5 g/dL or transfusion dependence

3.2.1.1.2 Response Criteria - AML

- **Complete Remission (CR)** -
  - Peripheral Blood Counts -Neutrophil count 1 x 10^9/L.
  - Platelet count ≥ 100 x 10^9/L.
  - Reduced hemoglobin concentration or hematocrit has no bearing on remission status.
  - Leukemic blasts must not be present in the peripheral blood.
  - Cellularity of bone marrow biopsy must be > 20% with maturation of all cell lines with < 5% blasts.
  - Extramedullary leukemia, such as CNS or soft tissue involvement, must not be present.
- **Complete Response in the absence of total platelet recovery (CRp**)
  - Bone marrow (<5% blasts) with adequate bone marrow cellularity, no evidence of circulating blasts or extramedullary disease and normalization of peripheral blood counts except for platelets (neutrophil count =1,000/µL).
- **Partial Remission (PR)**
  - Requires that all of the criteria for complete remission be satisfied except that the bone marrow may contain > 5% blasts but < 25% blasts. A marrow with <5% blasts that contain Auer rods will also be considered a PR
- **Progressive Disease (PD)**
  - An increase of at least 25% in the absolute number of leukemic cells in peripheral blood or bone marrow/aspirate, the development of extramedullary disease, or other evidence of increased tumor burden.
- **Relapse**
  - Relapse following complete remission is defined as: Reappearance of blasts in the blood.
  - Presence of 5% blasts, not attributable to another cause (e.g., bone marrow regeneration).
  - If there are no circulating blasts and the bone marrow contains 5% to 20% blasts, then a repeat bone marrow performed 1 week later documenting more than 5% blasts is necessary to meet the criteria for relapse.

Patients achieving a CR, CRp, or PR will be considered responders. Given the lag between first administration of azacitidine and clinical response, bone marrow examinations will be performed before entry into study, at 6 months, and then at least 6-month intervals afterwards. The biopsies will help

document response to treatment for both MDS and AML patients and to determine endpoints where necessary. Best response will be used for study endpoint.

3.2.2 Secondary Endpoints

3.2.2.1 Safety and tolerability

- The combination of these drugs will be deemed safe if the number of adverse events is no more that 10% greater than the additive number of events of azacitidine and sirolimus if administrated separately. This will be based upon data in the original phase 2 trials of azacitidine demonstrating an 8% toxic death rate and therefore be 18% of the total number enrolled (approx. 40 x18% = 7).
- Toxicity will be graded according to the NIH CTC version 4.0 (http://ctep.cancer.gov/reporting/ctc.html). Toxicity refers to toxic events during the full course of treatment (through day 21) that are attributed as possibly, probably or definitely due to treatment.

The following will be considered toxicities

- Non-hematologic toxicity is defined as Any Grade III or Grade IV non-hematologic toxicity attributable to the investigational drug with the specific exclusion of:
  a. Grade III nausea and vomiting responsive primarily to non-parenteral anti-emetics, intermittent use of parenteral anti-emetics is acceptable.
  b. Grade III fever or infection
  c. Splenic Infarction
  d. Grade III/IV Tumor lysis syndrome
  e. Grade III/IV metabolic abnormalities attributable to anti-fungal medication or tumor lysis syndrome that is correctable with IV or PO supplementation.
  f. Grade III/IV stomatitis that resolves within 14 days of neutrophil recovery
  g. Grade III/IV hypercholesteremia / hypertriglyceridemia
  h. Grade III hyperbilirubinemia and/or transaminitis that resolves to less than or equal to a grade II toxicity within 14 days.

Hematologic toxicity: Marrow aplasia (defined as <10% cellularity with <10% blast) > 4 weeks not attributable to disease. All unanticipated> Grade 2 non-hematologic AE's will be reported. The only hematologic event which will be considered an AE/SAE for this study is marrow aplasia (defined as <10% cellularity with <10% blast) > 6 weeks not attributable to disease and this will be reported. There is no degree of neutropenia, anemia, or thrombocytopenia that would qualify as an AE/SAE as the purpose of the treatment is to create a period of complete aplasia in the marrow.

- Blood product transfusion will not be considered a limiting toxicity, since these patients require frequent transfusions secondary to disease process.
- Toxicity requiring discontinuation of therapy or removal of the patient from the study.
- Death resulting from toxicity attributed to the combination of sirolimus and azacitidine.

3.2.2.2 Pharmacokinetic assessment

Pharmacokinetic assessment at Day 4 will assess levels of the drug in vivo. The Day 4 levels will be drawn prior to initiation of azacitidine to allow for a PK/PD correlation study. No dose adjustment is planned on the basis of these studies.

3.2.2.3 Pharmacodynamic assessment

The secondary pharmacodynamic endpoint is to determine if rapamycin effectively inhibits mTOR signaling at levels achieved in this regimen.

We want to define in vivo, the percentage of baseline samples that have activation of the S6 ribosomal protein and the percentage of those that were found to be activated which are subsequently inhibited by sirolimus in the post treatment sample. [S6 positive blasts post-treatment minus % S6 positive blasts at baseline]

We will determine the ability of oral sirolimus to inhibit mTOR in leukemic blasts. This will be measured by intracellular flow cytometry for phosphorylation of the downstream signaling target S6 ribosomal protein as a surrogate for mTOR activity. Flow results will be confirmed by Western blot as appropriate. The in vivo effects of sirolimus will be compared to a whole blood *ex vivo* rapamycin dose response curve for each sample as well as to sirolimus levels obtained at the time of pharmacodynamic measurements. Finally, S6 inhibition will be correlated with clinical outcome. We will draw PD datapoints on day 1 before treatment, Day 4 before azacitidine administration. If patient have less than 5000/μl of blasts in the periphery, than a bone marrow aspirate will be performed for analysis.

We hypothesize that oral sirolimus will fully inhibit S6 in sampled leukemic blasts if concentrations exceed a threshold established by ex-vivo rapamycin therapy of the baseline sample. We will therefore expose this sample to increasing doses of rapamycin ex vivo and then measure mTOR activation by flow cytometry.

3.2.2.4 Quality of Life assessment

To determine if patient's quality of life is improved while on this combination regimen, we will ask patients to complete two, previously validated instruments designed to assess physical symptoms and functioning, psychological state, social functioning, and demographic characteristics. The European Organization for Research and Treatment of Cancer (EORTC) QOL and the Mental Health Inventory (MHI) will be administered prior to treatment on day 1, on day 84 (after three cycles), and on day 164 (after six cycles) ± 30 days.

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

1. Patients must have a diagnosis of one of the following:
    - MDS (Arm A)
        i. High-risk MDS defined as: >5% blasts in bone marrow and/or the following cytogenetic categories: presence of inv(3)/t(3q)/del(3q), -7/del(7q), complex cytogenetics (3 or more abnormalities)
    - AML (Arm B)
        i. Relapsed/refractory/unable to tolerate conventional chemotherapy
    - MDS or AML clinical diagnosis with prior therapy with azacitadine (Arm C)

    ***Note:** As of July 2018, only high-risk MDS patients will be eligible as Arm B is closed. As of October 2017, those patients with MDS who have received prior treatment will now be enrolled on Arm A as Arm C is closed.
2. Patients must be ≥ 18 years old
3. Patients must have an ECOG performance status of ≤ 2 (see Attachment 1).
4. Patients must have a life expectancy of at least 4 weeks.
5. Patients must be able to consume oral medication.
6. Patients must have completed any radiotherapy four weeks prior to study entry, 0-2 weeks for local palliative XRT (small port).
7. Patients must have recovered from the toxic effects of any prior chemotherapy to < Grade 2 (except for alopecia).
8. Required initial laboratory values: Creatinine≤ 2.0mg/dL; total or direct bilirubin ≤ 1.5mg/dL (if not due to the leukemia itself or known Gilbert's Syndrome); (as documented by treating physician) SGPT (ALT) ≤ 3xULN; glucose <200 mg/dL, negative pregnancy test for women of child-bearing potential.
9. Patients must be able to sign consent and be willing and able to comply with scheduled visits, treatment plan and laboratory testing.
10. Patients may have had a prior stem cell transplant (autologous or allogeneic), however they may not have active GvHD, nor be on any immunosuppression

4.2 Exclusion Criteria

1. Patients must not be currently receiving any chemotherapy agents (except Hydroxyurea)
    a. Intrathecal ARA-C and intrathecal methotrexate are permissible (as they are not systemic and only isolated to the central nervous system).

   b. Patients cannot have received more than 3 prior lines of therapy for their hematologic malignancy. Patient may have previously had azacitidine or decitabine will be eligible to enroll on Arm A (MDS).
2. Patients must not be receiving growth factors.
3. Patients with a current second malignancy requiring systemic therapy, other than non-melanoma skin cancers, are not eligible. If a patient has had a prior second malignancy that is not currently requiring active treatment, the patient will be considered eligible.
4. Patients with uncontrolled high blood pressure, unstable angina, symptomatic congestive heart failure, myocardial infarction within the past 6 months or serious uncontrolled cardiac arrhythmia are not eligible.
5. Patients may not take any of the following medications while on study, but will be considered eligible if medication is discontinued 72 hours prior to first dose of Sirolimus:
   - Carbamazepine (e.g. Tegretol)
   - Rifabutin (e.g. Mycobutin)
   - Rifampin (e.g. Rifadin)
   - Rifapentine (e.g. Priftin)
   - St. John's Wort- may decrease effects of sirolimus by decreasing the amount of sirolimus in the body
   - Clarithromycin (e.g. Biaxin)
   - Cyclosporin e.g. (Neorla or Sandimmune)
   - Diltiazem (e.g. Cardizem)
   - Erythromycin (e.g. Akne-Mycin, Ery-Tab)
   - Itraconazole (e.g. Sporonox)
   - Fluconazole (e.g. Diflucan)
   - Ketoconazole (e.g. Nizoral)
   - Telithromycin (e.g. Ketek)
   - Verapamil (e.g. Calan SR, Isoptin, Verelan)
   - Voriconazole (e.g. VFEND) - May increase the effects of sirolimus by increasing the amount of this medicine in the body. Can take 72 hours after last dose of Sirolimus
   - Tacrolimus (e.g. Prograf) – May cause liver transplant rejection or serious side effects in patients on sirolimus.
6. Patients with known HIV positivity or AIDS-related illness are not eligible.
7. Patients with other severe concurrent disease which in the judgment of the investigator would make the patient inappropriate for entry into this study are ineligible.
8. Patients must not have received any investigational agents within 21days of study entry.
9. Patients must not be pregnant or breastfeeding. Pregnancy tests must be obtained for all females of child-bearing potential. Pregnant or lactating patients are ineligible for this study due to the unknown human fetal or teratogenic toxicities of rapamycin. Males or females of reproductive age may not participate unless they have agreed to use an effective contraceptive method.
10. Patients who have uncontrolled infection are not eligible. Patients must have any active infections under control. Fungal disease must be stable for at least 2 weeks before study entry. Patients with bacteremia must have documented negative blood cultures prior to study entry.

4.3 Subject Recruitment and Screening

Subjects will be recruited from the practices of the Department of Medical Oncology, Thomas Jefferson University. The patients are not excluded based on gender, race or economic status. The patient must be at least 18 years of age. Patients who meet eligibility criteria will be invited by their physician to participate in the study. All therapeutic options will be discussed with the patient and the patient's questions will be answered to the patient's satisfaction. Patients will be asked to read, comment/ask questions about the study and then sign the informed consent form before study procedures are to take place.

Bone marrow biopsy/aspiration and/or tissue biopsy will be utilized to determine eligibility for the study. Screening laboratory work to be performed will include blood chemistries, pregnancy testing (if applicable) and a physical assessment, including a neurological exam. Patients will be offered an option to complete the standard of care chemotherapy treatment at the following Jefferson Health network

hospitals: Center City Hospital, Abington Hospital, Aria Hospital, Methodist Hospital and NJ Division (Kennedy) Hospital. All research related procedures are to be done at Jefferson Center City campus.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Subjects will continue on study treatment unless the following occurs:

1. Unacceptable toxicity. See section 3.2.2.1 for details.
2. Non-compliance by the patient with protocol requirements
3. Changes in medical status of the patient such that the patient no longer meets eligibility requirements or the investigator believes that the treatment is no longer in the patient's best interest.
4. Patient refusal
5. Disease Progression
6. Alternative therapy is started as per the discretion of the physician, for example, bone marrow transplant.

The discontinuation of study treatment will not constitute study withdrawal or study completion. In the event of a decision to discontinue treatment, the treatment phase will be considered complete and the follow-up phase will begin.

If a subject discontinues treatment and actively withdraws consent, no additional data will be collected.

5 Study Drug

5.1 Sirolimus

Drug Name: Sirolimus

Other Names: Rapamycin, Rapamune®

Classification: Immunosuppressant Agent

Mode of Action: Rapamycin inhibits T-lymphocyte activation and proliferation in response to antigenic and cytokine stimulation. Its mechanism differs from other immunosuppressants.

Storage and Stability: Store at room temperature, 20ºC to 25ºC (68ºF to 77ºF); protect from light.

Dose Specifics: Assigned dose level. Patients will receive a 12mg loading dose of sirolimus. They will then receive a 4mg daily dose of sirolimus beginning 24 hours after the loading dose for the number of days indicated by protocol regimen.

Preparation 1 and 2 mg tablet

Route of Administration: *All drug is to be taken by mouth. Doses should be given every 24 hours ± 2 hours. The drug should be consistently* **without** *food. Rapamycin will be given every 24 hours for number of days indicated by protocol regimen.*

Incompatibilities: Diltiazem increases serum concentrations of sirolimus. Sirolimus serum concentrations may be increased with use of voriconazole, itraconazole , ketoconazole and fluconazole. Concurrent use of these drugs is contraindicated.

Availability: Available as Rapamune® 1mg tablet and 2mg tablets. Will be available through the Investigational Drug Services.

Side Effects

Common (21-100% Frequency) Immediate: Nausea, Vomiting Prompt: Within 2-3 weeks, prior to next course. Diarrhea, Hypertension, Increased creatinine Delayed: Any time after above. Hypercholesteremia, Hypertriglyceridemia
Occasional (5-20% Frequency) Immediate: Rash Prompt: Within 2-3 weeks, prior to next course. Infections; edema; weight gain, arthralgia; tremor; acne; myelosuppression; fever; abdominal pain; myalgia; hypokalemia; hypophosphatemia Delayed: Any time after above. Stomatitis
Rare (< 5% Frequency) Hepatotoxicity Delayed: Any time after above. Hirsutism, Secondary Lymphoma, Inflammation of the lungs

Nursing/Patient Implications: Monitor blood pressure, and serum creatinine.

5.2 Azacitidine

Other Names: Vidaza

Classification: Hypomethylating Agent

Mode of Action: Azacitidine is a nucleoside analogue of cytidine that is thought to cause hypomethylation of DNA in rapidly dividing cells. Hypomethylation of the DNA of cancerous hematopoetic cells is thought to inhibit uncontrolled cell cycle by restoring the ability for differentiation and normal growth factor responses. The drug has little effect on non-proliferating cells.

Storage and Stability: Store unreconstituted vials at 25ºC (77ºF) with acceptable variation including 15º - 30ºC (59ºF-86ºF). Reconstituted samples for subcutaneous injection are stable for up to 1 hour at 25°C (77°F) or for up to 8 hours between 2°C and 8°C (36°F and 46°F). Intravenous samples are stable at 25°C (77°F) but must be administered completely 1 hour after reconstitution.

Dose Specifics: 75mg/m^2 for 7 days in patients with normal renal function. Not studied in patients with reduced renal function. After 2 cycles, may increase dose to 100 mg/m^2 if no beneficial effect is seen and no other toxicity other than nausea and vomiting has occurred.

Preparation: 100mg: Injection, powder for suspension. Reconstitute with 4ml of sterile water for subcutaneous administration and 10ml of sterile water for intravenous. For intravenous usage, inject reconstituted solution in 50-100ml of normal saline solution or lactated ringers depending on desired final dosage/concentration.

Administration: I.V. infusions must administered within 1 hour of reconstitution and ideally should be administered in 10-40 minutes. Subcutaneous doses must be administered at least 1 inch from prior site of injection and doses greater than 2ml should be injected into two separate sites.

Compatibilities: Do not mix with Dextrose-containing fluids. Not compatible with hetastarch or fluids containing bicarbonate. Caution is urged when combining azacitidine with other immunosuppressing drugs.

Availability: Vials of powder for reconstitution, 100mg.

Side Effects:

Hematologic: Anemia, febrile neutropenia, leucopenia, neutropenia, thrombocytopenia.
Gastrointestinal: Abdominal pain, constipation, diarrhea, nausea, vomiting
General: Fatigue, injection site reactions, pyrexia
Infections: Rhinitis, upper respiratory tract infection, urinary tract infection
Metabolic: Hypokalemia
Renal: Hematuria, serum creatinine elevation, renal failure
Respiratory: Dyspnea, exertional dyspnea, pharyngeal pain
Skin: Erythema, petichiae, pruritis, rash

Nursing/ Instructions: Assess results of laboratory tests for rising creatinine as azacitidine is primarily cleared by the kidneys. Use with caution in patients with chronic liver disease, as azacitidine has shown to worsen hepatic encephalopathy. Assess other pharmacological or herbal products patients may be taking for potential interactions. Infusion site must be monitored for signs of extravasation.

5.3 Treatment Regimen

5.3.1 Chemotherapy

- Chemotherapy will consist of a sirolimus 12mg loading dose on day 1 followed by either 9 or 11 consecutive days of administration of sirolimus 4mg. If a split-dose schedule of azacitidine is followed, two additional doses of sirolimus will be given on days 11 and 12 of the 28 day cycles. If the alternate dose schedule is followed, sirolimus will be given on days 1-10. Concurrently, azacitidine 75mg/m^2 will be given on days 4-8,11 and 12 with cycles repeating every 28 days. Alternately, seven consecutive days of infusion is allowable, i.e. Day 4-10. The schedule, with split dosing or consecutive day dosing, are both frequently used in the outpatient setting given depending on availability of weekend infusion services. Given evidence of near equal efficacy, the decision for consecutive day or split dosing will be decided on the basis of availability of services and the treating physician's discretion. Patients will be offered an option to complete the standard of care chemotherapy treatment at the following Jefferson Health network hospitals: Center City Hospital, Abington Hospital, Aria Hospital, Methodist Hospital and NJ Division (Kennedy) Hospital. All research related procedures are to be done at Jefferson Center City campus. After 2 cycles, may increase dose to 100 mg/m^2 if no beneficial effect is seen and no other toxicity other than nausea and vomiting has occurred.

EXPERIMENTAL DESIGN SCHEMA*:*

DAY:	1	2	3	4	5	6	7	8	9	10	11	12
Sirolimus:	12mg	4mg	4mg	4mg	4mg	4mg	4mg	4mg	4mg	4mg	4mg	4mg
Azacitidine:				X	X	X	X	X			X	X
Azacitidine-Alt:				X	X	X	X	X	X	X		
PD:	X[1]			X[2]								
PK:				X[3]								

PD[1]-Sample drawn before sirolimus dosing.
PD[2]- Sample drawn before azacitidine administration
PK[3]- Sample drawn before sirolimus and azacitidine administration

Plasma Sirolimus (PK): Sirolimus levels will be determined by commercially available assay. Rapamycin levels will be obtained **prior** to the dosing on Day 4. 3-4 cc of peripheral blood will be collected in a purple top (EDTA) tube to be sent to the local lab. Record the exact time that the sample was drawn along with the exact time that the last dose of drug was administered.

Biologic Studies (PD)

Pretreatment bone marrow aspirate: 5-10 cc of Bone Marrow Aspirate in preservative-free heparin (green top) to be sent to the Stem Cell Core at the University of Pennsylvania. Aspirates should be drawn through a heparinized syringe. Peripheral Blood: 20 ml in a green top tube to be sent to the Stem Cell Core (if peripheral blast are ≥5000/μl then a Bone Marrow Aspirate is not required). Both marrow and blood will be sent for any patient with a peripheral blast count less than 5000/μl and greater than or equal to 200/μl.

Day 4 Bone Marrow: 5-10 cc of Bone Marrow Aspirate in preservative-free heparin (green top) to be sent to the Stem Cell Core only if pretreatment blast count is ≤ 200/μl otherwise 10cc peripheral blood may be substituted.

We will be determining the ability of oral sirolimus to inhibit mTOR in leukemic blasts. This will be measured by intracellular flow cytometry for phosphorylation of the downstream signaling target S6 ribosomal protein as a surrogate for mTOR activity. Flow results will be confirmed by Western blot as appropriate. The in vivo effects of sirolimus will be compared to a whole blood *ex vivo* rapamycin dose

response curve for each sample as well as to rapamycin levels obtained at the time of pharmacodynamic measurements. Finally, inhibition of S6 phosphorylation will be correlated with clinical outcome.

5.3.2 Dose Reductions

Standard dose reductions for Azacitidine will occur as follows:

If blood counts at time of baseline PD draw WBC ≥3.0 x10^9/L, ANC ≥1.5 x10^9/L, and platelets ≥75.0 x10^9/L adjust the dose as follows, based on nadir counts for any given cycle:

Nadir Counts		% Dose in the Next Course
ANC (x10^9/L)	Platelets (x10^9/L)	
<0.5	<25.0	50%
0.5 –1.5	25.0-50.0	67%
>1.5	>50.0	100%

For patients whose baseline counts are WBC <3.0 x10^9/L, ANC<1.5 x10^9/L, or platelets<75.0 x10^9/L, dose reductions will be at the discretion of the treating physician.

At the discretion of the treating physician cycles may be spaced out to up to 42 days apart based upon hematologic response and recovery between cycles.

Renal Toxicity - If unexplained reductions in serum bicarbonate levels to <20 mEq/L occur, the dosage should be reduced by 50% on the next course. If increases in BUN or serum creatinine (unexplained) occur, delay next cycle until values reach baseline or normal, then reduce dose by 50% for next treatment course.

5.3.3 Antibiotic Prophylaxis

- All patients should be treated with acyclovir 400mg BID unless tested and found to be HSV antibody negative.
- The recommended antibiotic prophylaxis is as follows, (but may be determined by physician discretion): ciprofloxacin and augmentin for severely neutropenic patients; fluconazole 400mg daily; and acyclovir 400mg twice daily.
- Voriconazole, fluconazole or posaconazole may NOT be used within 72 hours (prior to or after receiving) Rapamycin. If suspected or proven Aspergillosis infection occurs- Anidulofungin, Caspofungin or Amphoteracin B may be used, as per the physician's discretion.

5.3.4 Supportive Care

- Growth factor support and transfusion of blood products will be performed as per physician discretion.
- Supportive care will be administered at the discretion of the patient's supervising attending.

5.3.5 Method for Assigning Subjects to Treatment Groups

This is an open label study of a single therapy regimen.

Once eligibility has been established and confirmed by signature of the Jefferson principal investigator, the Research Coordinator at TJU will then be assigned a registration number according to the subject's diagnosis (MDS or refractory AML). This number is unique to the participant on this trial and must be used moving forward (i.e. for CRF completion, SAE reporting, etc). As of October 2017 and July 2018, both Arm C and Arm B are closed to enrollment respectively. Patients with MDS who have received prior treatment will be allowed to enroll onto Arm A.

A master study enrollment log will be maintained by the study team at the Thomas Jefferson University.

Patients cannot be registered to this study on the weekends. The Carroll lab at UPenn cannot accept samples after 1pm.

5.4 Preparation and Administration of Study Drug

Sirolimus will be purchased from Wyeth and then stored and dispensed from the Investigational Drug Service (IDS). It will be dispensed in pill bottles containing a full cycle's amount of Sirolimus and the labeling will contain explicit dosing instructions. Subjects may start a cycle as an outpatient and will self-administer their Days 1 and 2 doses at home or in the clinic prior to azacitidine administration. At time of enrollment, the subject, the subject will receive a pill diary to record time of pill administration. The subject will be asked to bring their pill bottle as well as their pill diary to the infusion center or hospital depending on location of treatment with azacitidine.

5.5 Subject Compliance Monitoring

Subjects will be asked to keep a pill diary to record the time of each dose of Sirolimus taken. If subject starts a cycle as an outpatient, they will self-administer their Days 1 and 2 doses. The subject will be asked to bring their pill bottle as well as their pill diary to the hospital at the time of admission. For the rest of the cycle, each daily dose will be administered by nurses in the inpatient setting.

Please see Attachment # 3 for emergent admission guidelines.

5.6 Prior and Concomitant Therapy

1. Information of prior cytotoxic regimens will be collected.
2. All concomitant medical therapy will be collected as part of the history.
3. All concomitant medicines/therapies are permitted during the study except for those noted in the exclusion criteria in section 4.2.
4. No concomitant voriconizole, posaconazole, itraconazole, ketokonazole, or diltiazem within 72 hours prior to or after receiving a dose of rapamycin.
5. All patients should receive acyclovir unless documented to be HSV negative.

5.7 Packaging

Available as Rapamune® 1mg tablet and 2mg tablets. Will be available through the Investigational Drug Services

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5.8 Receiving, Storage, Dispensing and Return

5.8.1 Receipt of Drug Supplies

Upon receipt of the of the study treatment supplies, an inventory must be performed and a drug receipt log filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment (active drug or comparator) will be documented in the study files. The investigator must notify study sponsor of any damaged or unusable study treatments that were supplied to the investigator's site.

5.8.2 Storage

See section 5.1 for details.

6 Study Procedures

At enrollment each subject will have a medical history taken, a physical exam (including neurological exam), vital signs, ECOG performance status, and laboratory studies including CBC with differential, liver function tests, electrolytes, uric acid, and glucose as well as a pregnancy test if applicable. An HSV titer is recommended. Patients who have not had a bone marrow biopsy sample within the last 4 weeks will require a bone marrow biopsy and aspirate.

Patients will be offered an option to complete the standard of care chemotherapy treatment at the following Jefferson Health network hospitals: Center City Hospital, Abington Hospital, Aria Hospital, Methodist Hospital and NJ Division (Kennedy) Hospital. All research related procedures are to be done at Jefferson Center City campus.

Patients will be followed for disease status and survival for 5 years following the enrollment of the last patient. These follow-ups will be via phone call, chart review, or in conjunction with their routine office visits every 3 months.

See Attachment #2 for Study Procedures Table.

6.1 Correlative Studies

6.1.1 Biologic studies

Within 4 weeks of beginning on study drugs, patients must have 5-10 ml of Bone Marrow Aspirate or 20ml of peripheral blood (if ≥5000/μl peripheral blasts (WBC x blast percentage >0.5 thousand/uL)), in a preservative-free heparin (green top) tube, sent to the Stem Cell Core at the University of Pennsylvania.

Peripheral blood samples will also be collected before the first sirolimus administration, and within 4 hours prior to Sirolimus dose on Day 4 of CYCLE 1 only (please see section 5.3.1 for details).

Both marrow and blood will be sent for any patient with a peripheral blast count ≤ 5000/μl and ≥200/μl.

Samples will then be analyzed by Dr. Martin Carroll's lab. Samples are to be delivered to Dr. Carroll's laboratory (Room 732 Biomedical Research Building II/III, 421 Curie Blvd University of Pennsylvania Philadelphia PA 19104).

Patient samples will be washed and lysed in 1% Triton X-100 lysis buffer. 100 μg protein per lane will be analyzed using SDS-PAGE and Western blotting. Protein phosphorylation and expression will be examined using p70S6 kinase antibodies. Optical densitometry will be used to quantify the protein.

1-2 ml will be obtained from the whole blood or marrow samples drawn at baseline and day 4. These samples must contain at least ≥200/ml (WBC x blast percentage >0.2 thousand/uL) Samples will be aliquotted as 100 uL sample/tube into 5 ml tubes and a subset will be inhibited with 1 uMrapamycin x 30 min or stimulated with the protein kinase C agonist phorybolmyristyl acetate (PMA) 40 nM x 10 min at 37^{o}C to provide negative and positive S6 controls. Following this incubation, cells will be fixed with 4% formaldehyde for 10 minutes, followed by red blood cell lysis and permeabilization with 0.1% triton X-100 for 30 minutes at room temperature. Cells will be washed with cold PBS and 4% BSA and cell pellets frozen in an isotonic medium containing 20% serum and 10% glycerol. After baseline and day 4 samples are obtained, these samples will be thawed, washed with cold PBS and 4% BSA and proteins denatured by exposure to 50% methanol in 0.9% NaCL prior to antibody staining and data acquisition on a multi-color flow cytometer. Expression of cell surface epitopes CD45, 13, and 33 and intracellular phospho-S6 kinase will be acquired using CellQuest or DiVA software and data stored as ListMode files. FlowJo software will be used to analyze these data.

Gating upon cells with characteristics of myeloid blasts (CD45 dim positive, intermediate side scatter) and confirming surface expression of CD13 and/or 33 in comparison to internal negative controls for these epitopes (CD45 bright positive, low side scatter lymphocytes), identified leukemic blasts will be examined for phosphorylation of S6 kinase at baseline and day 4. A positive gate containing PMA stimulated blasts and a negative gate containing unstained ("fluorescence minus one") blasts will be used to score samples as to the percentage of blasts that are positive or negative for S6 phosphorylation. Baseline and day 4 samples will be compared for the relative percentages of cells within these gates as an indication of in vivo S6 inhibition in leukemic blasts by rapamycin. Median fluorescence intensity will also be acquired for S6 phosphorylation and compared for the baseline and day 4 samples.

6.1.2 Pharmacokinetic studies

Plasma Sirolimus: Levels will be determined by commercially available assay. Sirolimus levels will be obtained **just prior** to the dosing on Day 4

7 Statistical Plan

7.1 Sample Size and Study Duration

This study is considered a phase II study.

For the MDS Arm A:
The optimal two-stage design to test the null hypothesis that P≤0.230 versus the alternative that P≥0.400 has an expected sample size of 24.60 and a probability of early termination of 0.592. If the drug is actually not effective, there is a 0.090 probability of concluding that it is (the target for this value was 0.100). If the drug is actually effective, there is a 0.200 probability of concluding that it is not (the target for this value was 0.200). After testing the drug on 14 patients in the first stage, the trial will be terminated if 3 or fewer respond. If the trial goes on to the second stage, a total of **40 patients** will be studied. If the total number responding is less than or equal to 12, the drug is rejected.

For the AML Arm B:
Note: As of July 2018, Arm B was closed after accruing 27 patients.

The optimal two-stage design to test the null hypothesis that P≤0.200 versus the alternative that P≥0.400 has an expected sample size of 20.58 and a probability of early termination of 0.747. If the drug is actually not effective, there is a 0.050 probability of concluding that it is (the target for this value was 0.050). If the drug is actually effective, there is a 0.200 probability of concluding that it is not (the target for this value was 0.200). After testing the drug on 13 patients in the first stage, the trial will be terminated if 3 or fewer respond. If the trial goes on to the second stage, a total of **43 patients** will be studied. If the total number responding is less than or equal to 12, the drug is rejected.

For the pre treated Arm C:
Note: As of October 2017, Arm C was closed after accruing 7 patients.

The optima two stage design to test the null hypothesis that P≤0.05 versus the alternative that ≥02.00 has an expected samples size of 10 and a probability of early termination of 0.508. If the drug is actually not effective, there is a 0.050 probability of concluding that it is (the target for this value was 0.050). If the drug is actually effective, there is a 0.200 probability of concluding that it is not (the target of this value was 0.200). After testing the drug on 10 patients in the first stage, the trial will be terminated if zero patients respond. If the trial goes on the second stage, a total of **29 patients** will be studied. If the total number responding is less than or equal to 3, the drug is rejected.

7.2 Statistical Analysis

We will estimate the distribution of time to death, time to relapse, and time to progression by the Kaplan-Meier method. Baseline patient and disease characteristics will be summarized using descriptive statistics (e.g. mean, median, standard deviation, range, frequency and percentage), histograms and box plots.

The key PD variable is percentage change in S6 inhibition from baseline to post-treatment, defined as 100*([% S6-positive blasts post-treatment minus % S6-positive blasts at baseline] divided by % S6-positive blasts at baseline). We will examine distributional characteristics by: histograms, box plots and descriptive statistics (e.g., mean, median, standard deviation, range). Variability will be of particular interest. We will conduct within-patient comparison of baseline versus posts-treatment percentages by Student's paired *t* test. A nonparametric Wilcoxon signed ranks test will be employed if normality cannot be assumed or achieved by simple transformation. We will also investigate association of S6 response and clinical response. We will make box plots of the percentage change in S6 inhibition by complete response (e.g., CR/non-CR) category. Mean and standard deviation estimates will be of particular interest for the design of future hypothesis-testing studies. With the small number of subjects these analyses are likely to have only modest power, and we will not perform formal significance tests.

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others

Unanticipated problems (UAPs) include, in general, any incident, experience, or outcome that meets the following criteria:

- unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;

UAPs are considered to pose risk to participants or others when they suggest that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Adverse Event

An adverse event is any untoward or unfavorable medical occurrence in a human participant, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the participant's participation in the research, whether or not considered related to the participant's participation in the research.

Serious Adverse Event

Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period

The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality is a grade 3 or above based on CTC 4.0

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

8.2.1 Safety Assessment and Follow-Up

The relationship to study intervention or study participation must be assessed and documented for all adverse events. Evaluation of relatedness must consider etiologies such as natural history of the underlying disease, concurrent illness, concomitant therapy, study-related procedures, accidents, and other external factors.

The following guidelines are used to assess relationship of an event to study intervention:

1. Related (Possible, Probable, Definite)

    a. The event is known to occur with the study intervention.

    b. There is a temporal relationship between the intervention and event onset.

c. The event abates when the intervention is discontinued.

d. The event reappears upon a re-challenge with the intervention.

2. Not Related (Unlikely, Not Related)

a. There is no temporal relationship between the intervention and event onset.

b. An alternate etiology has been established.

8.2.2 Expectedness

The PI is responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the intervention. Risk information to assess expectedness can be obtained from preclinical studies, the investigator's brochure, published medical literature, the protocol, or the informed consent document.

8.2.3 Intervention

Any intervention implemented to treat the adverse event must be documented for all adverse events.

8.3 Safety Reporting

8.3.1 Reporting to IRB

8.3.1.1 Unanticipated Problems

All incidents or events that meet criteria for unanticipated problems (UAPs) as defined in Section 9.1 require the creation and completion of an unanticipated problem report form (OHR-20).

UAPs that pose risk to participants or others, and that are not AEs, will be submitted to the IRB on an OHR-20 form via the eazUP system within 5 working days of the investigator becoming aware of the event.

UAPs that do not pose risk to participants or others will be submitted to the IRB at the next continuing review.

8.3.1.2 Adverse Events

Grade 1 AEs will be reported to the IRB at continuing review.

Grade 2 AEs will be reported to the IRB at the time of continuing review.

8.3.1.3 Serious Adverse Events

SAEs will be reported to the IRB on OHR-10 forms via the electronic reporting system (eSAEy) according to the required time frames described below.

Grade 3-4 AEs that are unexpected and deemed to be at least possibly related to the study will be reported to the IRB within 2 working days of knowledge of the event.

Grade 3-4 AEs that are deemed unrelated to the study will be reported to the IRB within 5 working days.

Grade 5 AEs will be reported to the IRB within one working day of knowledge of the event.

All SAEs will be submitted to the IRB at continuing review, including those that were reported previously.

8.3.2 Reporting to SKCC DSMC

All AEs and SAEs, safety and toxicity data, and any corrective actions will be submitted to the DSMC per the frequency described in the SKCC DSMP. The report to the SKCC DSMC will also include any unanticipated problems that in the opinion of the PI should be reported to the DSMC.

All emergent ≥ Grade 2 non-hematologic adverse events (AEs) will be reported to the DSMC. The only hematologic event which will be considered an AE/SAE for this study and will be reported is marrow aplasia (defined as <10% cellularity with <10% blast) > 4 weeks not attributable to disease. Grades 1 to 4 of neutropenia, anemia, or thrombocytopenia would not qualify as an AE/SAE as the purpose of the treatment is to create a period of complete aplasia in the marrow. However, all hematologic Grade 5 SAEs will be reported to the DSMC regardless of causality.

For expedited reporting requirements, see table below:

DSMC AE/SAE Reporting Requirements

	Grade 1	Grade 2		Grade 3				Grades 4 and 5
				Unexpected		Expected		
	Unexpected and Expected	Unexpected	Expected	With Hospitalization	Without Hospitalization	With Hospitalization	Without Hospitalization	Unexpected and Expected
Unrelated Unlikely	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	5 Working Days	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	5 Working Days	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Phase I - 48 Hours (Death: 24 Hours) Phase II - 5 working days
Possible Probably Definite	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	48 Hours (Death: 24 Hours)	Phase I - 48 Hours Phase II - 5 working days	48 Hours (Death: 24 Hours)	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Phase I and Phase II - 48 Hours (Death: 24 Hours)

8.3.3 Protocol Deviations/Exceptions

An accidental or unintentional deviation from the approved protocol, that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects, will be reported to the IRB and DSMC within 10 working days of notification of investigator. Deviations to protect subjects from immediate harm/danger should be reported immediately following the event to both the IRB and DSMC.

There may be times, when a planned deviation from the protocol seems warranted. Such deviations should only occur with the **prior** assessment of the DSMC and final approval of the IRB. All entities should be given sufficient time to evaluate the request.

8.3.4 Study Oversight

In addition to the PI's responsibility for oversight, study oversight will be under the direction of the SKCC's Data and Safety Monitoring Committee (DSMC). The SKCC DSMC operates in compliance with a Data and Safety Monitoring Plan (DSMP) that is approved by the NCI.

8.4 Stopping Rules

See section 7.2.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR

WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

9.4 Records Retention
Upon completion and termination of protocol, records will be kept for a period of 5 years

10 Clinical Site Monitoring and Auditing
Clinical site monitoring and auditing is conducted to ensure that the rights of human participants are protected, that the study is implemented in accordance with the protocol and/or other operating procedures, and that the quality and integrity of study data and data collection methods are maintained. Monitoring and auditing for this study will be performed in accordance with the SKCC's Data and Safety Monitoring Plan (DSMP) developed by the SKCC Data and Safety Monitoring Committee (DSMC). The DSMP specifies the frequency of monitoring, monitoring procedures, the level of clinical site monitoring activities (e.g., the percentage of participant data to be reviewed), and the distribution of monitoring reports. Some monitoring activities may be performed remotely, while others will take place at the study site(s). Appropriate staff will conduct monitoring activities and provide reports of the findings and associated action items in accordance with the details described in the SKCC DSMP.

11 Ethical Considerations
This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. See Attachment for a copy of the Subject Informed Consent Form. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

12 Study Finances
12.1 Funding Source:
The Department of Medical Oncology will be paying for this study.

12.2 Conflict of Interest
Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study.

13 Publication Plan
Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed

on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

We intend to publish our results in peer-reviewed journals. The primary responsibility for publication lies with the PI, co-investigator, and statistician

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15 Attachments

Attachment 1: ECOG Performance Status
Attachment 2: Study Schedule
Attachment 3: Admissions Plan

Attachment 1:

ECOG PERFORMANCE STATUS

Grade	ECOG
0	Fully active, able to carry on all pre disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

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Attachment #2: Study Procedures*

	Baseline[1]	Day 1 of Cycle 1	Day 4 of Cycle 1	Daily[2]	All cycles	Follow Up[12]
Informed Consent	X[11]					
Medical History	X[11]					
Physical Exam incl. weight and body surface area	X			X	X[7]	
Neuro exam	X					
ECOG/Performance Status	X			X		
Vital Signs	X		X	X	X[19]	
Height	X					
Adverse Event Assessment	X			X	X[3]	
LAB STUDIES						
CBC, Diff, Plts	X[10]	X[10]		X	X[17,18]	
Serum chemistry, electrolytes[6]	X[10]	X[10]		X	X[17,18]	
Liver function tests[6]	X[10]	X[10]		X[16]	X[17,18]	
Herpes Simplex Titer	X[14]					
Pregnancy Test[4]	X[4]					
Pharmacokinetics[5]			X[5]			
Pharmacodynamics	X[13,11]		X[13]			
TESTS & STUDIES						
Bone Marrow Biopsy	X[8]					X[9]
INVESTIGATIONAL AGENT						
Dispense Study Drug					X[20]	
Pill Diary Given to Patient		X			X	
Quality of Life Assessment		X[15]			X[15]	

*Note:
Patients will be offered an option to complete the standard of care chemotherapy treatment at the following Jefferson Health network hospitals: Center City Hospital, Abington Hospital, Aria Hospital, Methodist Hospital and NJ Division (Kennedy) Hospital. All research related procedures are to be done at Jefferson Center City campus.

1. To be done within ten days prior to study entry unless otherwise indicated.
2. If in Hospital
3. Twice a week while out of Hospital
4. For females of child bearing potential
5. Blood to be drawn prior to Sirolimus administration for trough level on Day 4.
6. Serum chemistry and electrolytes labs should include: creatinine, BUN, glucose, magnesium, C02, phosphate, and urate. Liver function labs should include: bilirubin, SGOT (AST), SGPT (ALT), alk phos.
7. One exam during Day 1- 10 of each cycle
8. If a diagnostic bone marrow biopsy has been done within 28 days of study enrollment, or the subject has a peripheral blast count of $\geq$5000/μl, and a sample is available in the stem cell core registry, a repeat bone marrow biopsy and aspirate is not necessary.
9. Every 6 months from enrollment (plus or minus 10 days), until there is evidence of progression of disease or if the treating physician wants to remove the patients from the study treatment and proceed to transplant. Can be performed more frequently at investigator's discretion.
10. Labs must be done within 1 week of Day 1 of each cycle.
11. Can be done within 4 weeks of study entry.

12. Patients will be followed every 3 months for 5 years for disease status and future therapy until relapse/progression**.** Patients who progress/relapse will continue to be followed for survival.
13. 20 mL of peripheral blood to be collected prior to first dose of Sirolimus. Samples will also be collected within 4 hours prior to Sirolimus dose on Day 4. This will be a bone marrow aspirate if there are insufficient circulating blasts (see section 6 for details)
14. Herpes simplex titer is recommended within 4 weeks of starting.
15. Quality of life assessment will take place prior to treatment on day 1, on day 84 (cycle 3) and 164 (cycle 6) ± 30 days
16. Twice weekly
17. On treatment (days 1-10): For cycles 1-6, twice weekly labs while on treatment. After cycle 6 (if stable disease), once weekly labs performed while on treatment. Labs can be performed more frequently at investigator's discretion, if needed.
18. Off treatment (days 11-28): During cycles 1-6 once weekly labs while off treatment. After 6 cycles, if disease is stable, interim labs will be performed at the investigator's discretion if needed.
19. Day 1 of azacitidine of each cycle
20. Within 1 week of Day 1
21. Patients who elect to have standard pf care Aza/Vidaza given at Abington Hospital, Aria Hospital, Methodist Hospital or NJ Division (Kennedy) Hospital will be seen C_XD_1 at Center City location for study procedures and for distribution of study drug Sirolimus.

Attachment # 3: Admissions Plan

Non Emergent and Emergent

Procedure for transitioning patients from outpatient to inpatient/ inpatient to outpatient on Dr. Kasner's Sirolimus trials:

1.) If participant begins the trial as an outpatient the investigator will handwrite prescription for Sirolimus.

2.) If planned admission occurs, the outpatient investigator will notify the inpatient team

(Preferably the attending or NP team) of the pending admission along with the following information:

a. The date that the intended transfer will take place

b. The day of treatment the patient is on

3.) The inpatient attending will be responsible for placing all orders* related to the patient's treatment while being treated in-patient on study, including but not limited to, sirolimus dosing, chemo administration and PK/PD orders. This may be delegated to a fellow/nurse practitioner under the direct management of the attending physician.

4.) Upon discharge, treating physician or designee must notify study coordinator. Hospital discharge planning must include outpatient follow up appointments for study required visit dates. Please note: weekend discharges may result in multiple protocol violations. Additionally, protocol prohibits use of azoles or dilitiazem until 72 hours following LAST dose of sirolimus.

***Patient Orders for Jeff Chart:**

Please put prompt in to notify that Azoles/ diltiazem

discontinued 72 hrs prior to first dose of Sirolimus until 72 hours post last sirolimus dose.

Patient own med order for Sirolimus

Study lab orders:

All required laboratory assessments per the protocol. PK/PD (blood or bone marrow) draws.

Nursing notes:

No azole drugs/diltiazem until 72 hours post last sirolimus dose.

Study coordinator cannot direct patients to start/stop any medications, including study therapy and azole drugs or other prohibited medications. This must be done by treating physician (in or outpatient) or nursing staff.