Title: Metabolically Optimized, Non-cytotoxic Low Dose Weekly Decitabine/Venetoclax in MDS and AML

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STUDY TITLE: Metabolically Optimized, Non-cytotoxic Low Dose Weekly Decitabine/Venetoclax in MDS and AML

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SPONSOR: Montefiore-Einstein Center for Cancer Care

SUPPLIED AGENT(S): Not applicable

IND #: IND Exempt

OTHER AGENTS: Venetoclax
Decitabine

Title: Metabolically Optimized, Non-cytotoxic Low Dose Weekly Decitabine/Venetoclax in MDS and AML

Principal Investigator: Mendel Goldfinger, MD

PRINCIPAL INVESTIGATOR SIGNATURE:

_____ Date: _____

Document History and SUMMARY OF CHANGES

Protocol Version	Version Date	Section	Change
5	June 17, 2024	Front Page	- Added MECCC co-investigators Ridhi Gupta, MD, David Levitz, MD, and Stephen Peeke, MD. - Added White Plains Hospital site PI name- Dr. Joshua Raff. - Removed Dr. Suman Kambhampati as the study will not be opened at Sarah Cannon, Kansas City, MO
		Study Schema	Updated study schema to reflect appropriate days for Decitabine administration.
		2.2	Secondary Objective (s) updated: -Removed the requirement to complete the Global Health Quality Of Life Questionnaire (GHS/QOL) as the IRB approval for the document was not obtained.
		3.0	Study Design updated: to remove the quality of life from the secondary objectives.
		4.2	Exclusion criteria to include other chemotherapy agents like cytarabine, cladribine and Gemtuzumab which we often use to cytoreduce new AML patients who have rapidly proliferative disease.
		Section 5	Registration: Removed Sarah Cannon Cancer Center and added White Plains Hospital Center for Cancer Care as the participating site.

		6	Added below to Concomitant Treatment section: - In patients with aggressive high bulk disease as determined by the investigator and PI, may receive debulking chemotherapy with either Gemtuzumab ozogamicin, Cladrabine or Cytarabine prior to starting decitabine/venetoclax. Also patients who have mutations in either FLT3 (ITD or TKD), JAK2, IDH1 or IDH2 mutation may receive concomitant inhibitors (as outlined in the table below) at any point during the protocol as determined by the PI. Other than HU and above-mentioned drugs, no other concomitant disease modifying therapy is permitted. - Added a table of the allowed mutation and concomitant medication. Updated Section 6.1.1 : - Decitabine dosing and schedule to on days 1, 8, 15, 28 (+/- 3 days). Added to section 6.1.4 – - Its at discretion of the treating physician based on clinical benefit to the patient, as to when to go back to the full dose of Decitabine or keep at reduced dose. Updated section 6.1.5 – Table 1to add some clarification and guidance. Updated section 6.4- Duration of Follow Up: from every 8 weeks to 4 weeks.
		7	Section 7.3 - SAE Report Form

			- the section updated to add "in Velos and reported to the DSMC. If needed, the SAE will also be reported to Einstein IRB and removed "on the FDA Form 3500A (MedWatch) but should only be reported as instructed below. The electronic FDA SAE reporting forms should not be used" Section 7.4.1 – SAE Reporting Requirements: - Added DSMC and removed FDA, external collaborator(s).
		9	Section 9.2 – Analytical Laboratory: - Dr. Amit Verma and Dr. Marina Konopleva Lab names added.
		10	Study Parameters and Calendar updated to provide clarification, remove the requirement to collect some unnecessary labs, allow buffer for visits to occur +/- 3 days. Added to foot note # 1 "Pre-study/screening starts from when patient signs the consent". Added foot note # 11 Bone marrow biopsy done before enrolling in the study done within 28-days of starting study therapy is acceptable
		Front page	Added Research Nurse Anne Munoz , Study Coordinator Aradhika Dhawan, and Regulatory Contact Rikin Gandhi Added 2 external Sites 1. Sarah Canon, Kansas City, MO 2. University of California, Davis, Sacramento, CA

4	April 11, 2023	Protocol Summary	Sample size updated to 75 patients
		3.1	Total patient to be enrolled updated and added Sarah Cannon, Kansas City, MO and University of California, Davis, Sacramento, CA as an external enrolling sites
		5.0 Registration	Added Sarah Cannon, Kansas City, MO and University of California, Davis, Sacramento, CA
		9.1 Methods	Added Bone marrow aspirates collection
		12.2.4	Added Montefiore Einstein Cancer Center to allow the Quality Assurance team to monitor and audit the study at external sites
		13.0	Sample Size Calculation : text added "After the 19 patients were enrolled and 16 patients were evaluable after a 12-week induction, there were with no safety concerns. An amendment will be added for additional 40 patients (above the originally planned 33 patients) to gather more data on safety and efficacy"
3	February 03, 2023	Front Page	Added Dr Konopleva as a Co-PI and removed Dr Braunschweig
		Protocol schema	Decitabine days changed from 2,9, 16,23 to 1,8, 15, 22
		Section 14.0	Funding: Added funding information
		Section 4.1	Added CMML into inclusion
2	April 21, 2022	Section 4.1	Change in Eligibility Criteria (inclusion #4 states subjects should have an ECOG PSD of ≥3 and was changed to ≤3)
1	November 09, 2021		Original Protocol

ABBREVIATIONS

AZA	5-zacitidine
AE	Adverse event
AEs	Adverse events
Allo-HCT	Allogeneic hematopoietic stem cell transplant
AML	Acute myeloid leukemia
AML-MRC	AML with myelodysplastic related changes
CML	Chronic myeloid leukemia
CMML	Chronic myelomonocytic leukemia
CR	Complete response
CRi	Complete remission with incomplete marrow recovery
DEC	Decitabine
DNMT1	DNA methytransferase 1
DOR	Duration of response
Dctp	Deoxycytidine triphosphate
EFS	Event-free survival
ELN	European Leukemia Net
FDA	Food and Drug Administration
FOCBP	Females of child bearing potential
GHS	Global health status
HI	Hematologic Improvement
HMA	Hypomethylating agents
ICF	Informed Consent Form
IRB	Institutional Review Board
IWG	International Working Group
NOS	Not otherwise specified
MDS	Myelodysplastic syndrome
MDS/MPN	Myelodysplastic and myeloproliferative overlap syndrome
MPN	Myeloproliferative neoplasm
MF	Myelofibrosis
ORR	Overall response rate
PR	Partial remission
QOL	Quality of life
RARS-T	Refractory anemia with ringed sideroblasts and essential thrombocytosis
R/R	Relapsed refractory
SAE	Severe adverse event
SOC	Standard of Care
ULN	Upper limit of normal
Ven	Venetoclax

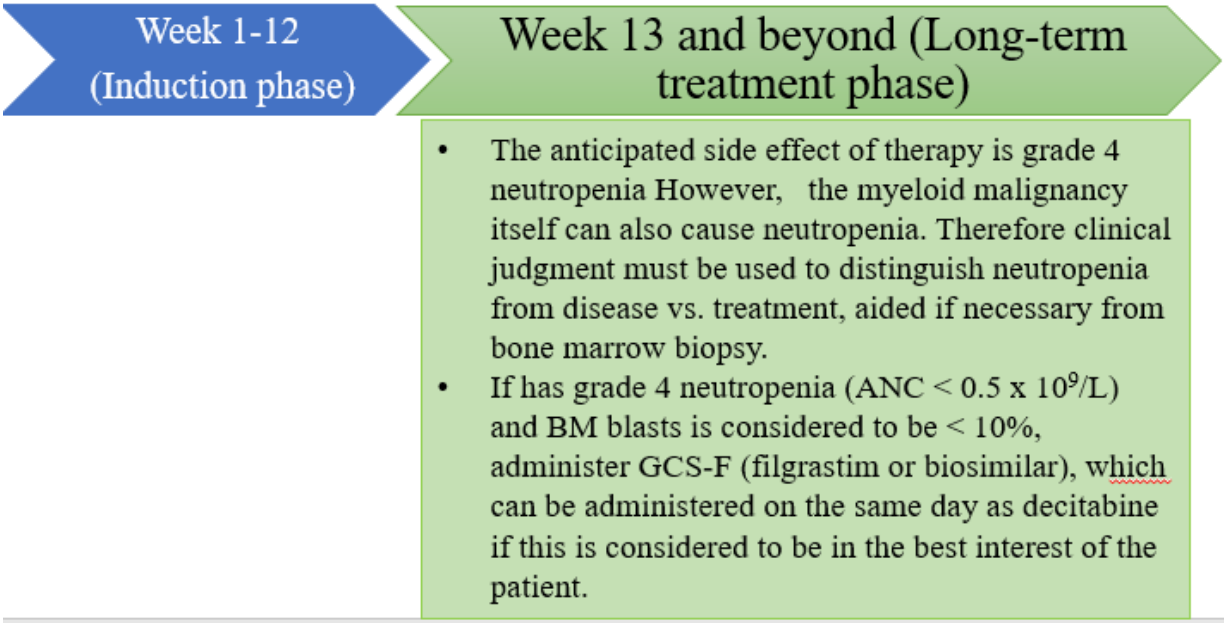
STUDY SCHEMA

Treatment (28 day cycles):

- Venetoclax 400 mg on day 1, 8, 15, 22 of every cycle
- Decitabine 0.2 mg/kg SQ on days 1, 8, 15, 22 (for aggressive disease will add decitabne on days 2, 9, 16, 23)
- If at baseline or during treatment ANC is < 1.5 x 10^9/L, administer GCS-F (filgrastim or biosimilar) on same day as decitabine if bone marrow blast count is considered to be < 10%. Hold G-CSF if ANC is > 1.5 x 10^9/L

Monitoring:

- CBC and type and screen as clinically indicated
- CBC with differential, CMP, LDH, reticulocyte count every four weeks
- Bone marrow biopsy and aspirate at screening, week 12 and week 24. Additional bone marrow as indicated



PROTOCOL SUMMARY

Protocol Number/Title	Proof-of-concept study of metabolically optimized, non-cytotoxic low-dose weekly decitabine and venetoclax in myeloid malignancies
Study Phase	Proof-of-concept clinical trial
Brief Background	The combination of Azacitidine and venetoclax is FDA approved for patients AML > 75 and/or unfit for induction chemotherapy. However, majority of patients receiving standard dosing of Aza/Ven require dose interruptions, treatment delays and dose reductions. In addition, Aza/ven has limited activity in various subgroups of myeloid malignancies such as P53 mutant MDS/AML. The severe cytopenias encountered with Aza/ven is particularly challenging for patients with poor hematopoietic bone marrow reserve such as MDS and MF. Also some elderly patients with comorbidities cannot tolerate the prolonged cytopenias caused by Aza/ven. This pilot clinical trial will evaluate the tolerability of a non-cytotoxic regimen for patients with myeloid malignancies who either cannot tolerate or are not known to benefit from standard Aza/ven dosing.
Rationale	Noncytotoxic dosing of decitabine given weekly at a dose of 0.2 mg/kg induces depletion of DNA methyltransferase 1 (DNMT1) while minimizing the toxicity and myelosuppression seen with standard HMA dosing regimens. This approach has been shown to be effective in a wide spectrum of patients with myeloid malignancies, including patients with resistant disease such as P53 mutations. Preclinical studies have demonstrated that adding a dose of venetoclax prior to administering decitabine enhances the anti-leukemic activity of HMAs by inhibiting de novo pyrimidine synthesis, which is a major mechanism of resistance to HMA in MDS/AML. Decreasing the dose of venetoclax to a weekly schedule also spares normal myelopoiesis. Therefore, a weekly dosing of decitabine/venetoclax is a very attractive option for a patient population who cannot tolerate or are not known to benefit standard Aza/ven.
Primary Objective	**Primary Endpoint** Percentage of participants who are able to continue on treatment without dose interruptions or delays. Defined as delaying or interrupting treatment due to toxicity or intolerability during the induction phase (weeks 1-12)
Secondary Objective(s)	**Secondary Endpoints**: (1) Cumulative incidence of response for both CR, CRi and overall response; (2) Event free survival

	(EFS); (3) Complete remission or complete remission with partial hematologic recovery rate (CR+CRh); (4) Post baseline transfusion dependence rate; (5) Fatigue/quality of life (QoL); (6) Rate of hospitalization; (7) Infection rate **Scientific Correlates**: Correlation of DNMT1 depletion with clinical response criteria
Sample Size	85 patients
Disease sites/Conditions	Myeloid malignancies: ≥MDS, AML, MDS/MPN overlap syndrome
• Interventions	• Venetoclax 400 mg po on days 1, 8, 15 and 28 of each cycle (28day cycle) (+/- 1 day allowed). • Decitabine 0.2 mg/kg SQ on days 1, 8, 15, 22 (for aggressive disease will add decitabine on days 2, 9, 16, 23)

TABLE OF CONTENTS

SUMMARY OF CHANGES **3**

ABBREVIATIONS **5**

STUDY SCHEMA **6**

PROTOCOL SUMMARY **7**

TABLE OF CONTENTS **9**

1.0 INTRODUCTION **12**

1.1 BACKGROUND 12

1.1.1 Diseases– Myeloid malignancies: Acute Myelogenous Leukemia (AML) and Myelodysplastic Syndromes (MDS) and Myeloproliferative neoplasms (MPN) *12*

1.1.2 Treatment of myeloid malignancies *12*

1.2 STUDY RATIONALE 14

1.2.1 Rationale for low dose non-cytotoxic weekly Decitabine dosing: *14*

1.2.2 Rationale for non-cytotoxic Venetoclax Priming of Decitabine: *15*

1.3 Rationale for subgroups of patients with myeloid malignancies who may benefit from a noncytotoxic HMA/ven regimen *16*

1.3.1 Patients who are unfit and cannot tolerate prolonged cytopenias from standard dosing of Aza/ven: *17*

1.3.2 Patients with antecedent Myeloproliferative disorders: *17*

1.3.3 Molecular subgroups that are not known to respond well to Aza/ven: *18*

1.3.4 High risk MDS: *18*

1.4 BACKGROUND AND RATIONALE OF CORRELATIVE STUDIES 19

1.4.1 DNMT1 expression *19*

2.0 OBJECTIVES **20**

2.1 PRIMARY OBJECTIVE 20

2.2 SECONDARY OBJECTIVE(S) 20

3.0 STUDY DESIGN **21**

3.1 NUMBER OF SUBJECTS 21

3.2 REPLACEMENT OF SUBJECTS 22

3.3 EXPECTED DURATION OF TREATMENT AND SUBJECT PARTICIPATION 22

4.0 PATIENT SELECTION **22**

4.1 INCLUSION CRITERIA 22

4.2 EXCLUSION CRITERIA 23

4.3 INCLUSION OF WOMEN AND MINORITIES 25

5.0 REGISTRATION **25**

6.0 TREATMENT PLAN **25**

6.1 **TREATMENT REGIMEN OVERVIEW** 25

6.1.1 ***Decitabine dosing and schedule*** *26*

All patients will receive at least one dose Decitabine every week. If decided by treating physician that the patient needs a more rapid debulking of high disease burden, a second dose can be added. If Decitabine is given twice a week, should preferably be given on two consecutive days. *26*

6.1.2 Venetoclax dosing and schedule *26*

Venetoclax is dosed at 400 mg by mouth one day a week a day prior to the first decitabine dose. If patients are taking another CYP3A4 inhibitor dose adjustments should be made as recommended by pharmacist for a goal dose of venetoclax of 400 mg. *26*

If patient receives two days of decitabine a week, they still only take venetoclax on the day prior to the first dose of decitabine. *27*

6.1.3 Treatment phase *27*
6.1.4 Dose adjustments for hematologic toxicity *27*
6.1.5 Adjustments for non-hematologic toxicity ≥ Grade 3 ..... *28*
6.2 General Concomitant Medications and Supportive Care Guidelines 32
6.3 Criteria for Removal from Study 32
6.4 Duration of Follow Up 34
6.5 Safety Run-In and Early Stopping Rule 34
6.6 Early Stopping Rule for Excessive Toxicity Following Safety Run-In 34
7.0 ADVERSE EVENTS AND POTENTIAL RISKS 35
7.1 Agent Adverse Events 35
7.2 Definitions 36
7.2.1 Adverse Event *36*
7.2.2 Serious Adverse Events *36*
7.2.3 Adverse Event Evaluation *38*
The investigator or designee is responsible for ensuring that all adverse events (both serious and non-serious) observed by the clinical team or reported by the subject which occur after the subject has signed the informed consent are fully recorded in the subject's medical records. Source documentation must be available to support all adverse events. 38
Any grade laboratory investigation abnormality considered clinically significant should be reported as an adverse event, with exceptions as noted above. 38
The investigator or sub-investigator (treating physician if applicable) will provide the following for all adverse events (both serious and non-serious): 38
- Event term (as per CTCAE) 38
- Description of the event 38
- Date of onset and resolution 38
- **Expectedness of the toxicity** 38
- **Grade of toxicity** 38
- **Attribution of relatedness to the investigational agent - (this must be assigned by an investigator, sub-investigator, or treating physician)** 38
- Action taken as a result of the event, including but not limited to; no changes, dose interrupted, reduced, discontinued, etc. or action taken with regard to the event, i.e. no action, received concomitant medication or other intervention, etc. 38
- Outcome of event 38

Descriptions and **grading scales** found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version **5.0** will be utilized for AE reporting. 38
An expected adverse event is an event previously known or anticipated to result from participation in the research study or any underlying disease, disorder, or condition of the subject. The event is usually listed in the package insert for Decitabine or venetoclax, consent form or research protocol. 38
An unexpected adverse event is an adverse event not previously known or anticipated to result from the research study or any underlying disease, disorder, or condition of the subject. 39
Attribution is the relationship between an adverse event or serious adverse event and the study drug. Attribution will be assigned as follows: 39
- Definite – The AE is clearly related to Decitabine/venetoclax. 39
- Probable – The AE is likely related to Decitabine/venetoclax. 39
- Possible – The AE may be related to Decitabine/venetoclax. 39
- Unlikely – The AE is doubtfully related to Decitabine/venetoclax. 39
- Unrelated – The AE is clearly NOT related to Decitabine/venetoclax. 39

Protocol must specify if attribution is required for individual components of the treatment regimen or the treatment regimen as a whole. 39
7.3 SAE Report Form 39
7.4 Reporting Procedures for Serious Adverse Events 39
7.4.1 SAE Reporting Requirements *39*
7.5 SAEs and OnCore 40

7.6 DATA SAFETY AND TOXICITY COMMITTEE 40
- **7.7 DATA AND SAFETY MONITORING PLAN (DSMP)** 40

8.0 PHARMACEUTICAL INFORMATION **41**

8.1 INVESTIGATIONAL AGENTS 41
8.1.1 Name of Agent: Decitabine *41*
8.2.1 Name of Agent: Venetoclax *42*

9.0 CORRELATIVE STUDIES **42**

9.1 METHODS 42
9.1 Analytical Laboratory *43*

10.0 STUDY PARAMETERS AND CALENDAR **43**

11.0 MEASUREMENT OF EFFECT **45**

12.0 DATA REPORTING / REGULATORY CONSIDERATIONS **51**

12.1 DATA REPORTING 51
12.2 REGULATORY CONSIDERATIONS 52
12.2.1 Written Informed consent *52*
12.2.2 Subject Data Protection *52*
12.2.3 Retention of records *52*
12.2.4 Audits and inspections *53*

13.0 STATISTICAL CONSIDERATIONS **53**

14. **FUNDING**

1.0 INTRODUCTION

1.1 Background

1.1.1 Diseases– Myeloid malignancies: Acute Myelogenous Leukemia (AML) and Myelodysplastic Syndromes (MDS) and Myeloproliferative neoplasms (MPN)

Myeloid malignancies are a heterogeneous group of clonal bone marrow disorders that include myeloproliferative neoplasms (MPN's), myelodysplastic syndromes (MDS), and acute myeloid leukemia (AML). Myelodysplastic syndromes are clonal malignancies characterized by dysplastic marrow and ineffective hematopoiesis. Myeloproliferative neoplasms (MPNs) are a group of disorders caused by overproliferation of bone marrow stem cells which can lead to development of myelofibrosis (MF), blast phase-MF and AML. The MDS/MPN overlap syndromes are a more heterogeneous group that have features of MDS, usually marrow dysplasia but also features such a proliferative component and marrow fibrosis. They include distinct entities such as chronic myelomonocytic leukemia (CMML), atypical or BCR-ABL negative chronic myelogenous leukemia (aCML), MDS with refractory anemia with ringed sideroblasts and thrombocytosis (MDS-RARS-T), and MDS/MPNs not-otherwise specified (NOS). Myeloid malignancies as a group lead to the clinical features of peripheral blood cytopenias resulting in transfusion dependence, infections, and bleeding. They have highly variable prognostic features based on cytogenetic changes and molecular mutations. Several validated prognostic classification systems are available, but no standardized guidance has been established on their usage. All types of MDS and MPN's are at increased risk of progression to AML, with some types such as p53 mutation and/or complex cytogenetics being at higher risk. Confirmed cases must present both clinical and morphologic criteria as defined by FAB and WHO guidelines.

1.1.2 Treatment of myeloid malignancies

Treatment options for myeloid malignancies are highly dependent on patient-related factors, such as comorbidities and the impact on quality of life and disease-related factors, particularly the higher incidence of adverse-risk cytogenetic abnormalities which are resistant to standard intensive chemotherapy regimens and elderly patients have relatively decreased tolerance to these agents. Using a number of validated prognostic schemes, of which the most commonly used is the

International Prognostic Scoring System (IPSS), patients with MDS are typically grouped into two major risk groups: higher risk and lower risk.
Treatment objectives for lower risk MDS are to reduce symptoms and improve quality of life. For higher risk MDS, the therapeutic objective is to aggressively modify the natural history of the disease, i.e., to increase survival, and decrease risk of progression to AML. Critical issues in determining therapeutic strategies for patients with MDS include disease heterogeneity, lack of clear pathogenic understanding, tolerability of therapy in the elderly population and a dearth of effective treatments. MDS therapies include: supportive care with transfusion therapy, growth factors (EPO, G-CSF), antibiotics and HMA's (Azacitidine and Decitabine). The only curative therapy for high risk MDS is allogeneic stem cell transplant which has a high morbidity/mortality and therefore not an option for elderly or patients with high comorbidities. Treatment for AML includes induction chemotherapy with cytarabine plus anthracycline, investigational drugs, hematopoietic stem cell transplantation (HSCT), FLT3 and IDH inhibitors in patients whose disease harbor these mutations. Lower intensity regimens using hypomethylating agents (HMA; azacitidine or decitabine) or low-dose cytarabine (LDAC), in combination with venetoclax are now FDA approved for patients over age 75 or younger patients who cannot tolerate cytotoxic chemotherapy. [1] In practice some clinicians will also use HMA/venetoclax for younger patients with diseases known to have a poor response to chemotherapy, such as patients who harbor P53 mutations and patients with MPN's that transform to AML for whom there is currently no universally accepted standard of care. The combination of Azacitidine/venetoclax received FDA approval for AML based the VIALE-A trial which randomized newly diagnosed AML patients older than age 75 or younger patients ineligible for standard induction therapy because of coexisting medical conditions, to Azacitidine/Venetoclax or Azacitidine/placebo and showed an improvement in OS (14.7 vs. 9.6 months), CR (36.7% vs 17.9%) and composite CR (complete remission or complete remission with incomplete hematologic recovery) of 66.4% vs. 28.3%. While the VIALE-A has established Azacitidine/ventoclax as a new standard for some patients > age 75 and for younger patient's ineligible for intensive chemotherapy; a major limitation of this regimen is dose limiting toxicities due to cytopenias requiring dose interruptions and delays leading to increased risk of disease progression. A modification of HMA/ven regimen which can limit dose interruptions due to cytoepanias has the

potential to increase the durability of responses. In addition, majority of responses seen with standard doses of HMA/ven are in patients with NPM1, IDH1, IDH2, or DNMT3A mutations; with limited activity in patients with P53, RAS and RUNX1 mutations. [2] An unmet need remains for patients with AML who either cannot tolerate standard dosing of Aza/ven or patients who have disease characteristics which have not shown to have a benefit from standard dosing Aza/ven.

1.2 Study Rationale

1.2.1 Rationale for low dose non-cytotoxic weekly Decitabine dosing:

Decitabine is a nucleoside analogue which depletes the epigenetic regulator DNA methyltransferase 1 (DNMT1). It is routinely dosed in myeloid malignancies to generate dose-dependent DNA damage in malignant cells that then triggers apoptosis (cytotoxicity). However, with this approach normal dividing hematopoietic cells are unfortunately also simultaneously destroyed causing prolonged cytopenia's and the myelotoxicity that result from it. In fact, in the VIALE-A trial where "standard" myelotoxic doses of HMA/ven were combined, 24% of patients had to discontinue treatment due to adverse events and more importantly 72% of patients required dose interruptions between cycles due to adverse events. [1] While some patients can get an initial response to high doses of HMA/ven, dose interruptions decrease the durability of many responses. An approach of continuous drug exposure for suppression of clones which spares preserved non-clonal hematopoietic stem cells may allow continuous dosing without interruptions due to cytoepnias and may lead to more durable remissions.

Weekly low doses of Decitabine administered as a subcutaneous injection has been shown to cause on target DNMT1-depletion (even cytoreduce p53-null myeloid malignancies) while sparing normal hematopoiesis. Sauntharajah et al identified the minimum doses of decitabine (0.1–0.2 mg/kg) can cause molecular targeted DNMT1 depletion via terminal differentiation, instead of apoptosis, while sparing normal hematopoiesis by limiting the off-target anti-metabolite effects/cytotoxicity.[3,4] These doses are given 1–2 times a week to increase S-phase dependent DNMT1-depletion. Long term followup (up to 6.5 years) using this approach (weekly SQ decitabine of 0.1-0.2 mg/kg), has demonstrated durable responses in myeloid malignancies across the clinicopathologic spectrum of diseases which include MDS, MDS/MPN overlap, MPN or AML, which is not surprising given the ability to continue weekly dosing without dose interruption due to

cytopenias. These responses were also in a diverse group of genetic abnormalities — consistent with scientific data implicating DNMT1 as a mutation-agnostic target that operates in a final common pathway of myeloid transformation.[4,5] In a cohort of 69 patients treated at the Cleveland clinic with low dose weekly decitabine for myeloid malignancies, Awada et al reported ~ 40% response rate despite approximately 50% of patients having disease that was relapsed/refractory to 5-azacytidine, lenalidomide and/or cytarabine. [5]

1.2.2 Rationale for non-cytotoxic Venetoclax Priming of Decitabine:

Decitabine is a pro-drug which is processed by pyrimidine metabolism into its active form - a deoxycytidine analog. Resistance to decitabine emerges from adaptive responses of the pyrimidine metabolism network causing shifts in expression of key pyrimidine metabolism enzymes in directions adverse to the pro-drug activation, such that decitabine processing into DNMT1-depleting nucleotide is forestalled (Figure 1). Continuous pro-drug exposure stabilizes these adaptive metabolic responses and thereby prevents DNMT1-depletion.[6] A key element in this auto-resistance is upregulated de novo pyrimidine synthesis which has an electron-transport dependent mitochondrial step executed by dihydroorotate dehydrogenase (DHODH) (see figure below). Venetoclax has shown to inhibit DHODH/pyrimidine synthesis and thereby inhibits de novo pyrimidine synthesis.[7] Therefore adding venetoclax as a single dose primer 24 hours prior to decitabine can counter this mode of resistance and enhance the activity of decitabine's anti-AML activity. This strategy overcomes and avoids the toxicity of myelosuppression seen with standard cytotoxic dosing of Aza/ven.

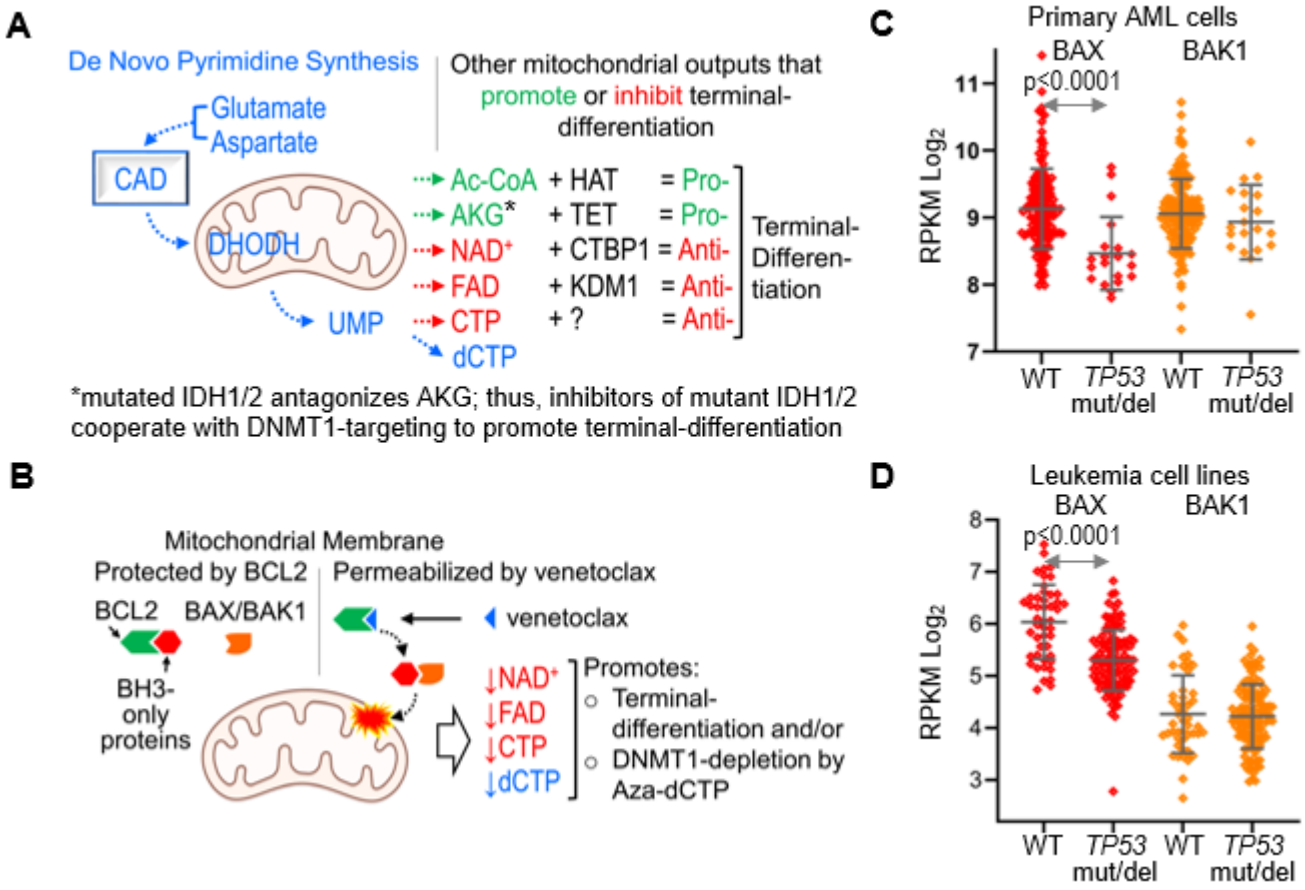


Figure 1. Mitochondrial-targeting by venetoclax and cooperation with DNMT1-targeting by Aza-dCTP.

A) De novo pyrimidine synthesis, that competes with Aza-dCTP, requires the mitochondrial enzyme dihydroorotate dehydrogenase (DHODH), powered by the membrane electron gradient. Also powered this way are mitochondrial outputs of epigenetic enzyme cofactors, that similar to DNMT1, inhibit terminal-differentiation of malignant cells (Ac-CoA=acetyl-CoA; HAT = histone acetyltransferase; AKG=alpha-ketoglutarate; TET=ten-eleven translocation methylcytosine dioxygenases; NAD=nicotinamide adenine dinucleotide; CTBP1=C-terminal binding protein 1 corepressor; FAD=flavin adenine dinucleotide; KDM1=lysine demethylase 1; CTP=cytidine triphosphate; dCTP=deoxycytidine triphosphate).

B) The anti-apoptotic BCL2 protein family (BCL2, BCL2L2, BCL2L1, MCL1) sequester BH2-only proteins (BID, BAD etc.) that otherwise activate mitochondrial membrane permeabilizing action of effector proteins BAX and BAK1.

C) BAX, a key effector protein in the pathway downstream of venetoclax, is significantly decreased in primary AML cells containing mutated or deleted *TP53*. Mean±SD. TCGA RNA-Seq, n=167, p-value 2-sided t-test.

D) This observation is recapitulated in hematopoietic malignancy cell lines. CCLE RNA-Seq, n= 175.

1.3 Rationale for subgroups of patients with myeloid malignancies who may benefit from a noncytotoxic HMA/ven regimen

Hypomethylating agents (decitabine and azacytidine) are approved for the treatment of MDS and AML. Venetoclax is approved for AML at and often used in the treatment of MDS. The combination of HMA/ventoclax at approved doses are cytotoxic causing severe and prolonged

cytopenias. However, MDS and AML are heterogenous diseases and there are many subgroups of patients for whom standard dosing of HMA +/- venetoclax have not been shown to be effective and therefore these patients currently do not have standard of care options available for them. In addition, there are subgroups of patients who are unable to tolerate the toxicities of standard HMA/venetoclax dosing and inevitably receive substandard doses of therapy due to dose delays and interruptions and therefore are unable to receive effective therapies. A non-cytotoxic weekly dosing schedule may be the only effective or practical therapy for these subgroups. Below we give a background on the rationale for each of these subgroups for who we plan to enrol in this study because they currently do not have a standard of care treatment option available to them either because there is no known benefit for their subtype of MDS/AML and/or they are unable to receive currently approved dosing schedules of HMA/venetoclax due to toxicity.

1.3.1 Patients who are unfit and cannot tolerate prolonged cytopenias from standard dosing of Aza/ven:

The standard dosing of Aza/ven used in the VIALE-A trial is Azacitidine 75 mg per square meter of body-surface area subcutaneously or intravenously on days 1 through 7 every 28-day cycle, venetoclax 400 mg orally once daily, in 28-day cycles.[1] A major challenge with "standard dosing" of Aza/ven is severe and prolonged cytopenias,[8] leading to transfusion dependence, increased risk of bleeding, infections, hospitalizations and poor quality of life. For example, in VIALE-A grade 3 neutropenia was observed in 42% of patients and over 50% required a reduction in the duration of venetoclax. Similarly, in clinical practice clinicians have been struggling with dose limiting cytopenias leading to dose reductions and treatment interruptions. Various algorithms are being suggested how to best dose, and interrupt regimens of Aza/ven, with majority suggesting shorter durations of venetoclax than was used in VIALE-A trial.[8] It's therefore not surprising that the real-world experience with standard dosing HMA/ven have reported outcomes to be inferior to the clinical trials.[9] This has been most challenging for elderly patients with multiple comorbidities and for patients with antecedent bone marrow disorders who have poor hematopoietic reserve, many of whom cannot tolerate standard Aza/ven dosing.

1.3.2 Patients with antecedent Myeloproliferative disorders:

Patients with MPN's who transform to MF, blast phase-MF or full blown AML have a very poor prognosis and there is currently no established standard of care for this population. The major challenge has been that these patients have marrow fibrosis

with poorly preserved hematopoietic cell function and treatments with cytotoxic doses of therapy are poorly tolerated. Like many AML trials, VIALE-A also excluded patients with any history of MPN's (including MF, PV, ET, CMML and CML) and the use of HMA/ven combination in this population has only been reported in a small single institution retrospective series demonstrating mild activity.[10] A noncytotoxic regimen for this population is an urgent unmet need.

1.3.3 Molecular subgroups that are not known to respond well to Aza/ven:

Responses to standard dosing of HMA/ven have been most robust in AML with NPM1, IDH1, IDH2, or DNMT3A mutations. Primary or early resistance to HMA/ven have been reported in patients with FLT3, C-KIT and TP53 mutations.[2] As described above, the non-cytotoxic regimen of weekly decitabine has shown to be effective in myeloid malignancies independent of mutations which are known to confer resistance to standard dosing which depends on cytotoxic effects.

1.3.4 High risk MDS:

Decitabine and Azactidine are the only drugs approved by the FDA for treatment of high risk MDS. Response to these agents occurs in ~50% of patients, duration of response is transient and all patients eventually progress.[11,12] Outcomes after HMA failure are particularly poor, with a median overall survival (OS) of 4 to 6 months.[13,14]More recently the addition of venetoclax to Azacitidine is being evaluated in a phase 1b trial for patients with R/R MDS with a reported 40% CR/marrow CR in R/R MDS patients in phase 1b trial. [15] However, ≥ grade 3 cytopenia's were seen in 50% of patients. In practice clinicians have been adding ventoclax to HMA for patients with HR-MDS (extrapolating from AML). A multi-institutional realworld experience using HMA/ven for MDS reported an ORR of 59% [14% CR, 27% marrow CR with hematologic improvement (HI), and 18% with a marrow CR without HI], with poor risk cytogenetics being associated with poor outcomes.[16] In this cohort delays in treatment were seen in 67% of patients, venetoclax interruption in 29% and discontinuation of ventoclax because of adverse events was 20%. While the exact causes for this were not reported, given the known major dose limiting toxicity of venEtoclax are cytopenias it's likely that this was the cause in the vast majority of patients. Therefore, a non-cytotoxic approach with the addition of venetoclax would be of great interest in this population.

1.3.5 AML with MDS Related Changes (AML-MRC):

AML-MRC is a distinct biologic subtype of AML that represents approximately 1/3 of the cases and is associated with e inferior outcomes compared to non-MRC

AML. Typically, patients with AML-MRC are older (median age 73) and experience low remission rates following intensive chemotherapy with a median overall survival of 9–12 months.[17] The only approved agent for this population is Vyxeos (CPX-351) which is a liposomal encapsulation of cytarabine and daunorubicin and is only an option for fit patients who can tolerate intensive chemotherapy. AML/MRC patients represented a 1/3 of the VIALE-A cohort and was one of the few subgroups who did not demonstrate a statistically significant survival benefit (HR 0.73, CI 0.48–1.11). The major challenge in the use of standard Aza/ven in this population is prolonged cytopenias requiring dose reductions and prolonged dose delays so a continuous noncytotoxic approach is a rationale approach for patients who cannot tolerate vyxeos.

1.4 Background and rationale of correlative studies

1.4.1 DNMT1 expression

The intended molecular target of decitabine therapy is the key epigenetic regulator DNMT1. In order to deplete DNMT1, decitabine, a pro-drug, is processed via pyrimidine metabolism into a nucleotide Aza-dCTP that incorporates into the newly synthesized DNA strand during cellular S-phase. Natural deoxycytidine (dCTP) directly competes with Aza-dCTP for incorporation into DNA, and accordingly, a major mechanism by which malignant cells evade DNMT1-depletion by decitabine is by upregulating de novo pyrimidine synthesis that synthesizes dCTP from glutamine and aspartate building blocks. We hypothesize that a single dose of venetoclax once a week prior to subcutaneous decitabine the next day counters this mechanism of resistance by temporarily inhibiting de novo pyrimidine synthesis, that requires intact mitochondrial membrane potential. Thus, the key correlative study in this study is measurement of DNMT1 levels in the bone marrow. By confirming the ability of to maintain DNMT1 depletion in a clinical setting, this study aims to be foundational in future studies by demonstrating appropriate testing intervals for biological correlates. Moreover, monitoring the DNMT1 repletion over time along with at the time of progression or relapse will indicate mechanisms of resistance to this therapeutic strategy. In other words, whether relapse was specifically associated with a failure to maintain DNMT1 depletion or another mechanism. DNMT1 protein levels will be measured in bone marrow biopsy specimens using immunohistochemistry (IHC), complemented by flow cytometry analysis of peripheral blood white cells that are in G1. The correlatives will be done at the Cleveland clinic laboratory of Dr Yogen Saunthararajah.

1.4 Rationale for cohort expansion (ammendment):

- In aa retrospective cohort of patients treated at Montefiore-Einstein with this regimen of weekly low dose decitabine/venetoclax (LDDec/VEN) wecompared the experience with the low dose regimen to a cohort of contemporaneous patients treated with standard dosing HMA/VEN per the VIALE-A trial (Levitz et al CCR in press) . The ORR for frontline AML and MDS patients for LDDec/VEN was 88% and 64% respectively, similar to the standard dosing regimen. Importantly, we showed that the LDDec/VEN cohort had a much longer time on therapy when compared to patients who received standard VIALE-A dosing (175 vs 78 days; p= 0.014) and a numerical trend toward a higher rate of transfusion independence (53% vs 26%), although this did not reach statistical significance (p=0.33). A preliminary analysis of the data from the initial 17 evaluable patients **enrolled** in this protocol (patients who completed 12 week of induction), demonstrated that only one patient had a dose interruption during induction (one dose held) and no patients required dose reductions. The majority of these patients had AML (n=11) with an ORR of 64% (7/11)and 6/7 responses are ongoing. Given the early high tolerability of the low dose regiment (primary endpoint) and encouraging activity (secondary endpoint), combined with our reported in retrospective data referenced above, adding additional patients in selected subsets of patients will allow further confirmation of the safety and tolerability of this novel low dose metronomic regimen as well as to to generate preliminary efficacy data (secondary endpoints) to guide sample size estimates for further clinical development Data from these additional patients will also help in defining the safety and efficacy profile of this regimen to allow for rationale design of future combination trials using the low dose metronomic HMA/VEN backbone with additional agentsto achieve further progress in clinical outcomes for patients with MDS and AML.

2.0 OBJECTIVES

2.1 Primary Objective

1. Percentage of MDS and AML participants who are able to continue on treatment without dose interruptions or delays, defined as delaying or interrupting treatment due to toxicity or intolerability for more than two weeks

2.2 Secondary Objective(s)

1. Percentage of MDS participants with complete remission (CR) and complete remission with incomplete marrow recovery (CRi)
2. Percentage of AML participants with complete remission (CR) and complete remission with incomplete marrow recovery (CRi)
3.

   This will be calculated based on current International Working Group (IWG) criteria. CR is defined as absolute neutrophil count > 1000/ microliter (mcL), platelets > 100k/mcL, red cell transfusion independence, and bone marrow with < 5% blasts. CRi is defined as bone marrow with less than 5% blasts, and absolute neutrophils of <= 10000/mcL or platelets <= 100k/mcL

4. Event-free survival (EFS)

   EFS will be defined as the number of days from randomization to the date of progressive disease, relapse from CR or CRi, treatment failure or death from any cause.

5. Complete remission or complete remission with partial hematologic recovery rate (CR+CRh)

   A response of CRh is defined as Bone marrow with <5% blasts, peripheral blood neutrophil count >0.5*10^3/mcL and peripheral blood platelet count >0.5*10^5/mcL.

6. Post baseline transfusion independence rate

Transfusion Independence is defined as a period of 56 days with no transfusion between first dose of study drug and the last dose of study drug + 30 days. The rate of conversion for red blood cells (RBC) and platelets is defined as percentage of participants being post-baseline transfusion independent from baseline transfusion dependence.

7. Rate of Hospitalization

   Defined as hospitalization for complication related to myeloid malignancy or treatment. Initial admission for diagnosis or initiation of therapy will not be considered an event.

8. Infection rate requiring hospitalization

   Defined as being hospitalized due to an infection or sepsis.

3.0 STUDY DESIGN

This will be a single arm, open label pilot study of weekly dosing of subcutaneous decitabine and venetoclax

Patients will be treated for a minimum of 12 weeks in the absence of clear evidence of progressive disease. Patients who have any response will be permitted to continue treatment until relapse or progression of disease. Primary endpoint will be the percentage of patients who will require dose interruptions due to cytopenias. Secondary endpoints will include assessment of response rates, transfusion dependence, rate of infections and hospitalizations.

Exploratory endpoints will include assessment of the ability of weekly low doses of Dec and Ven to cause DNMT1 depletion on bone marrow samples.

3.1 Number of Subjects

In the initial safety and tolerability phase of the study, 33 patients will be enrolled on this study, accounting for need for replacement subjects to evaluate endpoints. In the second expansion phase of the study up to 85 patients (including patients from the 1st stage), will be enrolled to obtain additional safety, tolerability and preliminary efficacy of the low dose regimen in selected subsets of patients with myeloid malignancies.

3.2 Replacement of Subjects

As the treatment with HMAs requires extended drug exposure for efficacy, patients who do not complete 12 weeks of therapy for reasons other than disease progression and who do not complete therapy due to toxicity will be replaced. Any patient who starts therapy will be evaluable for safety.

3.3 Expected Duration of Treatment and Subject Participation

In the absence of overt disease progression or dose limiting toxicity, patients would be anticipated to remain on treatment for at least 12 weeks. After 12 weeks, patient may continue therapy if felt to be experiencing clinical benefit.

4.0 PATIENT SELECTION

Each of the criteria in the sections that follow must be met for a subject to be considered eligible for this study.

Subject's Name _____

Medical Record # _____

Research Nurse / Study Coordinator Signature:

_____

Date _____

Treating Physician [Print] _____

Treating Physician Signature: _____

Date _____

4.1 Inclusion Criteria

Subjects must meet all of the following inclusion criteria to be eligible for enrollment:

1. Patient must have a diagnosis of MDS, AML, CMML or MDS/MPN with a histopathologic diagnosis confirmed by hematopathology review

AND

2. Indication for therapy with potential sensitivity to HMA therapy, defined as prior published evidence of response to HMA.

AND

3. Patients must be 18 years of age or older

AND

4. Patients must have an ECOG performance status of ≤ 3

AND

5. Patients must have adequate end organ function defined as.

   - AST and ALT < 4× the upper limit of normal (ULN)
   - Bilirubin ≤ 2× the ULN. If elevated bilirubin is due to impaired conjugation (e.g Gilbert's disease or concomitant medication) or disease related hemolysis, then direct bilirubin ≤ 1.5× the ULN.
   - As decitabine and venetoclax have little renal metabolism, and have proven safety even in dialysis patients, renal function with a creatinine clearance ≥30 mL/min or on dialysis is allowed.[18]

AND

6. Subjects must have the ability to understand and the willingness to sign a written informed consent document and complete study related procedures.

4.2 Exclusion Criteria

1. APL
2. Core binding factor AML who are candidates for intensive chemotherapy
3. Prior Treatment with azacitidine, decitabine or venetoclax

4. No other disease directed therapy, save for hydroxyurea, cytarabine, Cladribine, Gemtuzumab ozogamicin including experimental or investigational drug therapy for 14 days prior to study entry.

5. Currently pregnant or breast-feeding. Females of child bearing (FOCBP) potential must have negative serum pregnancy test within 72 hours from treatment start. (NOTE: FOCBP is any biologic female, regardless of sexual or gender orientation, having undergone tubal ligation, or remaining celibate by choice, who has not undergone a documented hysterectomy or bilateral oophorectomy or has had a menses any time in the preceding 12 months (therefore not naturally post-menopausal for > 12 months)

6. Uncontrolled intercurrent illness that could limit life expectancy or ability to complete study correlates. This includes, but is not limited to:

    a. Ongoing or active infection. As patients with myeloid malignancies are prone to infections, if patients are actively being treated with appropriate antibiotics or antifungal therapy with clinical evidence of infection control, then they will be considered eligible for study.

    b. Uncontrolled concurrent malignancy

    c. Congestive heart failure of NYHA class III/IV. Patients with compensated heart failure are permitted.

    d. Unstable angina pectoris

    e. New or unstable cardiac arrhythmia. Stable or controlled arrhythmias are permitted

    f. Decompensated liver cirrhosis (Child-Pugh score ≥12 or a MELD score ≥21

    g. Psychiatric illness/social situations that would limit compliance with study requirements.

    h. Any other prior or ongoing condition, in the opinion of the investigator, that could adversely affect the safety of the patient or impair the assessment of study results.

7. WOCBP and males that are unwilling to agree to use dual contraceptive measures (i.e., hormonal or barrier method of birth control; abstinence, condom) prior to study entry and for the duration of study participation.

Should a female subject become pregnant or suspect she is pregnant while participating in this study, she should inform the treating physician immediately

8. Sexually active male who is unwilling to use a condom when engaging in any sexual contact with a female with child-bearing potential, beginning at the screening visit and continuing until 4 weeks after taking the last dose of Decitabine/venetoclax.

9. Patients with uncontrolled active HIV infection, as this will further increase the risk for opportunistic infections. However, patients with HIV with undetectable viral load by PCR, without opportunistic infection, and on a stable regimen of antiretroviral therapy would be eligible.

10. Known allergy or hypersensitivity to any component of decitabine or venetoclax formulations

4.3 Inclusion of Women and Minorities

Men, women and members of all races and ethnic groups are eligible.

5.0 REGISTRATION

Subjects will be recruited at Montefiore/Einstein Cancer Center, University of California Davis, and White Plains Hospital Center for Cancer Care. All subjects who have been consented are to be registered in REDCap. Database. For those subjects who are consented, but not enrolled, the reason for exclusion must be recorded.

All subjects will be registered through Montefiore/Einstein Cancer Center and will be provided a study number by contacting the study coordinator listed on the cover page.

6.0 TREATMENT PLAN

6.1 Treatment Regimen Overview

Administration: Decitabine is reconstituted with 5 ml sterile water to facilitate subcutaneous administration. Decitaboine is given by subcutaneous injection. Venetoclax is taken as a tablet prepared by patients pharmacy.

Venetoclax is given at a dose of 400 mg po once per week concurrently with the Decitabine dose (+/- 1 day allowed).
All treatment regimens may be continued indefinitely.

Concomitant Treatment: Hydroxyurea is permitted to control leukocytosis and thrombocytosis. In patients with aggressive high bulk disease as determined by the investigator and PI, may receive debulking chemotherapy with either Gemtuzumab ozogamicin, Cladrabine or Cytarabine prior to starting decitabine/venetoclax. Also patients who have mutations in either FLT3 (ITD or TKD), JAK2, IDH1 or IDH2 mutation may receive concomitant inhibitors (as outlined in the table below) at any point during the protocol as determined by the PI. Other than HU and above mentioned drugs, no other concomitant disease modifying therapy is permitted. No additional investigational study is permitted and concurrent participation in other interventional trials are not permitted. Participation in registry or non-interventional studies is permitted.

Mutation	**Medication**
FLT3 ITD	Midostaurin Gilteritinib Quizartinib
FLT3 TKD	Midostaurin Gilteritinib
IDH1	Ivosidenib Olutasidenib
IDH2	Enasidenib
JAK2	Jakafi

Induction phase: The initial 12 weeks of therapy is considered an induction phase during which dose modifications or holds are discouraged except for severe toxicity severe, non-hematologic adverse events, as per figure study schema. However, if myelosuppression from medications are suspect, treatment holds and dose modifications may be considered in consultation with the study chairs. Desired treatment holds or dose modifications should be reviewed as soon as possible with the study chairs to determine whether dose hold/modification is acceptable. The purpose of the induction phase is to produce relatively rapid reduction of tumor burden to a level that will permit recovery by more functional hematopoiesis that can relieve cytopenias.

Long Term Treatment phase: After a 12 week induction phase, persistent treatment without holds could result in severe, treatment related cytopenias. Conceptually, targeted DNMT1 depletion may result in differentiation that could result in hematologic improvement with evidence of persistent marrow disease. Full response to therapy may not occur within 12 weeks as sometimes six months or longer is required for maximal response to HMAs. Thus, the goal should be to maintain dose while ensuring optimal hematologic improvement from therapy.

6.1.1 Decitabine dosing and schedule

Decitabine is given at a dose of 0.1–0.2 mg/kg/day on days 1, 8, 15, 28 (+/- 3 days)..

All patients will receive at least one dose Decitabine every week. If decided by treating physician that the patient needs a more rapid debulking of high disease burden, a second dose can be added. If Decitabine is given twice a week, should preferably be given on two consecutive days.

6.1.2 Venetoclax dosing and schedule

Venetoclax is dosed at 400 mg by mouth one day a week concurrently with the first decitabine dose (+/- 1 day allowed).. If patients are taking another CYP3A4 inhibitor dose adjustments should be made as recommended by pharmacist for a goal dose of venetoclax of 400 mg.

If patient receives two days of decitabine a week, they still only take venetoclax on the day ofthe first dose of decitabine (+/- 1 day allowed).

6.1.3 Treatment phase

Each treatment cycle is 28 days. Treatment is given weekly and continued without any interruptions

6.1.4 Dose adjustments for hematologic toxicity

The starting dose of decitabine being administered (0.2 mg/kg) and the dose of venetoclax of 400 mg have been identified by previous pharmacokinetic, pharmacodynamic and clinical studies (administering the drugs singly) as the minimal biologically active doses needed to produce the intended therapeutic molecular pharmacodynamic effect. Based on our experience using this regimen, an anticipated side-effect is neutropenia. However, neutropenia is frequently

caused by the myeloid malignancy being treated. Since doses are already minimal biologically active doses, it is important not to prematurely dose-reduce if neutropenia is from disease and not from therapy:

- To avoid premature dose reduction from already minimal biologically active doses, this protocol incorporates an Induction Phase (week 1-12) during which doses should not be held except for clinically exigent circumstances. After the induction phase, the treating clinical team will need to exercise judgement to distinguish between Grade 4 neutropenia (ANC < 0.5 $x10^9$/L) caused by disease *vs* treatment, *aided if necessary with repeat bone marrow aspirate and biopsy*.
- If Grade 4 neutropenia is judged to be from treatment, the first step is to hold treatment until recovery of the ANC to > 0.5 $x10^9$/L. If neutropenia recurs, then the next step is to decrease decitabine doses by 25% (0.15 mg/kg).
- If neutropenia recurs even on the reduced dose of decitabine, then decrease dose of decitabine by another 25% (0.1 mg/kg). If neutropenia persists even at this dose and is felt to be from treatment and not from disease, treatment should be held until recovery of the ANC to > 0.5 $x10^9$/L. Only continue therapy if it is considered to be in the best interests of the patient. Its at discretion of the treating physician based on clinical benefit to the patient, as to when to go back to the full dose of Decitabine or keep at reduced dose.
- To reduce risks from baseline neutropenia caused by the disease, that may deter or hinder administration of potentially beneficial therapy, this protocol permits concurrent administration of G-CSF (filgrastim or biosimilar ~5 µg/kg) if thought to be safe by the treating physician
- Venetoclax can also cause cytopenias but less so with once weekly dosing. If cytopenias are thought to be due to venetoclax, can first decrease dose by 50% (200 mg daily) and if cytopenias persist and is thought to be due to venetoclax, hold venetoclax and continue decitabine single agent. Can restart venetoclax based on the clinical judgment of the treating physician

6.1.5 Adjustments for non-hematologic toxicity ≥ Grade 3

Low doses of decitabine and weekly venetoclax have low risk of renal toxicity, and are not felt to be renally toxic. There is concern that decitabine has hepatotoxicity at standard dosing, but risk should be nominal in the setting of very low doses. In general, workup for alternative causes for non-hematologic toxicity should be strongly considered, and decitabine and venetoclax is only dose reduced in the absence of alternative explanation. However, doses should be reduced for toxicity considered *at least possibly related* to decitabine and venetoclax. This includes laboratory abnormalities.

Infections in patients with myeloid malignancies are common and should be treated accordingly. Infections do not require dose adjustments unless there is clear evidence of relation to decitabine or venetoclax toxicity leading to the associated infection. In other words, most infections should not warrant dose modification.

Table 1: Adjustments for non-hematologic toxicity

Serum creatinine (Renal Toxicity)	If pretreatment creatinine is < 0.5, and increased in creatinine to ≥ 1.5 If pre-treatment creatinine 0.6-0.8 mg/dL, an increase to ≥1.7 mg/dL For pretreatment creatinine of 0.9 to 3.0, and a doubling of creatinine, or > 5.0.	If no alternative cause identified and toxicity felt *at least possibly related*, hold decitabine and venetoclax until recovered below threshold and restarted with a 25% dose reduction. Can go back to full dose when felt safe to do so by treating physician. If toxicity recurs after dose reduction without an identifiable alternate cause and is thought to be related to decitabine or venetoclax, patient should be removed from protocol

Hepatotoxicity	AST or ALT ≥ 3× ULN OR Direct Bilirubin 2× ULN	If no alternative cause and toxicity felt *at least possibly related* to study drug: Hold until recovery to below threshold and restart decitabine and venetoclax with 25% dose reduction. Can go back to full dose when felt safe to do so by treating physician. If toxicity recurs after dose reduction without an identifiable alternate cause and felt to be related to Decitabine or Venetoclax, patient should be removed from protocol
Any other grade 3 and 4 adverse events at least possibly related to decitabine/venetoclax (No adjustments for alopecia)		If no alternative cause suspected or identified : Hold until recovery to grade 1 and restart at 25% dose reduction If toxicity recurs after dose reduction and thought to be related to Decitabine or Venetoclax, patient should be removed from protocol

6.1.5.1 Identification of differentiation syndrome

The differentiation syndrome is a potentially fatal complication observed in the treatment of acute promyelocytic leukemia with tretinoin, but has also been reported in therapies with epigenetic effects, such as IDH inhibitors.[19,20] Although rare, there are case reports of differentiation syndrome in patients with HMA therapy.[21] In general, differentiation syndrome has not been described in other low dose HMA studies, and is *not* an anticipated adverse event. However, with the proposed treatment schedule of decitabine/ven is effective in promoting differentiation of myeloblasts and cytokine release occurs from these cells, this is a potential complication that requires awareness by treating physicians.

The differentiation syndrome is characterized by fever, dyspnea, possible hypoxemia and respiratory distress with pulmonary infiltrates, peripheral edema, hypotension and renal and hepatic insufficiency. If differentiation syndrome is suspected, treatment with corticosteroids should be started promptly. The corticosteroid of choice is Dexamethasone 10mg IV twice daily, which should be continued until resolution of symptoms, signs and laboratory abnormalities related to differentiation syndrome. Hydroxyurea would be permitted in this setting to control leukocytosis.

Decitabine/venetoclax may be continued unless it is a severe case of differentiation syndrome as determined by the treating physician or if there is no improvement after treatment with corticosteroids. If discontinued, decitabine/ven may be resumed without dose adjustment after resolution of differentiation syndrome.

6.2 General Concomitant Medications and Supportive Care Guidelines

Antimicrobial prophylaxis:
Patients with hematologic illnesses should be offered antimicrobial prophylaxis (antiviral, antifungal, antibacterial) per institutional standards.

Supportive care:
G-CSF can may be considered for any patient with < 5% bone marrow blasts who has a baseline ANC < 1.5 x 10^9/L during the induction phase, but should be held if the most recent ANC is ≥ 1.5 x 10^9/L. During the long-term treatment phase, G-

CSF could be administered for neutropenia (ANC < 1 x 10^9/L), not felt to be due to active disease.

Concomitant erythropoietin analogues are permitted at physician discretion for transfusion dependent patients with EPO level < 500.

Patients with advanced myeloid malignancies often require frequent clinical visits and CBC evaluation for supportive transfusions. Patients should be monitored as per local standards and treating physicians' standard practice. A weekly CBC at minimum during the induction phase of treatment is suggested.

No antiemetic therapy is required per protocol, but any is permitted at treating physician's discretion.

At higher doses, decitabine is known to cause gastrointestinal toxicity, including constipation. Though not anticipated at provided doses, patients should be monitored and treated as indicated for gastrointestinal toxicity.

The need for central venous access can be determined on a case by case basis, per physician discretion, as central lines carry both benefits and risks to patients. However, decitabine should be given subcutaneously in this setting.

6.3 Criteria for Removal from Study

In the absence of treatment delays due to adverse events, treatment may continue as long as patient is receiving benefit from treatment (see section 6.4), or in the event of the following:

- Disease progression unresponsive to dose adjustment, or progression at maximum dose level
- Intercurrent illness that prevents further administration of treatment,
- The investigator considers it, for safety reasons, to be in the best interest of the subject.
- Any toxicity or adverse event that leads to delay in drug administration of greater than 6 weeks
- Subject decision to withdraw from treatment or from the study

- In accordance with the Declaration of Helsinki and the guidelines of the country of the participating Clinical Study Center, each subject is free to withdraw from the study at any time. An investigator also has the right to withdraw a subject from the study in the event of the patient suffering an intercurrent illness, adverse events, or other reasons concerning the health or well-being of the patient, or in the case of lack of cooperation by the patient. All serious adverse reactions need to be followed until resolution and information returned to study coordinators.

- Should a subject decide to withdraw after administration of study drug, or should the investigator decide to withdraw the subject, all efforts should be made to complete and report the observations up to the time of withdrawal as thoroughly as possible. A final evaluation, the reason for, and the date of withdrawal must be recorded on the CRF. The last visit for each subject will be defined as a study discontinuation/ end-of-study visit, which will occur 30 (±7 days) days after time of study drug withdrawal.

- Subjects with clinically significant abnormal laboratory values as determined by the investigator or who have ongoing clinically significant treatment related adverse events during their last scheduled clinical evaluation will be monitored and treated until resolution or stabilization is achieved; or, in the event that the subject's condition is not likely to improve because of disease progression, until the cause of the abnormal test result or adverse event can be determined.

- Pregnancy during the course of the study for a child-bearing participant

- Death

- Study chair reserves the right to temporarily suspend or prematurely discontinue this study. The date and reason for discontinuation must be documented. Every effort should be made to complete the appropriate assessments.

6.4 Duration of Follow Up

The evaluation of the primary and secondary endpoints of ORR, CIR, and DOR are continuous. If in the judgement of patients and the treating clinical team, patients are benefiting from this therapy, the therapy will be continued even after measurement of the primary end-point at week 12 but monitoring will continue every 4 weeks or more often if clinically indicated. Patients will be considered off-protocol monitoring if they develop relapsed or progressive disease.

6.5 Early Stopping Rule for Excessive Toxicity

Bayesian toxicity monitoring (https://trialdesign.org/one-page-shell.html#BTOX) will be used to terminate early for excessive toxicity. The maximum allowable probability of Grade ≥ 3 non-hematologic, non-infectious toxicity related to treatment during the induction phase (weeks 1-12) attributed to study treatment, is assumed to be 20% and prior distribution (1,1), then early stopping will occur if the posterior probability that the Grade ≥ 3 non-hematologic toxicity rate exceeds this maximum allowable rate is 80% or greater. The minimum number of patients before stopping 3, cohort size 3, then the study will be stopped early for example, if 2 out of 3, 4 out 9, 7 out of 18, and 12 out of 33 experience SAEs.

7.0 ADVERSE EVENTS AND POTENTIAL RISKS

7.1 Agent Adverse Events

The most commonly occurring adverse reactions for Decitabine and venetoclax are:

- Nausea
- Reduced red blood count (anemia)
- Low blood platelets count (thrombocytopenia)
- Vomiting
- Raised body temperature; fever
- Reduced white blood cell count (leukopenia)
- Diarrhea
- Weakness
- Reddening of the skin at Injection site (erythema)
- Constipation
- Abnormally low count of white blood cells (neutropenia)
- Discoloration of the skin resulting from bleeding underneath (ecchymosis)
- Round spots that appear on the skin as a result of bleeding (petechia)
- Low potassium level (hypokalemia)
- Shivering as a result of a high fever (rigors)

Leukopenia is a major toxicity of decitabine and nausea, or vomiting were common non-hematologic toxicities. The main side-effect was an increase in the platelet count (as opposed to the usual side-effect of thrombocytopenia seen with cytotoxic treatments) consistent with a non-cytotoxic mechanism of action. Cytotoxicity/DNA damage assays based on bone marrow morphological examination, bone marrow DNA content analysis, VDJ recombination assay,

erythrocyte micronucleus assay and gamma-H2AX assay were also negative. These assays did not reveal evidence of DNA damage or cytotoxicity. The increase in the platelet count was not associated with any clinical adverse events, and specifically, without evidence by correlative studies for an increase in thrombotic tendency.

7.2 Definitions

7.2.1 Adverse Event

An adverse event (AE) is any unfavorable or unintended event, physical or psychological, associated with a research study, which causes harm or injury to a research participant as a result of the participant's involvement in a research study. The event can include clinically significant abnormal laboratory findings, symptoms, or disease associated with the research study. The event does not necessarily have to have a causal relationship with the research, any risk associated with the research, the research intervention, or the research assessments.

Adverse events may be the result of the interventions and interactions used in the research; the collection of identifiable private information in the research; an underlying disease, disorder, or condition of the subject; and/or other circumstances unrelated to the research or any underlying disease, disorder, or condition of the subject

7.2.2 Serious Adverse Events

A **serious adverse event** (SAE) is any adverse experience occurring at any dose that results in any of the following outcomes:

- Results in **death.**
- Is a **life-threatening** adverse experience. The term life-threatening in the definition of serious refers to an adverse event in which the subject was at risk of death at the time of the event. It does not refer to an adverse event which hypothetically might have caused death if it were more severe.
- Requires **inpatient hospitalization or prolongation of existing hospitalization**. Any adverse event leading to hospitalization or prolongation of hospitalization will be considered as Serious, UNLESS at least one of the following expectations is met:
    - The admission results in a hospital stay of less than 24 hours OR
    - The admission is pre-planned (e.g., elective or scheduled surgery arranged prior to the start of the study) OR
    - The admission is not associated with an adverse event (e.g., social hospitalization for purposes of respite care.

However it should be noted that invasive treatment during any hospitalization may fulfill the criteria of "medically important" and as such may be reportable as a serious adverse event dependent on clinical judgment. In addition, where local regulatory authorities specifically require a more stringent definition, the local regulation takes precedent.

- Results in **persistent or significant disability/incapacity**. The definition of disability is a substantial disruption of a person's ability to conduct normal life's functions.
- Is a **congenital anomaly/birth defect**.
- Is an **important medical event**. Important medical events that may not result death, be life-threatening, or require hospitalization may be considered a serious adverse experience when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood disease or disorders, or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse. The development of a new cancer is always considered an important medical event.

Moreover, grade 3 and 4 hematologic laboratory investigations are common in myeloid malignancies and will not be considered SAEs regardless of clinical significance. Grade 3 and 4 laboratory investigations not felt clinically significant by the treating physician will not be considered SAEs.

Patients with hematologic malignancies are known to have high rates of neutropenic fever, admission for the administration of IV antibiotics for uncomplicated neutropenic fever will not be considered a Serious Adverse Event, but will be tabulated as grade 3 or grade 4 toxicity as appropriate.

7.2.3 Adverse Event Evaluation

The investigator or designee is responsible for ensuring that all adverse events (both serious and non-serious) observed by the clinical team or reported by the subject which occur after the subject has signed the informed consent are fully recorded in the subject's medical records. Source documentation must be available to support all adverse events.

Any grade laboratory investigation abnormality considered clinically significant should be reported as an adverse event, with exceptions as noted above.

The investigator or sub-investigator (treating physician if applicable) will provide the following for all adverse events (both serious and non-serious):

- Event term (as per CTCAE)
- Description of the event
- Date of onset and resolution
- **Expectedness of the toxicity**
- **Grade of toxicity**
- **Attribution of relatedness to the investigational agent - (this must be assigned by an investigator, sub-investigator, or treating physician)**
- Action taken as a result of the event, including but not limited to; no changes, dose interrupted, reduced, discontinued, etc. or action taken with regard to the event, i.e. no action, received concomitant medication or other intervention, etc.
- Outcome of event

Descriptions and **grading scales** found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version **5.0** will be utilized for AE reporting.

An expected adverse event is an event previously known or anticipated to result from participation in the research study or any underlying disease, disorder, or condition of the subject. The event is usually listed in the package insert for Decitabine or venetoclax, consent form or research protocol.

An unexpected adverse event is an adverse event not previously known or anticipated to result from the research study or any underlying disease, disorder, or condition of the subject.

Attribution is the relationship between an adverse event or serious adverse event and the study drug. Attribution will be assigned as follows:

- Definite – The AE is clearly related to Decitabine/venetoclax.
- Probable – The AE is likely related to Decitabine/venetoclax.
- Possible – The AE may be related to Decitabine/venetoclax.
- Unlikely – The AE is doubtfully related to Decitabine/venetoclax.
- Unrelated – The AE is clearly NOT related to Decitabine/venetoclax.

Protocol must specify if attribution is required for individual components of the treatment regimen or the treatment regimen as a whole.

7.3 SAE Report Form

SAEs will be recorded in Velos and reported to the DSMC. If needed, the SAE will also be reported to Einstein IRB

7.4 Reporting Procedures for Serious Adverse Events

For the purposes of safety reporting, all adverse events will be reported that occur on or after day 1 of cycle 1 through 30 days after the final dose of study drug. Adverse events, both serious and non-serious, and deaths that occur during this period will be recorded in the source documents. All SAEs should be monitored until they are resolved or are clearly determined to be due to a subject's stable or chronic condition or intercurrent illness(es). Related AEs will be followed until resolution to baseline or grade 1 or stabilization.

7.4.1 SAE Reporting Requirements

- Participating investigators (all sites) must report all serious adverse events to the Principal Investigator within **24 hours** of discovery or notification of the event. The participating investigator must also provide follow-up information on the SAE until final resolution.

- Mendel Goldfinger, MD, E-mail: mgoldfin@montefiore.org, 718-920-4826

The Principal Investigator will review the SAE and report the event to the DSMC and IRB as applicable.

It is the Principal Investigator's responsibility to ensure that ALL serious adverse events that occur on the study (e.g. ALL SAEs that occur at each enrolling institution) are reported to all participating sites.

Institutional Review Board Reporting Requirements:

- Principal investigator will report adverse events to the IRB according to the Einstein IRB's policies and procedures in reporting adverse events.

7.5 SAEs and REDcap

- All SAEs will be entered into the REDcap database.
- A copy of the SAE form(s) submitted to the investigator is also uploaded into the REDcap database.

7.6 Data Safety and Toxicity Committee

It is the responsibility of the PI to ensure that all SAEs occurring on this trial are reported to the Einsteins Cancer Center's Data and Safety Toxicity Committee. This submission is simultaneous with their submission to other regulatory bodies.

The principal investigator is responsible for submitting an annual report to the DSTC as per CCCC Data and Safety Monitoring Plan.

- **7.7 Data and Safety Monitoring Plan (DSMP)**

This protocol will adhere to the policies of the Montefiore/Einstein Cancer Center Data and Safety Monitoring Plan in accordance with NCI guidelines. The principal investigator will review with his co-investigators the data relevant to the safety run-in phase, stopping rules (see sections 6.5 and 6.6) and also for an interim analysis. As outlined in sections 6.5 and 6.6, if 3 or more of the first 6 patients develop Grade 3 or 4 non-hematologic, non-infectious toxicity possibly, probably or definitively related to decitabine or venetoclax , during the induction phase (weeks 1-8) of therapy, then the study will be permanently stopped. During the course of the clinical trial, an interim analysis will be done to monitor the safety

following the accrual of every 3 patients after run-in of 6 patients. The stopping criteria are described for the incidence of Grade 3 or 4 non-hematologic, non-infectious toxicity (SAE).Using Bayesian toxicity monitoring with maximum probability of Grade ≥ 3 non-hematologic toxicity of 0.2, prior distribution (1,1), maximum patients 33, minimum number of patients before stopping 3, cohort size 3, and posterior probability 0.8, we will pause the study for review if 2 out of 3, 4 out 9, 7 out of 18, and 12 out of 33 experience SAEs.

8.0 PHARMACEUTICAL INFORMATION

8.1 Investigational Agents

8.1.1 Name of Agent: Decitabine

Product description:

Decitabine is manufactured for Otsuka pharmaceuticals and is commercially available. It is supplied as a sterile, lyophilized white to almost white powder, in a single-dose vial, packaged in cartons of 1 vial. Each vial contains 50 mg of decitabine.

Solution preparation:

For Administration Within 15 Minutes of Preparation: If Decitabine is intended to be administered within 15 minutes from the time of preparation, dilute the reconstituted solution with room temperature (20˚C - 25˚C) 0.9% Sodium Chloride Injection or 5% Dextrose Injection to a volume of 5mL.

For Delayed Administration: If Decitabine is intended to be administered after 15 minutes of preparation, dilute the reconstituted solution with 5mL of cold (2˚C - 8˚C) 0.9% Sodium Chloride Injection or 5% Dextrose Injection. Store at 2˚C - 8˚C for up to 4 hours. Diluted stored solution must be used within 4 hours from the time of preparation. Use the diluted, refrigerated solution within 4 hours from the time of preparation or discard. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Do not use if there is evidence of particulate matter or discoloration.

Storage requirements:

Store vials at 25°C (77°F); excursions permitted to 15-30°C (59-86°F)

Route of administration:

To provide a homogeneous suspension, the contents of the syringe must be re-suspended by inverting the syringe 2–3 times and vigorously rolling the syringe between the palms for 30 seconds immediately prior to administration.

Decitabine is administered subcutaneously. Doses greater than 5 mL should be divided equally into 2 syringes and injected into 2 separate sites. Rotate sites for each injection (thigh, abdomen, or upper arm). New injections should be given at least 1 inch from an old site and never into areas where the site is tender, bruised, red, or hard

Drug Procurement:

Decitabine must be obtained from commercial sources. Cost of this agent will be subject's responsibility

8.2.1 Name of Agent: Venetoclax

Product description:
Venetoclax is manufactured by AbbVie Inc. and is commercially available. It is supplied as tablets

Route of administration:
Oral
Drug Procurement:
Venetoclax must be obtained from commercial sources. Cost of this agent will be subject's responsibility

9.0 CORRELATIVE STUDIES

9.1 Methods

Immuno-detection and quantitation: As part of standard of care, all patients on this trial will have a bone marrow biopsy at baseline (pre-treatment), then every three months and as clinically indicated. For each bone marrow sample, the paraffin block be stored at the pathology department for testing in formalin-fixed paraffin-embed embedded tissue. For quantitative analysis of DNMT1, paraffin blocks from different time-points will be cut onto the same slide for staining. The Pathology department will send 4 micron section slides to the Cleveland Clinic lab of Dr. Yogen Sauntharajah where immunostaining will be performed on bone marrow biopsy slides (4μm), positive and negative controls. Nuclei positive for the targeted

biomarker are identified and quantified in high resolution, large field-of-view images per ImageIQ algorithms (Image IQ Inc., Cleveland, OH) after subtraction of bone from the original image. DNMT1 quantification is correlated with bone marrow morphology parameters and hematologic response as previously described.[4]

Peripheral blood or Bone marrow aspirate collection: Peripheral blood or bone marrow aspirates for correlate analyses will be collected at three months intervals. When patients come in for routine blood draws or bone marrow aspirates done as part of their routine clinical care, an extra tubes with ~ 8 ml will be collected every three months. These samples will be cryopreserved for future analysis and stored at the Einstein lab of Dr Amit Verma. Blood or bone marrow will be collected in a one 8mL (purple or green top) tube at pre-treatment baseline and every three months if patient is having blood drawn or a Bone marrow biopsy done at Montefiore cancer center or any other site where the protocol is open. These samples will be used for flow cytometry analysis of DNMT1 protein levels in non-G0 cells if needed to confirm results of DNMT1-protein analyses in the bone marrow biopsies: Cryopreserved mononuclear cells from patient blood or marrow are thawed and fixed using formaldehyde (1%) and methanol (90%). Fixed cells are stained for DNMT1 and cell surface antigens. Cell cycle analysis is performed using DAPI staining. The expression of DNMT1 in white blood cells in S-phase is measured as mean fluorescent intensity (MFI) normalized to a control as previously described.[22] Samples may also be used for preclinical research analyzing mechanisms of resistance to HMA and/or venetoclax treatment.

9.2 Analytical Laboratory

Specimens will be analyzed in the labs of Dr. Yogen Sauntharajah, Dr Amit Vermaand Dr Marina Konopleva. Delivery will be arranged by the primary investigator:

Mendel Goldfinger
Montefiore Medical Center
111 East 210 street
Bronx, NY 10952
Telephone: 718-920-4826
E-mail: mgoldfin@montefiore.org

10.0 STUDY PARAMETERS AND CALENDAR

STUDY CALENDAR

Study Days:	Pre-study/Screening[1]	Induction Phase, Day 1 (+/- 4 days)	Long-term Treatment phase – 4-week treatment intervals – Day 1 (+/- 4 days)	End of Treatment[9]
REQQUIRED ASSESSMENTS				
Informed consent	X			
Demographics	X			
Medical History	X			
Height	X			
Weight	X	X		X
Vitals	X	X	X	X
Physical Examination	X	X	X	
Concomitant Medications	X	X		
Performance status	X	X		
Baseline Symptoms	X	X		
SAE and > grade 2 AE Assessment	X	X	X[2]	X
CBC w/diff	X	X[3]	X[3]	X
CMP	X	X[4]	X[4]	X
LDH, uric acid	X	X[4]		
Pregnancy test[10]	X			
DISEASE ASSESSMENT				
Bone Marrow Biopsy or peripheral blood pathology	X[11]		X[5]	X[6]
TREATMENT				
Decitabine/venetoclax [7]		X	X	
CORRELATIVE STUDIES (Refer to section 9 for details)				
Peripheral blood and/or Marrow Samples[8]	X	X	X[5]	X[6]

[1]Pre-study assessments for screening should be completed within 21 days of registration. All other assessments are ± 3 days unless otherwise noted. Pre-study/screening starts from when patient signs the consent.
[2]May be captured continuously
[3]During induction phase weeks 1-12, CBC needs to be checked a minimum of weekly. Study only *requires* CBC to be collected at start of each treatment interval to evaluate toxicity and hematologic response in the long-term phase and beyond; however, treating physicians should assess CBC as clinically indicated depending on disease status and need for potential transfusion support or other supportive care, as per their judgement and standard clinical practice. Any CBC results collected should be considered data for efficacy analysis. See section 6.2.
[4]During induction phase weeks 1-12, CMP, LDH, uric acid are *required* a minimum of every 3 weeks. During the long-term treatment phase, CMP, LDH, uric acid are only *required* at day 1 of treatment. Treating physicians should consider increased frequency as clinically indicated for supportive as per their judgement and standard clinical practice.
[5]Marrow should be obtained at the end of cycle 3 and every three months for the first two years. After two years BM biopsy every six months. Marrow timing may be considered ± 14 days. IHC will be completed from core biopsy samples processed in the department of pathology per standard of care.. Peripheral blood samples should be sent the same day as any marrow samples when able.
[6]If feasible but not mandated, but especially in cases where EOT is due to progressive disease.
[7]See Treatment Schema and section 6 for details.
[8] Correlative samples are not mandatory and are obtained when able
[9] End-of-treatment evaluations should be done when able but is not mandatory
[10] For women of childbearing age
11 Bone marrow biopsy done before enrolling in the study done within 28-days of study therapy is acceptable.

11.0 MEASUREMENT OF EFFECT

Responses for CR, PR and HI will be described per IWG criteria for MDS and MPN[23,24]. For AML will use ELN response criteria. Additionally, in order to focus the most objective outcomes, additional MDS/MPN response criteria of spleen response and symptoms response will not be part of the measurement of effect of response, although will be collected as available for descriptive purposes.

Table 2: Criteria for Hematologic Improvement (HI)

Hematologic Improvement (HI)	Response Criteria (Must last at least 8 weeks)
Erythroid Response (pretreatment < 11g/dL	• Hgb increase by ≥ g/dL • Relevant reduction of units of RBC transfusions by an absolute number of at least 4 RBC transfusions/8 weeks comparted with the pretreatment transfusion number in the previous 8 weeks.
Platelet response (pretreatment, < 100 ×10^9/L)	• Absolute increase of ≥ 30×10^9/L for patients with > 20×10^9/L platelets • Increase from < 20×10^9/L to > 20×10^9/L and by at least 100%
Neutrophil response (pretreatment < 1.0×10^9/L)	• At least 100% increase and an absolute increase > 0.5×10^9/L

*Pretreatment values should not be influenced by transfusion, if they are considered transfusion dependent

Table 3: Response criteria for patients with primary diagnosis of MDS[23]

Category	Response Criteria (response must last at least 4 weeks)

Complete Remission	• Bone marrow: ≤ 5% myeloblasts with normal maturation of all cell lines. (Note persistence of dysplasia)
	• Peripheral Blood
	  ○ Hgb ≥ 11g/dL
	  ○ Platelets ≥ 100×10^9/L
	  ○ Neutrophils ≥ 1.0×10^9/L
	  ○ Blasts 0%

Partial Remission	• All CR criteria if abnormal before treatment except:
	  ○ Bone marrow blasts decreased by ≥ 50% over pretreatment but still > 5%
	  ○ Cellularity and morphology no relevant

Marrow CR	• Bone marrow: ≤ 5% myeloblasts and decrease by ≥ 50% over pretreatment • Peripheral blood: if HI responses, should be noted in addition to marrow CR. (*for this protocol, marrow CR absent HI is NOT considered a response*)
Stable Disease	• Failure to achieve at least PR, but no evidence of progression for > 8 weeks
Failure	• Death during treatment or disease progression characterized by worsening of cytopenias, increase in percentage of bone marrow blasts, or progression to a more advanced MDS subtype than pretreatment

Relapse after CR or PR	• At least one 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 ≥ 1.5g/dL or transfusion dependence

Cytogenetic response	• Complete: Disappearance of the chromosomal abnormality without appearance of the new ones • Partial: at least 50% reduction of the chromosomal abnormality

Disease Progression	• For patients with:
	  ○ Less than 5% blasts: ≥ 50% increase to > 5% blasts
	  ○ 5%-10% blasts: ≥ 50% increase to > 10% blasts
	  ○ 10-20% blasts: : ≥ 50% increase to > 20% blasts
	• Any of the following:
	  ○ At least 50% decrement from maximum remission/response in granulocytes or platelets

	○ Reduction in hgb by ≥ 2g/dL ○ Transfusion dependence

In general, all reductions in to define progression counts must not be attributable to treatment. If treatment causes transient reductions in counts, response should be considered durable through therapy. Other concomitant causes such as infection or bleeding need to be considered to ensure there is not concomitant causes.

Table 4: Response Criteria for patient with a primary diagnosis of MDS/MPN[24]

Category	Response Criteria (response must last at least 4 weeks)

Complete Remission (presence of all of the following)	• Bone marrow: ≤ 5% myeloblasts with normal maturation of all cell lines, and return to normal cellularity
	• Osteomyelofibrosis absent or equal to "mild reticulin fibrosis" (≤ grade 1 fibrosis)
	• Peripheral Blood
	  ○ WBC ≤ 10×10^9/L
	  ○ Hgb ≥ 11g/dL
	  ○ Platelets ≥ 100×10^9/L; ≤ 450×10^9/L
	  ○ Neutrophils ≥ 1.0×10^9/L
	  ○ Blasts 0%
	  ○ Neutrophil precursors reduced to ≤ 2%
	  ○ Monocytes ≤ 1×10^9/L
	• Extramedullary disease: complete resolution of extramedullary disease present before therapy (e.g. cutaneous disease, disease-related serous effusions) including palpable splenomegaly
	• Persistent low-level dysplasia is permitted given subjectivity of assignment of dysplasia

Complete cytogenetic remission	• Resolution of previously present chromosomal abnormality (known to be associated with MDS, MPN, or MDS/MPN) as seen on classic karyotyping with minimal of 20 metaphases or FISH
Partial Remission	• Normalization of peripheral counts and hepatosplenomegaly with bone marrow blasts (and blasts equivalents) reduced by 50%, but remaining > 5% of cellularty, except in cases of MDS/MPN with ≤ bone marrow blasts at baseline
Marrow response	• Optimal marrow response: presence of all marrow criteria for CR without normalization of peripheral blood indices as per CR • Partial marrow response: bone marrow blasts (and blast equivalents) reduced by 50%, but remaining > 5% of cellularity, *or* reduction in grading of reticulin fibrosis from baseline on at least 2 bone marrow evaluations spaced at least 2 months apart.
Stable Disease	• Failure to achieve at least PR, but no evidence of progression for > 8 weeks
Failure	• Death during treatment or disease progression characterized by worsening of cytopenias, increase in percentage of bone marrow blasts, or progression to a more advanced MDS subtype than pretreatment

Relapse after CR or PR	• At least one 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 ≥ 1.5g/dL or transfusion dependence
Cytogenetic response	• Complete: Disappearance of the chromosomal abnormality without appearance of the new ones • Partial: at least 50% reduction of the chromosomal abnormality
Disease Progression: (Combination of 2 major criteria, 1 major and 2 minor criteria, or 3 minor criteria	• Major Criteria: ○ Increase in marrow blast count ▪ Less than 5% blasts: ≥ 50% increase to > 5% blasts ▪ 5%-10% blasts: ≥ 50% increase to > 10% blasts ▪ 10-20% blasts: : ≥ 50% increase to > 20% blasts ○ New extramedullary disease ▪ worsening splenomegaly ▪ Extramedullary disease outside the spleen (new hepatomegaly • Minor Criteria ○ Transfusion dependence (at least 2 units of red blood cell transfusion in past month for hgb < 8.5g/dL not associated with clinically overt bleeding nor secondary to therapy) ○ Significant loss of maximal response on cytopenias ≥ 50% decrement from maximum remission/response in granulocytes or platelets ○ Reduction in Hgb by ≥ 1.5g/dL from best response or from baseline as noted on complete blood count

Table 5: Response Criteria for patient with AML

Category	Definition	Comment
Response		
CR without minimal residual disease (CR_{MRD})	• If studied pretreatment, CR with negativity for a genetic marker by RT–qPCR, or CR with negativity by MFC	• Sensitivities vary by marker tested, and by method used; therefore, test used and sensitivity of the assay should be reported; analyses should be done in experienced laboratories (centralized diagnostics)
Complete remission (CR)	• Bone marrow blasts <5%; absence of circulating blasts and blasts with Auer rods; absence of extramedullary disease; ANC ≥1.0 × 10^9/L (1000/microL); platelet count ≥100 × 10^9/L (100,000/microL)	• MRD^+ or unknown
CR with incomplete	• All CR criteria except for residual neutropenia (<1.0 × 10^9/L	•

hematologic recovery (CR_i)	[1000/microL]) or thrombocytopenia (<100 × 10^9/L [100,000/microL])	
Morphologic leukemia-free state (MLFS)	• Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required	Marrow should not merely be "aplastic"; at least 200 cells should be enumerated or cellularity should be at least 10%
Partial remission (PR)	• All hematologic criteria of CR; decrease of bone marrow blast percentage to 5 to 25%; and decrease of pretreatment bone marrow blast percentage by at least 50%	• Especially important in the context of phase 1/2 clinical trials
Treatment Failure		
Primary-refractory disease	• No CR or CR_i after 2 courses of intensive induction treatment; excluding patients with death in aplasia or death due to indeterminate cause	• Regimens containing higher doses of cytarabine are generally considered as the best option for patients not responding to a first cycle of 7+3; the likelihood of responding to such regimens is lower after failure of a first
Death in aplasia	• Deaths occurring ≥7 days following completion of initial treatment while cytopenic; with an aplastic or hypoplastic bone marrow obtained within 7 days of death, without evidence of persistent leukemia	
Death from indeterminate cause	• Deaths occurring before completion of therapy, or <7 days following its completion; or deaths occurring ≥7 days following completion of initial therapy with no blasts in the blood, but no bone marrow examination available	
Response criteria for clinical trials only		
Stable disease	• Absence of CR_{MRD-}, CR, CR_i, PR, MLFS; and criteria for PD not met	• Period of stable disease should last at least 3 months
Progressive disease (PD)*¶	Evidence for an increase in bone marrow blast percentage and/or increase of absolute blast counts in the blood: • >50% increase in marrow blasts over baseline (a minimum 15% point increase is required in cases with <30% blasts at baseline; or persistent marrow blast percentage of >70% over at least 3 months; without at least a 100% improvement in ANC to an absolute	Category mainly applies for older patient given low-intensity or single-agent "targeted therapies" in clinical trials In general, at least 2 cycles of a novel agent should be administered Some protocols may require blast increase in 2 consecutive marrow assessments at least 4 weeks apart; the date of progression should then be defined as of the first observation date

	level (>0.5 × 10^9/L [500/microL], and/or platelet count to >50 × 10^9/L [50,000/microL] nontransfused); or • >50% increase in peripheral blasts (WBC × % blasts) to >25 × 10^9/L (>25,000/microL) (in the absence of differentiation syndrome)¶; or • New extramedullary disease	Some protocols may allow transient addition of hydroxyurea to lower blast counts "Progressive disease" is usually accompanied by a decline in ANC and platelets and increased transfusion requirement and decline in performance status or increase in symptoms
Relapse		
Hematologic relapse (after CR_{MRD-}, CR, CR_i)	Bone marrow blasts ≥5%; or reappearance of blasts in the blood; or development of extramedullary disease	
Molecular relapse (after CR_{MRD-})	If studied pretreatment, reoccurrence of MRD as assessed by RT–qPCR or by MFC	Test applied, sensitivity of the assay, and cutoff values used must be reported; analyses should be done in experienced laboratories (centralized diagnostics)

CR: complete response; MRD: measurable residual disease (also known as minimal residual disease); ANC: absolute neutrophil count; MLFS: morphologic leukemia-free state; WBC: white blood cell; IDH: isocitrate dehydrogenase; RT-qPCR: real-time quantitative polymerase chain reaction; MFC: multiparameter flow cytometry.
* The authors acknowledge that this new provisional category is arbitrarily defined; the category aims at harmonizing the various definitions used in different clinical trials.
¶ Certain targeted therapies, for example, those inhibiting mutant IDH proteins, may cause a differentiation syndrome, that is, a transient increase in the percentage of bone marrow blasts and an absolute increase in blood blasts; in the setting of therapy with such compounds, an increase in blasts may not necessarily indicate PD.

12.0 DATA REPORTING / REGULATORY CONSIDERATIONS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events and potential risks).

12.1 Data Reporting

The REDCap database will be utilized, as required by the Montefiore/Einstein Cancer Center, to provide data collection for both accrual entry and trial data management. REDCap is a Clinical Trials Management System housed on secure servers. Access to data through REDCap is restricted by user accounts and assigned roles. Once logged into the REDCap systems with a user ID and password, defined roles for each user limit access to appropriate data. User information and password can be obtained by contacting the REDCap administrator. REDCap is designed with

the capability for study setup, activation, tracking, reporting, data monitoring and review, and eligibility verification. This study will utilize electronic Case Report Forms. A calendar of events and required forms will be available on REDCaps.

12.2 Regulatory Considerations

The study will be conducted in compliance with ICH guidelines and with all applicable federal state or local laws.

12.2.1 Written Informed consent

Provision of written informed consent must be obtained prior to any study-related procedures. The Principal Investigator will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study as well as the subject's financial responsibility. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and be allowed time to consider the information provided.

The original, signed written Informed Consent Form must be kept with the Research Chart in conformance with the institution's standard operating procedures. A copy of the signed written Informed Consent Form must be given to the subject. Additionally, documentation of the consenting process should be located in the research chart.

12.2.2 Subject Data Protection

In accordance with the Health Information Portability and Accountability Act (HIPAA), a subject must sign an authorization to release medical information to the regulatory authority, or Institutional Review Board access to subject's medical information that includes all hospital records relevant to the study, including subjects' medical history.

12.2.3 Retention of records

The Principal Investigator will supervise the retention of all documentation of adverse events, records of study drug receipt and dispensation, and all IRB correspondence for as long as needed to comply with local, national and international regulations. No records will be destroyed until the Principal Investigator confirms destruction is permitted. Records will be retained for 5 years after the last accrual.

12.2.4 Audits and inspections

The Montefiore Einstein Cancer Center Cancer Clinical Trials Office (MECC CCTO) quality assurance team will perform monitoring visits and audits of regulatory documents and participant charts either in person or remotely for all sites which includes but is not limited to eligibility verification, drug accountability, and source data verification. The MECC quality assurance team will follow the MECC DSMP timeline of quality assurance program monitoring and audit frequency based on the protocol risk category.

The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonization (ICH), and any applicable regulatory requirements.

13.0 STATISTICAL CONSIDERATIONS

The combination of HMA (decitabine or azacytidine) and Venetoclax are approved to treat elderly patients with AML. The FDA-approved regimens of HMA/venetoclax is with pulse-cycle drug administration of HMA and venetoclax requires continuous dosing. However, with this approach the myelosuppression from treatment leads to frequent dose-reductions, and/or cycle delays especially for many older patients and patients who have poor bone marrow reserve. In addition, some subgroups of myeloid malignancies have not been shown to benefit from standard dosing of decitabine/venetoclax as outlined above in section 1 study rationale. The registration trial which lead to FDA approval of Azacitidine in combination with venetoclax (VIALE-A) used standard dosing (Aza 75 mg/m2 days 1-7, venetoclax 400 mg days 1-28 of a 28 days cycle). In this trial 24% of patients had to discontinue azacitidine–venetoclax due to adverse events and more importantly the dose interruption of azacitidine–venetoclax between cycles owing to adverse events occurred in 72% of patients.

While venetoclax enhances the activity of HMA, the inability to get the required doses due to myelosuppression limits the benefits. Our hypothesis is that the weekly low dose venetoclax/decitabie regimen avoids severe myelosuppression by preserving functional hematopoietic cells, while maximizing suppression of clonal cell population by decitabine's S-phase dependent DNMT1-targeting while the venetoclax priming inhibits de novo pyrimidine synthesis which counters a major mechanism of resistance to decitabine in myeloid malignancies. This regimen will allow for continuous dosing without interruptions due to myelosuppression and may

lead to more durable responses. The doses and total amounts of decitabine/venetoclax administered int this trial are known to be safe and effective in this patient population based on our experience at Montefiore medical center using this regimen. Therefore, in this proof-of-concept study, the primary end-point relates to the ability to administer the treatment continuously without interruptions compared to standard dosing used in VIALE-A trial

***Considerations in choice of primary end-point*:** The primary end-point is the overall percent of patients who can receive weekly low dose decitabine/ven during the induction period (weeks 1-12) without dose interruptions. The reason for the 12-week time period was used is because responses to HMA/ven are gradual and more so with the low dose regimen. For example, in VILAE-A, the median time to first response (either complete remission or complete remission with incomplete hematologic recovery) was 1.3 months (range, 0.6 to 9.9) and 2.8 months (range, 0.8 to 13.2), respectively.

Considerations in sample-size calculations: As mentioned above, in VIALE-A dose interruptions were required in 72% of patients receiving standard currently approved dosing of Aza/ven . Therefore, the sample size has been calculated to detect a 50% reduction in treatment interruptions, which would suggest that the proposed regimen has a high likelihood of being an advance over the current FDA approved regimen.

***Sample size calculations*:** A Simon 2-stage Optimal design is used to test the null hypothesis of 60% or more patients requiring a dose reduction versus the alternative hypothesis of 36% or less of patients requiring dose interruptions with a one-sided alpha of 5%, power of 80%, and a probability of early termination of 0.77. After assessing dosing of decitabine/ven in 13 patients in the first stage, the trial will be terminated if 7 or more patients require dose reduction. If the trial goes on to the second stage, a total of 33 patients will be studied. If the total number of dose reduction is greater than or equal to 16, the treatment regimen is rejected.

After the 19 patients were enrolled into the study and 17 patients were evaluable after a 12-week induction, there were with no safety concerns. If after a total of 33 patients have been evaluated after the 12-week induction period and show satisfactory results (i.e., fewer than 16 dose reductions during the induction period, so the treatment was not rejected), the proposed amendment for an expansion phase will be implemented in which additional patients will be enrolled to gather more data on the secondary endpoints of efficacy using this regimen in MDS and AML. Background for the rationale for this amendment is outlined in section 1.4. Given the early efficacy seen in the first 11 evaluable patients with AML (64% response rate) and very low toxicity, more subjects will be required to establish the secondary

endpoints of efficacy in MDS and AML. This additional data may help in the design subsequent studies which will require adding an additional agent to this regimen as a backbone as it's becoming increasingly clear the HMA/VEN does not have a cure rate[25] and the way forward will requires a third effective agent with multiple trials now evaluating triplets and using HMA/VEN as a backbone. The comparator arm for HMA/VEN in all trials is the current standard of single agent HMA (Azacitidine or Decitabine).

The expansion phase will be stratified by disease type, i.e., MDS and AML.

Single agent HMA has a 16% CR rate in MDS. [26] The CR rate with HAM/VEN is expected to be at least 36%. The target sample size for the MDS group will be a total of 32 patients (from both initial and expansion phases). With this sample size, we can estimate the CR rate with standard error no greater than 9%.

In AML, single agent HMA has a CR rate of 17.9%. [1] The CR rate with HAM/VEN is expected to be at least 38%. With a total of 38 AML patients from both phases, we can estimate the CR rate with standard error no greater than 8%.

In summary, an amendment will be added for additional 42 patients (above the originally planned 33 patients) with either MDS or AML after accounting for drop-outs. For example, if we assume among the first stage of 33 patients, we have 16 MDS, 17 AML, then the study needs enroll additional 16 MDS and 21 AML, a total of 37 patients. Allowing up to 5 patients to drop out from the study and the study may also enroll approximately 10 patients with other rare myeloid malignancies such as CMML and MDS/MPN. We therefore expect the total number of patients needed to be enrolled in the study to be not more than 85 (33+37+5+10) patients.

***Safety*:** Incidence of treatment-emergent adverse events will be tabulated severity/grade, and whether the AE resulted in death or discontinuation of treatment.

Early Stopping Rule for Excessive Toxicity in Expansion Phase: We will continue using Bayesian toxicity monitoring in the expansion phase, similar to what was described in the initial stage of the study (Sec 6.6), to allow for early stopping due to excessive toxicity.

Laboratory data will be summarized in tables that show changes from pre-treatment values and frequencies of abnormal values. Descriptive statistics will also be provided.

***Efficacy*:** The primary end-point of a decrease in dose interruptions will be described as proportion of patients point-estimates, together with the corresponding 95% confidence interval. Kaplan-Meier method will be used to summarize time to event variables.

Secondary end-points and scientific correlative studies: For the secondary end-points and scientific correlates (secondary objectives), either logistic regression or correlation analysis will be utilized to examine and assess the relationships and predictive nature of these biological parameters on hematologic improvement and ability of decitabine/ven to deplete DNMT1 in malignant cells, and the alteration of pyrimidine metabolism. Descriptive summaries of the data will be provided.

***Accrual rate*:** Estimated accrual rate of 1-3 patients per month with approximately 18-24 months to meet total accrual goals, and additional 3 months to observe primary outcome measures. DOR of response will be followed for up to two years, thus a total of three to four years is anticipated to meet all study endpoints.

14.0 FUNDING

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