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STUDY TITLE: Targeting TET2 mutations in Myelodysplastic Syndromes and Acute Myeloid Leukemia with Azacitidine and Ascorbic Acid

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SUPPLIED AGENTS: Azacitidine, Ascorbic Acid

AMENDMENT SUMMARY: Original Protocol: 10/13/2017
Amendment 1, Protocol Version 2: 11/20/2017
Amendment 2, Protocol Version 3: 05/24/2018

PROTOCOL SUMMARY

Protocol Number/Title	Case 1917: Targeting TET2 mutations in Myelodysplastic Syndromes and Acute Myeloid Leukemia with Azacitidine and Ascorbic Acid
Study Phase	II
Brief Background/Rationale	Ten eleven translocation 2 (*TET2*) mutations are among the most common genomic alterations in myeloid malignancies. Somatic mutations in *TET2* can be identified in 25-30% of patients with myelodysplastic syndromes (MDS) and 50-60% of patients with chronic myelomonocytic leukemia (CMML), an overlapping MDS/myeloprolifrative neoplasm (MPN) disorder, and 15-20% of patients with acute myeloid leukemia (AML). Moreover, somatic mutations in *TET2* gene were identified in healthy individuals. The presence of these mutations increases with age and can increase the risk of these individuals to develop hematologic malignancies and all-cause mortality. Because synthetic lethal approaches cannot be applied for the therapy of $TET2^{MUT}$ and because *TET2* gene is very pleomorphic, identification of targeted therapies for patients with *TET2* mutations remains challenging. The dioxygenase TET2 forms hydroxyl radicals that hydroxylate methyl groups on DNA to remove them. Thus, indirectly TET2 functions as a DNA demethylase that ultimately affects gene expression. Consequently, mutations in TET2 result in increased methylation of tumor suppressor genes and also possibly cytidine deamination leading to C>T transitions at CpG sites. To split O_2, TET2 needs 3 electrons, 2 from a-ketoglutarate (aKG) and 1 from ascorbic acid. Ascorbic acid is therefore an activator of TET2. Indeed, in preliminary results with purified TET2, ascorbic acid increased the enzymatic activity of mutant *TET2* indicating that ascorbic acid supplementation may increase the efficiency of dioxygenation but this increase was only seen in cells with heterozygous mutations. Further, in vitro studies have shown that high dose ascorbic acid with plasma concentration of 1000 µmol/L is toxic to some cancer cells but not normal cells. Although trials with oral ascorbic acid in mid 1970s did not show any benefit of it in cancer patients, recent trials have shown that the addition of high dose IV ascorbic acid to chemotherapy is well tolerated, nontoxic, and may improve responses to therapy in some patients and can also improve symptoms.

	Azacitidine (AZA), decitabine (DAC), and lenalidomide are the only FDA approved drugs in MDS. Treatment with AZA and DAC improves cytopenias and prolongs survival in patients with MDS, though only 30-35% of patients respond to therapy. Studies have shown that MDS patients with *TET2* mutations have a higher response rate to AZA. In a study of 82 patients with MDS and oligoblastic AML (20-30% BM blasts) treated with AZA, *TET2* mutations were found in 15% of the patients. Patients with *TET2* mutations had a higher response to AZA compared to wild type patients (82% vs 45%, p = 0.04, respectively). In a larger retrospective study, samples from 213 patients with MDS were sequenced prior to their treatment with hypomethylating agents (HMAs) for the presence of 40 recurrent gene mutations. *TET2* mutations with variant allelic frequency (VAF) >10% were associated with a significantly higher overall response rate (ORR) compared with WT (60% vs. 43%; OR, 1.99; 95%CI, 1.05-3.80, *P*=0.036). Further, mice with transplanted TET2-null hematopoietic cells exhibit decrease in white blood counts when they are treated with AZA compared to vehicle. In another in vitro study, adding vitamin C at physiological levels to low doses azacitidine showed synergistic inhibition of cancer-cell proliferation and increased apoptosis. This synergistic effect is likely the result of both passive DNA demethylation by DNMTi and active conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) by TET enzymes at LTR regions of endogenous retrovirus, because vitamin C acts as a cofactor for TET proteins. In addition, TET2 knockout reduces the synergy between the two compounds. Taken together, we hypothesize that the combination of standard dose AZA with oral dose of ascorbic acid will improve overall response rate (ORR) in MDS and AML patients with *TET2* mutations.
Primary Objective	Primary Endpoint(s) To estimate the overall response rate (ORR) of the combination of standard dose azacitidine and oral dose of ascorbic acid in patients with MDS, MDS/MPN overlap, and AML with *TET2* mutations
Secondary Objective(s)	Secondary Endpoint(s) 1. The safety profile of the combination in the targeted patient population 2. Response duration 3. Overall survival of the treated population (compared to matched historical cohort of patients treated with single agent Azacitidine) 4. The identification of biomarkers that predict response to

	the combination
Correlative Objective(s)	Correlative Endpoint(s) Identify gnomonic biomarkers for response. Evaluate methylation profile and after treatment to determine its relation with response.
Sample Size	28
Disease sites/Conditions	Myelodysplastic Syndromes (MDS), MDS/MPN syndromes, and AML
Interventions	Azacitidine 75 mg/m^2, SC / IV day (1-7) every 28 days cycle
	Ascorbic acid 1 g PO every day (daily) for every 28 days cycle

ABBREVIATIONS

CCCC	Case Comprehensive Cancer Center
CRF	Case Report Form
DCRU	Dahm's Clinical Research Unit
DSTC	Data Safety and Toxicity Committee
FDA	Food and Drug Administration
ICF	Informed Consent Form
IRB	Institutional Review Board
PRMC	Protocol Review and Monitoring Committee
SOC	Standard of Care
CCF	Cleveland Clinic Foundation
UH	University Hospitals
MDS	Myelodysplastic Syndromes
CMML	Chronic Myelomonocytic Leukemia
MPN	Myeloprolifrative Neoplasm
AML	Acute Myeloid Leukemia
DNA	Deoxyribonucleic acid
AZA	Azacitidine
DAC	Decitabine
FDA	The Food and Drug Administration
HMAs	Hypomethylating Agents
VAF	Variant Allelic Frequency
ORR	Overall Response Rate
TET2	Ten eleven translocation 2
5mC	5-methylcytosine
5hmC	5-hydroxymethylcytosine
SC	Subcutaneous
CALGB	Cancer and Leukemia Group B
NCI	National Cancer Institute
AA	Ascorbic Acid

PO	Orally
Sp	Specificity protein
SVCT-2	Sodium -dependent Ascorbic Acid transporter 2
EGCG	Epigallocatechin-3-gallate
ETP	Extended Treatment Period
EOT	End of Treatment
CR	Complete Remission
PR	Partial Remission
HI	Hematologic Improvement
IWG	International Working Group
WHO	World Health Organization
APL	Acute promyelocytic leukemia
CNS	Central Nervous System
PI	Principal Investigator
WBC	White Blood Cell
CSF	Colony-Stimulating Factor
CTCAE	Common Terminology Criteria for Adverse Events
AE	Adverse Event
SAE	Serious Adverse Event
Co-I	Co-Investigator
DSMP	Data and Safety Monitoring Plan
PRBC	Packed Red Blood Cell
ALT	Alanine transaminase
AST	Aspartate aminotransferase
ESA	erythropoiesis-stimulating agent
ECOG	Eastern Cooperative Oncology Group
RBC	Red Blood Cells
CRi	Complete Response (CR) with incomplete hematologic recovery
CRp	Complete Response (CR) with incomplete platelets
IEC	Independent Ethics Committee
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
HIPAA	Health Insurance Portability and Accountability Act of 1996
OR	Odds Ratio
CI	Confidence Intervals
EOS	End of Study
BUN	Blood Urea Nitrogen
MCV	Mean Corpuscular Volume
MCHC	Mean Cell Hemoglobin Concentration
TP	Treating Physician

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1.0 Introduction

1.1 Background of Study Disease

Myelodysplastic syndromes (MDS) are clonal hematopoietic stem-cell disorders characterized by ineffective hematopoiesis, peripheral-blood cytopenias, and increased tendency to progress to acute myeloid leukemia (AML) [1]. The median age of patients with MDS is approximately 70 years[2]. This patient population is frequently affected by other comorbid conditions, a factor that often influences treatment decisions[3]. Treatment of MDS is based on prognostic factors that predict survival and progression to AML. The most widely used prognostic system for therapeutic decision-making is still the International Prognostic Scoring System[3]. This system stratifies patients into the following four groups: low, intermediate-1, intermediate-2, and high risk. Risk is based on number of cytopenias, percentage of bone marrow blasts, and karyotype. Low risk and intermediate-1 risk are usually grouped together as lower-risk disease, whereas intermediate-2 risk and high risk are grouped together as higher-risk disease[3]. Recently, genome sequencing technologies such as whole genome sequencing, whole exome sequencing, and next-generation targeted deep sequencing have identified several recurrent somatic mutations that play an important role in the disease pathophysiology and prognosis[4, 5].

The discovery of new somatic mutations and the newly attained ability to use mutational screens as a diagnostic tool opened new opportunities for the development of new targeted therapies. Ten eleven translocation 2 (*TET2*) gene mutations are among the most common genomic alterations in myeloid malignancies[4, 5]. Somatic mutations in *TET2* can be identified in 25-30% of patients with myelodysplastic syndromes (MDS), 50-60% of patients with chronic myelomonocytic leukemia (CMML) an overlapping MDS/myeloprolifrative neoplasm (MPN) disorder, and 15-20% of patients with AML[4-6]. The *TET2* gene is very polymorphic, with hundreds of single nucleotide polymorphisms (SNPs) of unknown clinical impact, but with some variants that may be pathogenically important. Similarly, somatic mutations affect all portions of the gene, and can be missense or truncating, homo-, hemi- and heterozygous. While the majority of *TET2* mutations are ancestral, they can also be subclonal, implicating the clonal architecture in the consequences of *TET2* lesions. Further, somatic mutations in *TET2* gene were identified in healthy individuals[7, 8]. The presence of these mutations increases with age and can increase their

risk of developing hematologic malignancies and death[7, 8]. *TET2* protein is one of the three proteins in TET family that are conserved dioxygenases that catalyzes the conversion of 5-methyl-cytosine (5-mc) to 5-hydroxymethyl-cytosine (5-hmc) and promotes DNA methylation. Dioxygenases function of *TET2* enzyme requires a-ketoglutarate (αKG), oxygen, and Fe (II) which is enhanced with the presence of ascorbic acid. Ascorbic acid interact with the catalytic domain of *TET2* enzymes which may promote their folding and recycling of Fe (II) [10]. Further, in mouse models, ascorbic acid can promote rapid and global increase in 5-hmc followed by DNA demethylation for many promoter genes, a change that are TET2 dependent[10]. Ascorbic acid is therefore an activator of TET2. Indeed, in preliminary results with purified TET2, ascorbic acid increased the enzymatic activity of WT TET2 and partially overcome the effects of the mixture of WT and mutant TET2 ($TET2^{mut}$) indicating that ascorbic acid supplementation may increase the efficiency of dioxygenation and ultimately TET2 activity.

TET2 belongs to a family of Fe^{2+} and α-ketoglutarate (αKG) dependent TET (TET1, TET2 and TET3) DNA-dioxygenases that catalyze the oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), this to 5-formylcytosine (5fC), and this to 5-carboxylcytosine (5caC). Ascorbic acid (Vitamin C) is a known enhancer of TET2 activity. We and others have shown that Vitmine C enhances the basal TET activity. Higher TET activity results in DNA demethylation either actively, by base excision repair of fC and caC, or passively, via replication, due to DNA methyltransferase's inability to recognize/read hmC. The resulting DNA demethylation increases gene transcription of context dependent differentiation promoting genes. To access the level of TET2, we screened several cell lines with different TET2 genotype using Cosmic (https://cancer.sanger.ac.uk/cell_lines/cbrowse/all) and cancer cell encyclopedia (https://portals.broadinstitute.org/ccle) see Table 1. To confirm the expression level or protein in each cells we grew them to 70-80% confluence in indicated cell culture conditions. The cells were harvested and western blot analysis were performed on the nuclear fraction utilizing highly specific TET2 antibodies. The data is presented in **Fig. 1 A** and quantification in Fig. **1B.** CMK, a $TET2^{+/+}$ cell line established from the peripheral blood of a 10-month-old boy with down's syndrome and acute megakaryocytic leukemia (AML M7) at relapse, showed highest level of TET2 while HEL cells have a moderate level of TET2 and carries a mono allelic missense mutation in catalytic domain and SIGM5 a $TET2^{-/-}$ cell established from the bone marrow of a

63-year-old man with acute myeloid leukemia of monocytic origin (AML FAB M5a) at diagnosis shows no TET2 protein expression in western blot analysis. To ascertain the role of ascorbic acid (Vitamin C) in epigenetic modifications, TET2 mutant and wild type MDS and MDS/AML we used these three cells. CMK, HEL and SIGM5 cells were propagated as described previously. Cells were treated at 300,000 cells/ml (sub confluent) for 12 hours with 100 µM of Vitamin C and the genomic DNA were extracted as described previously and 100 ng of DNA was spotted on PVDF membrane and cross-linked using UV crosslinking, blocked using BSA and probed with 5hmC and 5mC specific antibodies respectively. The spot analysis was performed on a Biorad ChemiDoc™ MP Imaging System. The data presented in Fig 2 is representative of three independent experiments. Consistent with the TET2 status, the ratio of 5hmC/5mC was maximum in CMK followed by HEL and SIGM5. Thus we conclude that the effect of vitamin C will be similar for mono- and bi-allelic mutant MDS. Based on this data that was generated in our lab, we wanted to amend the protocol to include patients with any TET2 mutations.

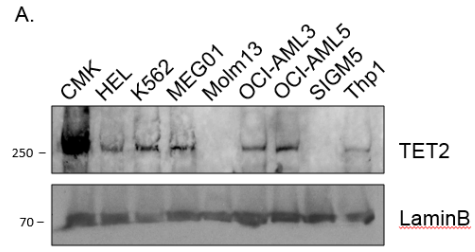

Table1.

Cell Line	Source	TET2 Status	
		CCLE	COSMIC
CMK	ATCC	wild type	wild type
HL-60	DMSZ	wild type	wild type
K-562	ATCC	wild type	wild type
MEG-01	DMSZ	wild type	wild type
Molm-13	DMSZ	wild type	wild type
MV-4-11	ATCC	wild type	wild type
OCI-AML3	ATCC	wild type	wild type
Thp1	ATCC	wild type	--
U937	ATCC	wild type	--
HEL	DMSZ	wild type	p.P1575L
OCI-AML5	DMSZ	p.S825* p.Y1148C	p.S825*
SIG-M5	DMSZ	p.F1041fs p.I1181fs	p.F1041fs*2 p.Y1182fs*44 p.S1203R

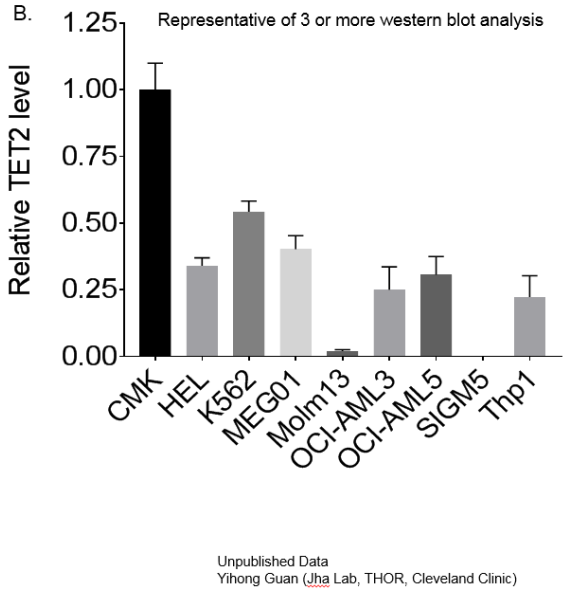

Figure 1A: Western blot analysis results/ **Figure 1B**: Western blot analysis quantification

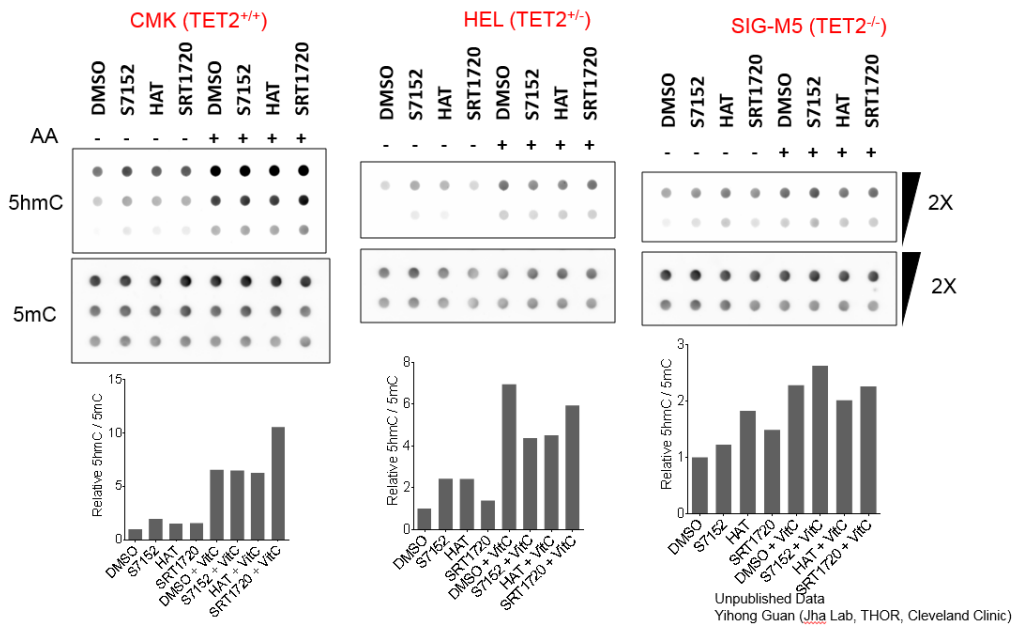

Figure 2: Results of three independent TET2 status experiments

Azacitidine, decitabine, and lenalidomide are the only FDA approved drugs in MDS. Treatment with AZA and DAC improves cytopenias and prolongs survival in patients with MDS, though only 30-40% of patients respond to therapy[3]. Azacitidine was approved in USA based on phase III clinical trial that compared azacitidine to best supportive care. Overall response rate to azacitidine was 34% with significant delay in AML transformation but no overall survival benefit. In a phase III clinical trial conducted in Europe, azacitidine was compared to conventional care arm that included best supportive care, low dose cytarabine, and intensive chemotherapy[11]. The median OS for patients treated with azacitidine was 24.5 months compared to 15 months for patients treated with best supportive care (HR .58, p = .00001) [11].

Studies have shown that MDS patients with *TET2* mutations have a higher response rate to AZA. In a study of 82 patients with MDS and oligoblastic AML (20-30% BM blasts) treated with AZA, *TET2* mutations were found in 15% of the patients. Patients with *TET2* mutations had a higher response to AZA compared to wild type patients (82% vs 45%, p = 0.04, respectively) [12]. In a larger retrospective study, samples from 213 patients with MDS were sequenced prior to their treatment with hypomethylating agents (HMAs) for the presence of 40 recurrent gene mutations[13]. *TET2* mutations with variant allelic frequency (VAF) >10% were associated with a significantly higher overall response rate (ORR) compared with WT (60% vs. 43%; OR, 1.99;

95%CI, 1.05-3.80, *P*=0.036) [13]. Further, mice with transplanted TET2-null hematopoietic cells exhibit decrease in white blood counts when they are treated with AZA compared to vehicle.

Recent in vitro study have shown that the addition of Ascorbic Acid at physiological levels to low doses of azacitidine is synergistic and can increase the inhibition of cancer-cell proliferation and increased apoptosis[14]. This synergistic was related to both passive DNA demethylation by DNMTi and active conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) by TET2 enzymes[14]. More importantly, TET2 knockout reduces the synergy between the two compounds. Further, evidence to suggest that treatment with multiagent combination can be beneficial and feasible in patients with MDS/MPN syndromes was also shown in a study of the combination of (thalidomide, arsenic trioxide, dexamethasone, and ascorbic acid [TADA] [15]. With a median on-study follow-up of 5.7 months, 21 patients (75%) completed the entire 12-week course of therapy, and 6 patients (29%) responded to TADA [15]. With a median extended follow-up of 24.1 months for 15 evaluable patients, the median progression-free survival was 14.4 months, and the median overall survival was 21.4 months.

Taken together, the combination of standard dose AZA with ascorbic acid represent a rational, combination that may improve overall response rate (ORR) in MDS and AML patients with *TET2* mutations.

1.2 Name and Description of Azacitidine

Azacitidine, a ring analog of the pyrimidine nucleoside cytidine, has effects on cell differentiation, gene expression, and DNA synthesis and metabolism. Since the early 1970s, azacitidine has been investigated in the US for the treatment of acute leukemia. Clinical trials have focused primarily on patients with disease refractory to conventional chemotherapy. These investigations indicated azacitidine has activity in the treatment of acute myeloid leukemia (AML). Clinical trials subsequently have been conducted to evaluate the effects of azacitidine in a variety of other malignant and hematologic disorders, including solid tumors, hemoglobinopathies (thalassemia and sickle cell anemia), and myelodysplastic syndromes (MDS).

Toxicology studies have been conducted in mice, rats, dogs, and Rhesus monkeys. Most

of the studies were performed during the 1970s and early 1980s according to existing guidelines and standards in place during that period. The nonclinical studies identified the bone marrow, liver, kidneys, and lymphoid tissues (spleen, lymph nodes) as the main target organs of toxicity. In single-dose studies, the lethal dose of azacitidine after intravenous (IV) administration in mice, rats, and dogs was approximately 250 mg/m^2. Repeated daily dosing appears to increase the toxicity of azacitidine. The genotoxicity of azacitidine is consistent with that of other nucleoside analogs that interact with nucleic acids. Likewise, similar to other agents with cytostatic properties, azacitidine was embryotoxic and reduced the reproductive performance in mice and rats. It is important to note that animal study data is superseded in many respects by the extensive clinical safety data collected in the last two decades.

Limited azacitidine pharmacokinetic data are currently available. Based on plasma concentrations of total radioactivity (which represent parent drug plus circulating metabolites), azacitidine is rapidly absorbed when given subcutaneously (SC), with maximum plasma concentrations found 0.5 to 2 hours after dosing. Azacitidine and/or its by-products are rapidly cleared by the kidneys. The half-lives and percent radioactivity recovered in urine are similar for the IV and SC routes of administration.

The effects of renal or hepatic impairment, gender, age, and race on the pharmacokinetics of azacitidine have not been studied. In 1985, the Cancer and Leukemia Group B (CALGB) study investigators began clinical trials with azacitidine in MDS patients under the auspices of the National Cancer Institute (NCI). Results from the three studies conducted by the CALGB (Protocols 8421, 8921, and 9221) have been published. The first two CALGB studies (Protocol 8421 and Protocol 8921) were uncontrolled Phase 2 investigations. The most recent CALGB study (Protocol 9221) was a Phase 3 investigation that compared azacitidine to supportive care alone. The azacitidine dose investigated in the CALGB studies was 75 mg/m^2/day for 7 days, repeated on a 28-day cycle. Azacitidine was administered by continuous IV infusion in the first study (Protocol 8421), and by SC injection in the two studies that followed. The dose was adjusted based on toxicity and clinical response. In the Phase 3 investigation (Protocol 9221), azacitidine produced higher response rates than supportive care alone. In addition, azacitidine prolonged the time to transformation to AML or death.

The efficacy of azacitidine to treat MDS also was evaluated in 7 open-label studies conducted outside the CALGB protocols. The dosage regimen used in 6 of these studies was 75 mg/m^2 given daily for 7 days every 3-4 weeks by SC injection in 4 studies, SC or IV in 1 study, and the route was not specified in the last study. The dosage regimen used in the seventh study was 5-35 mg/m^2/day given by continuous IV infusion for 14 days. The lowest response rate was found in this seventh study, which suggests the mechanism of 5-azacitidine's activity requires repeated administration of a minimally effective dose to achieve improvement in hematologic parameters.

As with other antimetabolites, bone marrow suppression (leukopenia, thrombocytopenia) is a common adverse event associated with azacitidine. However, myelosuppression generally occurs more often and with greater severity at doses higher than those used to treat MDS. Gastrointestinal toxicity (i.e., nausea, vomiting, and diarrhea) can limit the dose of azacitidine in any patient population. Infrequent adverse effects include neuromuscular aches, generalized weakness, renal tubular acidosis, and liver enzyme abnormalities. Erythema and burning at the injection site can occur following SC administration, which usually resolves within 24-72 hours.

1.3 Name and Description of Ascorbic Acid

Ascorbic acid is a water-soluble vitamin. Ascorbic acid has few strictly pharmacological actions. Administration in amounts greatly in excess of physiologic requirements causes no demonstrable effects. The vitamin is an essential coenzyme for collagen formation, tissue repair and synthesis of lipids and proteins. It acts both as a reducing agent and as an antioxidant and is necessary for many physiologic functions, e.g., metabolism of iron and folic acid, resistance to infection, and preservation of blood vessel integrity. Signs and symptoms of early AA deficiency include malaise, irritability, and arthralgia, hyperkeratosis of hair follicles, nosebleed, and petechial hemorrhages. Prolonged deficiency leads to clinical scurvy.

Ascorbic acid is normally present in both plasma and cells. The absorbed vitamin is ubiquitous in all body tissues [16]. The highest concentrations are found in glandular tissue, the lowest in muscle and stored fat. Ascorbic acid is partially destroyed and partially excreted by the body. There is a renal threshold for AA; the vitamin is excreted by the kidney in large amounts

only when the plasma concentration exceeds this threshold, which is approximately 1.4 mg/100 mL[16]. When the body is saturated with ascorbic acid, the plasma concentration will be about the same as that of the renal threshold; if further amounts are then administered, most of it escapes into the urine. When body tissues are not saturated and plasma concentration is low, administration of ascorbic acid results in little or no renal excretion[16].

A major route of metabolism of ascorbic acid involves its conversion to urinary oxalate, presumably through intermediate formation of its oxidized product, dehydro-ascorbic acid. In a study of healthy volunteers at the NIH, an in-hospital depletion-repletion study was conducted. Seven healthy volunteers were hospitalized for 4-6 months and consumed a diet containing <5 mg of AA daily[16]. Steady-state plasma and tissue concentrations were determined at seven daily doses of vitamin C from 30 to 2500 mg. AA steady-state plasma concentrations as a function of dose displayed sigmoid kinetics. Complete plasma saturation with AA occurred at 1000 mg PO daily[16].

Numerous studies have demonstrated that pharmacological doses of ascorbic acid (0.1–100 mM) decrease cell proliferation in a variety of cancer cell lines [17, 22]. Specifically, decreases in cell proliferation after ascorbic acid treatment have been reported for prostate, pancreatic, hepatocellular, colon, mesothelioma, and neuroblastoma cell lines. The potential mechanisms through which treatment with high-dose ascorbic acid may exert its effects on cancer cells have been extensively investigated. Several studies have demonstrated that the *in vitro* direct cytotoxic effect of ascorbic acid on various types of cancer cells is mediated through a chemical reaction that generates hydrogen peroxide [17,22]. Treating colon cancer cells with 2 mM to 3 mM of ascorbic acid resulted in down regulation of specificity protein (Sp) transcription factors and Sp-regulated genes involved in cancer progression. One study suggested that Ascorbic Acid-mediated prostate cancer cell death may occur through activation of an autophagy pathway. Differences in chemosensitivity to Ascorbic Acid treatment in breast cancer cell lines may depend on expression of the sodium -dependent Ascorbic Acid transporter 2 (SVCT-2) [22]. Research has suggested that pharmacological doses of ascorbic acid enhance the effects of arsenic trioxide on ovarian cancer cells gemcitabine on pancreatic cancer cells, and combination treatment of gemcitabine and epigallocatechin-3-gallate (EGCG) on mesothelioma cells.

Ascorbic Acid has also been investigated in clinical trials. One study reported three case reports of cancer patients who received IV ascorbic acid as their main therapy [18]. During ascorbic acid therapy, the patients used additional treatments, including vitamins, minerals, and botanicals. According to the authors, the cases were reviewed in accordance with the NCI Best Case Series guidelines. Histopathologic examination suggested poor prognoses for these patients, but they had long survival times after being treated with IV ascorbic acid [18]. Ascorbic Acid was given at doses ranging from 15 g to 65 g, initially once or twice a week for several months; two patients then received it less frequently for 1 to 4 years. Two studies demonstrated that IV ascorbic acid treatment resulted in improved quality of life and decreases in cancer-related side effects in cancer patients [18]. Studies have shown that ascorbic acid can be safely administered to healthy volunteers or cancer patients at doses up to 1.5 g/kg and with screening to eliminate treating individuals with risk factors for toxicity (e.g., glucose-6-phosphate dehydrogenase deficiency, renal diseases, or urolithiasis). These studies have also found that plasma concentrations of ascorbic acid are higher with IV administration than with oral administration and are maintained for more than 4 hours [18].

Ascorbic Acid was also investigated in combination with other cancer treatments. A phase I study published in 2012 examined the safety and efficacy of combining IV ascorbic acid with gemcitabine and erlotinib in stage IV pancreatic cancer patients [19]. Fourteen subjects entered the study and planned to receive IV gemcitabine (1,000 mg /m2 over 30 minutes, once a week for 7 weeks), oral erlotinib (100 mg daily for 8 weeks), and IV ascorbic acid (50 g/infusion, 75 g/infusion, or 100 g/infusion 3 times per week for 8 weeks). Minimal adverse effects were reported for ascorbic acid treatment[19]. Five subjects received fewer than 18 of the planned 24 ascorbic acid infusions and thus did not have follow-up imaging to assess response. Three of those patients had clinically determined progressive disease [19]. All of the other nine patients had repeat imaging to assess tumor size, and each met the criteria for having stable disease. A 2013 phase I clinical study evaluated the safety of combining pharmacological ascorbic acid with gemcitabine in treating stage IV pancreatic cancer patients [20]. During each 4-week cycle, patients received gemcitabine weekly for 3 weeks (1,000 mg/m2 over 30 minutes) and twice weekly ascorbic acid infusions for 4 weeks (15 g over 30 minutes during the first week, followed by weekly escalations in dose until plasma levels reached at least 350 mg/dL [20 mM]) [20]. Among nine patients, mean progression-free survival was 26 weeks and overall

survival was 12 months [20]. The combination treatment was well tolerated, and no significant adverse events were reported. In 2014, a phase I/IIA clinical trial evaluated the toxicities of combining IV ascorbic acid with carboplatin and paclitaxel in stage III /IV ovarian cancer. Twenty-seven patients were randomly assigned to receive either chemotherapy alone or chemotherapy and IV ascorbic acid concurrently [21]. Chemotherapy was given for 6 months, and IV ascorbic acid was given for 12 months. The addition of IV ascorbic acid was associated with reduced chemotherapy-related toxicities. Trials of high-dose IV ascorbic acid with other drugs are ongoing [21]. A number of studies have included IV ascorbic acid treatment (1,000 mg) with arsenic trioxide regimens, with mixed results. The combination therapies were well tolerated and suggested beneficial effects in multiple myeloma patients, although the specific contribution of ascorbic acid could not be determined. However, similar combination regimens resulted in severe side effects and disease progression in patients with acute myeloid leukemia, refractory metastatic colorectal cancer, and metastatic melanoma [21].

2.0 Objectives

2.1 Primary Objective

To estimate the overall response rate (ORR) of the combination of standard dose azacitidine and ascorbic acid in patients with MDS, AML, and MDS/MPN overlap with *TET2* mutations.

2.2 Secondary Objective(s)

To evaluate the following:

1. The safety profile of the combination of azacitidine and ascorbic acid in the targeted patient population.
2. The response duration for study patients.
3. The overall survival of the treated population (compared to matched historical cohort of patients treated with single agent Azacitidine)
4. The identification of genomic biomarkers that may predict response to the combination treatment.

5. Evaluation of changes in methylation prior and during therapy

3.0 Study Design

This is an open-label, phase II study that will be conducted at Cleveland Clinic, Taussig Cancer Institute.

Azacitidine will be administered intravenously or subcutaneously at a fixed dose of 75mg/m2/day for 7 consecutive days, (allowing for weekends, and holidays) of each 28-day cycle. Ascorbic acid will be administered orally daily at 1 g/day three days prior to start azacitidine and then continues daily for a total of 28 days of each 28 day cycle.

3.1 Number of Subjects

Approximately 28 patients will be enrolled in this trial.

3.2 Expected Duration of Treatment and Subject Participation

Upon signing consent, patients will be enrolled onto the study. Patients will undergo a screening period. The screening period is defined as the time period between the signing of the consent to the day the patient receives the first dose of study drug. During this period the patient will be assessed to determine if the patient meets all inclusion and exclusion criteria to be eligible for the study (Appendix IV).

The treatment period is defined as the time between first day of study drug dosage (Day -3) to C6D28. At this time the patient will be assessed and eligible to move into the Extended Treatment Period (ETP). The extended treatment period is defined as C7D1 to the patient meets EOT criteria (Section 6: Table 1; Appendix IV)

Study treatment is defined as the treatment period and if applicable the extended treatment period (Appendix IV). Study drug will be continued during the duration of the study treatment unless patients exhibit any EOT criteria (Section 6: Table 1; Appendix IV).

Treatment should be continued for at least 6 cycles in the absence of toxicities that require discontinuation and/or any EOT criteria (Section 6: Table 1; Appendix IV). After 6 cycles of treatment, a bone marrow biopsy and aspiration will be obtained to evaluate response. If patient has achieved either of the below response criteria (Appendix IV) based on their disease diagnosis patient will be eligible continue onto the extended treatment period (ETP). The patient is eligible to remain on treatment during the ETP until disease progression or patient meets EOT criteria (Section 6: Table 1; Appendix IV).

Response Criteria for extended treatment eligibility

- complete remission, partial remission, or hematologic improvement (CR/PR/HI) per IWG 2006 criteria for patients with MDS
- (CR/PR/marrow CR) per IWG 2003 criteria for patients with AML
- If the response is stable disease and the investigator determines the patient is gaining clinical benefit, patient may continue on treatment until progression.

At the point in which the patient meets the any EOT criteria (Section 6) the patient will enter into the follow up period to follow for survival status. The patient will be followed for up to 1 year and/or death, which ever were to occur first. End of study (EOS) is defined as the time the patient completes 1 year of survival follow up and/or patient's death, whichever were to occur first.

4.1 Subject Selection

4.2 Inclusion Criteria

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

4.2.1 Patients must have a confirmed *TET2* mutations identified by next generation targeted deep sequencing.

4.2.2 Patients must have MDS, or MDS/MPN overlap defined by 2016 WHO criteria. Both newly diagnosed or previously treated MDS or MDS/MPN patients are eligible as long as the patient has never received prior treatment with azacitidine or decitabine.

4.2.3 Patients with Leukemic/blast phase transformation MPN.

4.2.4 Patient with AML according to 2016 WHO criteria (excluding APL [AML-M3]).

4.2.4.1 Newly diagnosed patients who are ineligible or declined to receive intensive chemotherapy after discussion of risks and benefits of that approach or patients with primary refractory/relapsed AML.

4.2.4.2 Patients with active central nervous system (CNS) leukemia eligible at the discretion of treating physician.

4.2.4.3 Relapse/Refractory is defined as at least 1 course of treatment for AML excluding any patients treated with azacitidine or decitabine.

4.2.4.3.1 Patients should be off any prior treatment or line of therapy for 2 weeks prior to start study with the exception of hydrea (Hydroxyurea).

4.2.5 Prior therapy with hydroxyurea, biological or targeted therapy (e.g. flt3 inhibitors, other kinase inhibitors) or hematopoietic growth factors is allowed.

4.2.6 Age $\geq$18 years.

4.2.7 ECOG performance status $\leq$ 3.

4.2.8 Patients must have normal organ and marrow function as defined at the discretion of the treating physician and PI.

4.2.9 Women of childbearing potential must have a negative serum or urine pregnancy test within 10-14 days prior to enrollment.

4.2.10 Patients must have the ability to understand and the willingness to sign a written informed consent document.

4.2.11 Patient must be willing to comply with all aspects of the protocol including completing the drug diary.

4.2.12 Patient must discontinue any and all use of multivitamin and/or vitamin c medication 24 hours before first dose of Ascorbic Acid.

4.2 Exclusion Criteria

The presence of any of the following will exclude a subject from study enrollment:

4.2.1 Any prior treatment with azacitidine or decitabine.

4.2.2 Patients diagnosed with APL, AML-M3.

4.2.3 Patients receiving other active treatment for their myeloid malignancy including investigational agents with the exception of hydrea for WBC control.

4.2.4 Nursing or pregnant women.

4.2.5 History of allergic reactions to either azacitidine or ascorbic acid.

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

4.2.7 Patients with higher risk of bleeding (deemed by the treating physician) or on anticoagulation.

4.2.8 Patients who are unwilling or unable to comply with all study requirements.

4.3 Inclusion of Women and Minorities

Men, women and members of all races and ethnic groups are eligible and encouraged to participate in this trial.

5.0 Registration

All subjects who have been consented are to be registered in the OnCore™ Database. For those subjects who are consented, but not enrolled, the reason for exclusion be recorded.

All subjects will be registered through Cleveland Clinic and will be provided a study number by contacting the study coordinator once the patient signs the consent.

6.0 Treatment Plan

6.1 Treatment Regimen Overview

All azacitidine courses will be given at Cleveland Clinic Taussig Cancer Institute or an affiliate hospital approved to treat patients.

6.1.1 Azacitidine

Azacitidine will be administered intravenously or subcutaneously at a fixed dose of 75mg/m2/day for 7 consecutive days, allowing interruptions for weekends and holidays within each 28-day cycle. No dose modifications will be permitted during the treatment period.

6.1.2 Ascorbic Acid

Ascorbic acid will be administered orally daily at 1 g/day three days prior to start azacitidine and then continues daily for a total of 28 days of each 28 day cycle. No dose modifications will be permitted during the treatment period.

6.2 Treatment Duration

The length of the treatment will vary per patient. The typical treatment period will be 6 months for the course of the required treatment. If the patient is eligible to continue treatment during the ETP, the patient is able to receive treatment until the patient meets one or more of the EOT criteria.

6.3 General Concomitant Medications and Supportive Care Guidelines

Patients should be off any prior treatment or line of therapy for 2 weeks prior to start study with the exception of hydrea.

Necessary supportive measures for optimal medical care will be given throughout the treatment as determined by the treating physician and the patient's medical need. No concomitant chemotherapy, immunotherapy, or therapy with monoclonal antibodies will be allowed during the study with the exception of hydroxyurea or corticosteroids for control of blood counts.

Use of a colony-stimulating factor or combinations thereof (e.g. G-CSF, GM-CSF, or erythropoietin) are at the discretion of the treating physician and is permitted if judged in the patient's best medical interest.

Prophylactic antibiotics, antifungals, and antiviral agents (e.g. levofloxacin, itraconazole, valacyclovir, etc.) are recommended; however, the use of these or other drugs will be left to the treating physician's discretion.

6.4 Criteria for Removal from Study Treatment (EOT)

In the absence of treatment delays due to adverse events, treatment may continue for 6 cycles or be extended after 6 cycles unless l one of the following criteria applies:

End of Treatment Criteria

• Disease progression defined as no response after 6 months of treatment or loss of response during treatment as defined in section 12
• Intercurrent illness that prevents further administration of treatment
• The investigator and/or treating physician considers it to be in the best interest of the subject to permanently discontinue study treatment
  o This includes if the treatment is deemed to be of excessive toxicity to the patient by the treating physician
• Patient decision to withdraw from treatment (partial consent) or from the study (full consent)
• Pregnancy during the course of the study for a child-bearing participant
• Death
• Unacceptable adverse events defined as following:
  o Unacceptable treatment related toxicity, NCI CTC AE version 4.0 Grade 3 or 4 that fails to recover to baseline or < Grade 3 in the absence of treatment within 4 weeks
  o Any toxicity or other issue that causes a delay of study drug administration by more than 4 weeks

Table 1: End of Treatment Criteria

6.5 Duration of Follow Up

The clinical course of each adverse event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause.

Serious adverse events that are still ongoing at the end of the study period will necessitate follow-up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported immediately.

Following completion of active treatment (EOT) and while still on study (Follow up), patients will be followed for survival as of standard of care for up to 1 year. This follow up can either be done as a visit to the study doctor's clinic or via telephone call to the patient.

7.0 Dose Delays/Dose Modifications

Patients will receive a course of their treatment every 4weeks, but not exceeding more 8 weeks between treatment courses (4 weeks of treatment + a window of 4 weeks between cycles). Patients should receive their second course of therapy without interruption, regardless of their degree of myelosuppression. After the first two courses of therapy, the interval between subsequent cycles of Azacitidine therapy could be prolonged (with a maximum of 4 weeks prolongation between each 28 day cycle) at the discretion of the investigator. During any dose delays of Azacitidine, Ascorpic Acid therapy should not be delayed. Subsequent cycles can be given prior to baseline peripheral blood count recovery if considered to be in the best interest for the patient and after discussion with the principal investigator and the discussion documented in the patient's medical record.

No dose modification to azacitidine or ascorbic acid can occur during the treatment course.

8.0 Potential Risks and Adverse events

8.1 Potential Risks

8.1.1 Azacitidine

COMMON, SOME MAY BE SERIOUS
In 100 people receiving azacitidine more than 20 and up to 100 may have:

COMMON, SOME MAY BE SERIOUS
In 100 people receiving azacitidine more than 20 and up to 100 may have:
▪ Fever ▪ Fatigue ▪ Headache ▪ Nausea ▪ Vomiting ▪ Diarrhea ▪ Constipation ▪ Loss of appetite ▪ Bruising ▪ Low blood cell counts ▪ Weakness ▪ Joint pain ▪ Shivering ▪ Cough ▪ Difficulty Breathing

OCCASIONAL, SOME MAY BE SERIOUS
In 100 people receiving azacitidine from 4 to 20 may have:
▪ Chest pain ▪ Pale skin ▪ Swelling (such as in arms and legs) ▪ Abnormal heart sound ▪ Fast heart beat ▪ Low blood pressure ▪ High blood pressure ▪ Fainting ▪ Dizziness ▪ Anxiety ▪ Depression ▪ Difficulty sleeping ▪ Lack of energy ▪ Numbness ▪ Pain ▪ Skin hives and /or rash ▪ Dry skin and /or itching ▪ Sweating ▪ Low blood levels of potassium ▪ Weight loss ▪ Abdominal pain, tenderness, and/or swelling ▪ Mouth blisters ▪ Upset stomach ▪ Hemorrhoid bleeding ▪ Difficulty swallowing ▪ Sore or painful throat

OCCASIONAL, SOME MAY BE SERIOUS
In 100 people receiving azacitidine from 4 to 20 may have:
▪ Difficult and/or painful urination ▪ Blood in urine ▪ Pain ▪ Stuffy and/or runny nose ▪ Wheezing ▪ Lymph node swelling ▪ Infection ▪ Hair loss

RARE, AND SERIOUS
In 100 people receiving azacitidine 3 or fewer may have:
▪ Irregular heartbeat ▪ Heart failure ▪ Stoppage of heart and lung function ▪ Bleeding in and/or around the brain ▪ Seizure ▪ Skin condition with fever and skin lesions ▪ Abnormal Blood acid/base balance (possible organ damage) ▪ Dehydration ▪ Inflammation of the gallbladder ▪ Terry or coffee ground-like blood in the stool ▪ Enlarged spleen ▪ Liver failure ▪ Kidney failure, even requiring dialysis ▪ Build-up of bodily waste products in the blood ▪ Coughing up blood ▪ Lung inflammation (possible difficulty breathing) ▪ Allergic reaction, which may be life-threatening ▪ Body-wide inflammation ▪ Breakdown products of the cancer cells entering the blood stream

Azacitidine may cause you to develop another type of cancer such as leukemia, a type of blood cancer

8.1.2 Ascorbic Acid

COMMON, SOME MAY BE SERIOUS
In 100 people receiving ascorbic acid more than 20 and up to 100 may have:
• Fatigue ▪ Headache ▪ Nausea ▪ Vomiting ▪ Diarrhea ▪ Constipation ▪ Loss of appetite ▪ Bruising ▪ Low blood cell counts ▪ Weakness ▪ Joint pain ▪ Shivering ▪ Cough ▪ Difficulty Breathing

OCCASIONAL, SOME MAY BE SERIOUS
In 100 people receiving ascorbic acid from 4 to 20 may have:
▪ Pale skin ▪ Swelling (such as in arms and legs) ▪ Abnormal heart sound ▪ Fast heart beat ▪ Low blood pressure ▪ High blood pressure ▪ Fainting ▪ Dizziness ▪ Anxiety ▪ Depression ▪ Lack of energy ▪ Numbness ▪ Pain ▪ Skin hives and /or rash ▪ Dry skin and /or itching ▪ Sweating

RARE, AND SERIOUS
In 100 people receiving ascorbic acid 3 or fewer may have:
▪ Kidney stones ▪ Kidney failure ▪ Bleeding that could be life threatening ▪ Loss of blood due to hemolysis (a process where red cell gets destructed)

8.2 Definitions

8.2.1 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 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.

Abnormal laboratory results will be assessed in real time by the physicians. Treatment for any clinically significant values will be completed in real documented in the physician's note. Documentation for the assessment and treatment will be provided in the physician's note as source documentation.

Clinical staff (i.e. nurses, clinical research coordinators) can be delegated to evaluate and assess all adverse events, with the exception of determining relationship to study treatment. These assessments will require documented review by either the treating physician or PI.

8.2.2 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.

8.2.3 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.

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

- Event term (as per CTCAE 4.1)
- 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 conmed or other intervention, etc.
- Outcome of event

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 Investigator Brochure, 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 of any adverse event can only be assessed by the PI and/or Co-I. Attribution will be assigned as follows:

- Definite – The AE is clearly related to the study drug.
- Probable – The AE is likely related to the study drug.
- Possible – The AE may be related to the study drug.
- Unlikely – The AE is doubtfully related to the study drug.
- Unrelated – The AE is clearly NOT related to the study drug.

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

8.3 SAE Report Form

SAEs will be recorded on the FDA Form 3500A (MedWatch) and Study SAE coversheet but should only be reported as instructed below. The electronic FDA SAE reporting forms should not be used.

8.4 Reporting Procedures for Serious Adverse Events

For the purposes of safety reporting, all adverse events will be reported that occur during treatment and 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.

8.4.1 SAE Reporting Requirements

- Participating investigators (all sites) must report all serious adverse events to the Lead Site Principal Investigator (e.g. Sponsor-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.
- The Lead Site Principal Investigator will review the SAE and report the event to the FDA, external collaborator(s), and IRB as applicable.
- It is the Sponsor-Investigator's responsibility (e.g. lead site PI) 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:

- Investigative sites will report adverse events to their respective IRB according to the local IRB's policies and procedures in reporting adverse events.

8.5 SAEs and OnCore

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

8.6 Data Safety and Toxicity Committee

It is the responsibility of each site PI to ensure that ALL SAEs occurring on this trial (internal or external) are reported to the Case Comprehensive Cancer Center's Data and Safety Toxicity Committee. This submission is simultaneous with their submission to the sponsor and/or other regulatory bodies.

8.7 Data and Safety Monitoring Plan (DSMP)

This protocol will adhere to the policies of the Case Comprehensive Cancer Center Data and Safety Monitoring Plan in accordance with NCI guidelines.

9.0 PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agents administered in this study can be found in Section 8.1.

9.1 Investigational Agents

9.1.1 Name of Agent Azacitidine

Other Names: Vidaza

Product description: Azacitidine is FDA approved and commercially available.

Preparation for Intravenous Administration: Reconstitute the appropriate number of azacitidine vials to achieve the desired dose. Reconstitute each vial with 10 mL sterile water for injection. Vigorously shake or roll the vial until all solids are dissolved. The resulting solution will contain azacitidine 10mg/mL. The solution should be clear. Parenteral drug product should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.

Withdraw the required amount of azacitidine solution to deliver the desired dose and inject into a 50-100 mL infusion bag of either 0.9% Sodium Chloride Infusion or Lactated Ringer's Infusion.

Azacitidine is incompatible with 5% Dextrose solutions, Hespan, or solutions that contain bicarbonate. These solutions have the potential to increase the rate of degradation of azacitidine and should therefore be avoided.

Intravenous Administration*:* Azacitidine solution is administered intravenously. Administer the total dose over a period of 10-40 minutes. The administration must be completed within 1 hour of reconstitution of the azacitidine vial.

Solution Stability: Azacitidine reconstituted for intravenous administration may be stored at 25°C (77°F), but administration must be completed within 1 hour of reconstitution. If reconstituted azacitidine comes into contact with the skin, immediately and thoroughly wash with soap and water. If it comes into contact with mucous membranes, flush thoroughly with water. Reconstituted azacitidine may be stored for up to 1 hour at 25°C (77°F) or for up to 8 hours between 2 and 8°C (36 and 46°F). The azacitidine suspension is single-use and does not contain any preservatives. Unused portions of each vial should be discarded properly. Do not save any unused portions for later administration. It is recommended that 5-azacitidine in solution is administered as soon as possible.

Storage: Store reconstituted vials at 25o C (77o F); excursions permitted to 15o-30o C (59o-86o F) (See USP Controlled Room Temperature). There is no need to protect azacitidine from

exposure to light.

Preparation for Subcutaneous Administration: Reconstitute VIDAZA aseptically with 4 mL sterile water for injection. Inject the diluent slowly into the vial. Vigorously shake or roll the vial until a uniform suspension is achieved. The suspension will be cloudy. The resulting suspension will contain azacitidine 25 mg/mL. Do not filter the suspension after reconstitution. Doing so could remove the active substance.

Preparation for Immediate Subcutaneous Administration: Doses greater than 4 mL should be divided equally into 2 syringes. The product may be held at room temperature for up to 1 hour, but must be administered within 1 hour after reconstitution.

Preparation for Delayed Subcutaneous Administration: The reconstituted product may be kept in the vial or drawn into a syringe. Doses greater than 4 mL should be divided equally into 2 syringes. The product must be refrigerated immediately. When VIDAZA is reconstituted using water for injection that has not been refrigerated, the reconstituted product may be held under refrigerated conditions (2ºC - 8ºC, 36ºF - 46ºF) for up to 8 hours. When VIDAZA is reconstituted using refrigerated (2ºC - 8ºC, 36ºF - 46ºF) water for injection, the reconstituted product may be stored under refrigerated conditions (2ºC - 8ºC, 36ºF - 46ºF) for up to 22 hours. After removal from refrigerated conditions, the suspension may be allowed to equilibrate to room temperature for up to 30 minutes prior to administration.

9.1.2 Name of Agent Ascorbic Acid

Other Names: Vitamin C, 1000 mg

Product description: Ascorbic Acid is a tablet of 1000 mg Patient is to take one tablet once a day.

Product Description

USP-Verified Dietary Supplement. Recommended by pharmacists. Antioxidant - Helps boost the immune system. Vitamin C helps neutralize free radicals and helps iron get absorbed in the body. UPS has tested and verified ingredients, potency and manufacturing process. USP sets official

standards for dietary supplements. No artificial colors. No artificial flavors. No preservatives. No chemical solvents, yeast or gluten. (These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure or prevent any disease).

Brand: Nature Made

This Nature Made product provides vitamin C, a versatile and essential vitamin critical for the proper functioning of our immune system. In addition, vitamin C helps manufacture certain nerve-transmitting substances and hormones while helping absorb and utilize other nutrients, such as vitamin E and iron. Vitamin C also makes collagen, which holds connective tissues together in skin, bone, teeth, and other parts of the body.

Vitamin C is an important and powerful antioxidant that works in the aqueous (water) environments of the body, such as the lungs and lens of the eye. Its primary antioxidant partners are vitamin E and the carotenes (such as beta-carotene), but it also works with the antioxidant enzymes. Vitamin C regenerates oxidized vitamin E and restores the antioxidant potential of vitamin E in the body.

Vitamin C is recommended as part of a daily regimen for women's and men's health, longevity, heart strength, and immunity.

- No artificial colors
- No artificial flavors
- No preservatives
- No chemical solvents, yeast or gluten. Recommended By Pharmacists.
- USP Standards plus Nature Made's Pure Ingredients guarantee optimum quality. Guaranteed purity, potency and dissolution.

<u>Safety Warning</u>

Do not use if imprinted seal under cap is broken or missing.

Ingredients

Cellulose Gel, Hypromellose, Croscarmellose Sodium, Stearic Acid, Magnesium Stearate, Silicon Dioxide, Polyethylene Glycol, Vitamin C

Directions

Suggested Use: Take one tablet daily with a meal. Keep bottle tightly closed. Store in a cool, dry place, out of reach of children.

10.0 EXPLORATORY or CORRELATIVE STUDIES

A blood sample will be obtained for a next generation targeted deep sequencing genomic panel of 170 gene mutations prior to start treatment. Quantification of 5hmc and 5mc in patient's derived mononuclear cells will be obtained during the screening period and on C6D15 to evaluate for changes in methylation. We will use the ELISA, dot-blot and mass spec assay to measure the level of 5hmC, a signature for TET2 activity in HSPCs and 5mC an indicator of the lack of TET2 activity in patient derived PBMCs (Peripheral blood mononuclear cells) and bone marrow cells. For this purpose total genomic DNA from whole cell nuclear lysates (PBMCs or Bone marrow) or from the nuclear lysate of $CD34^+$ cells will be extracted and used for ELISA assay using a known standard curve. The mass spec will be performed on the samples after digesting with NaOH (Ref: Nature 468, 839–843) and separated and quantified by LCMS-MS (Ref: Mo Cell; Volume 64, Issue 5, 1 December 2016, Pages 913–925) utilizing X-bridge reverse phase column. Recombinant TET2 protein will be used to prepare the standard reaction mixture for 5hmC determination and comparison to the patient samples.

All samples obtained will shipped to and stored at The Cleveland Clinic. Samples will remain at the Cleveland Clinic to be used for research purposes for this study as well as for future research projects approved by the IRB.

10.1 Collection of Samples

10 ML of whole blood or bone marrow aspirate whichever available will be collected in extra lab tube. Details and instructions about the collection, processing, and shipping will be explain in the Lab Manual. All samples should be delivered and/or shipped to:

Attn: Elise Lemelle, Cassie Hirsch, and/or Amy Graham

Principal Research Technologists
Translational Hematology and Oncology
Cleveland Clinic, LC – NE6 – 250
9620 Carnegie Ave.
Cleveland, OH 44195
Phone: (216) 444-0256

11.0 STUDY PARAMETERS AND CALENDAR

11.1 Study Parameters

11.1.1 Screening Evaluation (Consent signing to Day -4) & Baseline period (Day -17 to day -3)

1. A complete history and physical examination within 30 days prior to study entry.

2. A whole blood sample (10 ML) that will be used for next generation targeted deep sequencing of genes that are common in myeloid malignancies

3. Hematology exam (Appendix VI)

4. Serum Chemistry (Appendix VI)

5. Bone marrow biopsy and aspiration with conventional cytogenetic analysis within 30 days prior to start the study. 10 ML of whole blood or bone marrow aspirate whichever available will be collected in extra lab tube.

6. Documentation of transfusion frequency (PRBC and platelets only) prior to first cycle and prior to each additional cycle

7. Documentation of all medication including multivitamins and other over the counter medications

8. Dates of multivitamin/vitamin C discontinuation must be documented and occur before first dose of study drug. Pre-study laboratory tests must be obtained within 14 days of registration.

9. MDS Response Assessment, assessed by the treating physician

Treatment Period (Day -3 to C6D28)

1. Hematology exams (Appendix VI) at the start of each cycle (day 1) and the last day of cycle 6 (C6D28)

2. Serum Chemistry (Appendix VI), including Creatinine, bilirubin, ALT or AST at the start of each cycle (day 1)

3. Bone marrow aspirate and/or biopsy to confirm response, to be performed at the end of cycle 6. 10 ML of whole blood or bone marrow aspirate whichever available will be collected in extra lab tube. All bone marrows samples are to be stored at Cleveland Clinic

4. Conventional cytogenetics to be performed at the end of cycle 6

5. Documentation of frequency of transfusions (only PRBC and Platelets) prior to start of each new cycle.

6. Documentation in the medical record of any grade 2 or higher adverse events at each visit. For guidelines on SAE Reporting and AE entry for Case Report

7. Documentation of any prior administration of growth factors and ESA prior to start of each cycle

8. A 10 ML blood sample that will be used for next generation targeted deep sequencing of genes that are common in myeloid malignancies

9. Physical exam and ECOG performance status occurring at the end of each cycle

10. Vital signs occulting at each visit

11. Pregnancy test if applicable

12. MDS Response Assessment, assessed by the treating physician occurring at the end of cycle 6 or EOT, whichever occurs first.

13. Methylation panel only occurring in Cycle 1

14. Azacitidine will be administered intravenously or subcutaneously at a fixed dose of 75mg/m2/day for 7 consecutive days, (allowing interruptions for weekends, and holidays, of each 28-day cycle).

15. Ascorbic acid will be administered orally daily at 1 g/day three days prior to start azacitidine and then continues daily for a total of 28 days of each 28 days cycle.

Extended Treatment Period (Cycles 7+)

1. Documentation of frequency of transfusions (only PRBC and Platelets) prior to start of each new cycle

2. Documentation in the medical record of any grade 3 or higher adverse events at each visit. For guidelines on SAE Reporting and AE entry for Case Report

3. Physical exams and ECOG performance status occurring at the end of each cycle

4. Vital Signs at each visit

5. Hematology exams (Appendix VI) at the start of each cycle (day 1)

6. Serum Chemistry (Appendix VI), including Creatinine, bilirubin, ALT or AST at the start of each cycle (day 1).

7. Azacitidine will be administered intravenously or subcutaneously at a fixed dose of 75mg/m2/day for 7 consecutive days, (allowing interruptions for weekends, and holidays)

of each 28-day cycle

8. Ascorbic acid will be administered orally daily at 1 g/day for a total of 28 days of each 28 days cycle.

End of Treatment (EOT)

1. Documentation of frequency of transfusions (only PRBC and Platelets) prior to start of each new cycle

2. Documentation in the medical record of any grade 3 or higher adverse events at each visit. For guidelines on SAE Reporting and AE entry for Case Report

3. ECOG performance

4. Hematology exams (Appendix VI)

5. Serum Chemistry (Appendix VI), including Creatinine, bilirubin, ALT or AST

16. Optional bone marrow aspirate and/or biopsy to confirm response, to be performed at the end of cycle 6. All bone marrows samples are to be stored at Cleveland Clinic

11.2 Calendar

Period	Screening Period (Day-17 to Day -3)	Treatment Period															Extended Treatment Period Cycles		End Of Treat-ment (EOT)	Follow up[k]
		Cycle 1				Cycle 2		Cycle 3		Cycle 4		Cycle 5		Cycle 6						
Day		-3	1	7[c]	14[n]	1	7[c]	1	7[c]	1	7[c]	1	7[c]	1	7[c]	28[g]	1	7[c]		
Administration of Azacitidine			X	X[b]		X	X[b]	X	X[b]	X	X[b]	X	X[b]	X	X[b]		X	X[b]		
Ascorbic Acid Dispensement		X[a]				X		X		X		X		X			X			
disease history and prior therapies	X																			
Record RBC/ platelets transfusion	X	X				X		X		X		X		X		X	X			
History and physical exam[dm]	X	X				X		X		X		X		X		X	X			X[j]
Vital signs[d]	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X		X[j]
ECOG performance status[em]	X	X				X		X		X		X		X		X	X		X	
Hematology[d m]	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X[j]
Serum Chemistry[d m]	X	X				X		X		X		X		X		X	X		X	
Pregnancy test	X															X				
Disease response assessment[f]	X															X	X		X	
Bone marrow biopsy and aspiration[L]	X															X[h]			X[i]	
Mutational analysis blood draw[/L]	X[l]															X				
Methylation profile blood draw[L]	X		X	X	X															
Drug Diary Accountability		X				X		X		X		X		X		X	X			
Survival Follow up																				X[k]
Record adverse events[m]		← X →																		

A: For Cycle 1 only, patient will begin treatment with Ascorbic Acid 3 days prior to initial treatment of Azacitidine
B: Azacitidine administration has a +/- 3 day window to account for breaks in treatment for weekends, and holidays
C: Day 7 has a +/- 3 day window to account for breaks in treatment for weekends, holidays, and institutional standards
D: See Appendix VI for required tests
E: See Appendix I for performance status grading
F: See Section for 12.0 for MDS/AML response assessment criteria
G: If patient is eligible for the extended treatment period, and moving forward on the next cycle without a dosing delay, the C6D28 visit can serve as the visit of C7D1. If the patient is not eligible for the extended treatment period the patient's C6D28 visit can serve as the EOT visit.
H: The cycle 6 bone marrow biopsy can occur up to 14 days (C6D14) prior to the C6D28 visit.
I: EOT bone marrow biopsy is optional per discretion of the treating physician
J: These tests in follow up are optional pending discretion of the treating physician
K: Survival Follow Up will occur bimonthly (60 days +/- 7 days) for a duration of 1 year from patient's EOT visit.
L: 10 ML of whole blood or bone marrow aspirate whichever available will be collected in extra lab tube for these tests.
M: If these procedures require a physician visit, these can occur 3 calendar days prior to the day 1 of that cycle (window of – 3 days).
N: Cycle 1 Day 14 has a +/- 1 day window to account if the day falls on a holiday or weekend.

12.0 MEASUREMENT OF EFFECT

The response criteria recommended by the MDS International Working Group 2006. Responses must be verified, documented, and signed at cycle 6 in the local medical record by the local PI. (Patients are considered non evaluable if they have received less than 2 courses of treatment, unless there is clear disease progression.)

Definitions:

Responses for MDS patients:

Complete Response (CR): Bone marrow: ≤ 5% myeloblasts with normal maturation of all cell lines; persistent dysplasia will be noted. Peripheral blood: Hgb ≥ 11 g/dL Platelets ≥ 100×10^9/L Neutrophils ≥ 1.0×10^9/L, Blasts 0%

Partial response (PR): All CR criteria if abnormal before treatment except: bone marrow blasts decreased by ≥ 50% over pretreatment but still > 5%

Marrow CR: Blasts ≤ 5% and decrease by ≥ 50% from baseline (baseline blasts should be above 5% to be eligible for marrow CR)

Hematologic Improvement (HI): (responses must last at least 8 weeks) **Erythroid response** [HI-E; (if pretreatment Hgb < 11 g/dL)]; Hgb increase by ≥ 1.5 g/dL; Relevant reduction of units of RBC transfusions by an absolute number of at least 4 RBC transfusions/8 weeks compared with the pretreatment transfusion number in the previous 8 weeks. Only RBC transfusions given for an Hgb of ≤ 9.0 g/dL pretreatment will count in the RBC transfusion response evaluation.

Platelet response [HI-P; ((if pretreatment Platelet count < 100 x 10^9 /L)]: Absolute increase of ≥ 30×10^9/L for patients starting with > 20×10^9/L platelets, or increase from < 20×10^9/L to > 20×10^9/L and by at least 100%.

Neutrophil response [HI-N; (pretreatment, ANC < 1.0×10^9/L)]: At least 100% increase and an absolute increase > 0.5×10^9/L

Progression/Relapse after hematological improvement: ≥1 of the following: ≥50% decrement from maximum response levels in granulocytes or platelets; ↓ in Hgb by ≥1.5 g/dL; transfusion dependence

Stable Disease: Not satisfying criteria for Complete Remission, Partial Response, Marrow CR, Hematologic Improvement or Progression/Relapse.

Responses for AML patients:

Complete Response (CR): Bone marrow: ≤ 5% myeloblasts with normal maturation of all cell lines, ANC >/= 1.0 X 10^9/L, platelet count >/= 100 X 10^9/L, no detectable Auer rods and no extramedulary leukemia.

Complete Response (CR) with incomplete hematologic recovery (CRi): Responses as in CR but ANC < 1.0 X 10^9/L.

Complete Response (CR) with incomplete platelets recovery (CRp): Responses as in CR but platelets < 100 X 10^9/L.

Partial response (PR): All CR criteria if abnormal before treatment except: bone marrow blasts decreased by ≥ 50% over pretreatment but still > 5%.

13.0 DATA REPORTING / REGULATORY CONSIDERATIONS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 8.0 (Adverse Events: List and Reporting Requirements).

13.1 Data Reporting

The OnCore™ Database will be utilized, as required by the Case Comprehensive Cancer Center, to provide data collection for both accrual entry and trial data management. OnCore™ is a Clinical Trials Management System housed on secure servers maintained at Case Western Reserve University. OnCore™. Access to data through OnCore™ is restricted by user accounts and assigned roles. Once logged into the OnCore™ system with a user ID and password,

OnCore™ defines roles for each user which limits access to appropriate data. User information and password can be obtained by contacting the OnCore™ Administrator at OnCore-registration@case.edu. OnCore™ 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 Form completion in the OnCore™ database. A calendar of events and required forms are available in OnCore™.

13.2 **Regulatory Considerations**

The study will be conducted in compliance with ICH guidelines and with all applicable federal (including 21 CFR parts 56 & 50), state or local laws.

13.2.1 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.

13.2.2 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 sponsor and/or allow the sponsor, a 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.

13.2.3 Retention of records

The Principal Investigator of The Case Comprehensive Cancer Center supervises 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.

13.2.4 Audits and inspections

Authorized representatives of the sponsor, a regulatory authority, an Independent Ethics Committee (IEC) or an Institutional Review Board (IRB) may visit the site to perform audits or inspections, including source data verification. 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. For multi-center studies, participating sites must inform the sponsor-investigator of pending audits.

14.0 STATISTICAL CONSIDERATIONS

Definition of primary outcome/endpoint: A binary indicator of overall response, defined as achieving CR, PR, or HI) per 2006 IWG criteria for MDS and MDS/MPN overlap and (CR, CRp, CRi, or PR) per 2003 IWG criteria for AML after 6 cycles of treatment

Definition of secondary outcomes/endpoints:

1. Incidence of adverse events of the combination defined by CTCAE 4.1 criteria. Toxicity will be any drug related Grade 3 or 4 toxicity.
2. Response duration to the combination recorded from start of treatment to progression
3. Overall survival measured from start of treatment to death or last follow up

Sample size justification and study design: The study sample size was determined using Minimax Simon's two-stage design (Simon, 1989. The null hypothesis that the true response rate is 0.3 will be tested against a one-sided alternative that the true response rate is 0.5. In the first stage, 12 patients will be accrued. If there are 3 or fewer responses, the study will be stopped. Otherwise, 16 additional patients will be accrued for a total of 28. The null hypothesis will be

rejected if 11 or more responses are observed in 28 patients. This design yields a type I error rate of 0.1 and a power of 0.8 when the true response rate is 0.5.

Analysis plan: Descriptive statistics will be used for the primary endpoint and secondary endpoints 1,2, and 3. Overall survival will be estimated with Kaplan Meier method and will be compared to the survival of a historical cohort of MDS patients treated at our institution with single agent Azacitidine between 2006-2012 using a one-sample log rank test. A Cochran-Mantel-Haenszel test will be used to test for differences in response rate by mutational status controlling for treatment. Unadjusted and adjusted logistic regression models were used to predict response to therapy. Models will be adjusted for covariates including age, sex, and other prognostic factors. The odds ratio (OR) and 95% confidence intervals (CI) will be estimated for the risk group (mutated) and compared with the reference group (WT).

REFERENCES

REFERENCES

1. Tefferi A, Vardiman JW. Myelodysplastic syndromes. The New England journal of medicine 2009;361:1872-85.
2. Sekeres MA. Epidemiology, natural history, and practice patterns of patients with myelodysplastic syndromes in 2010. Journal of the National Comprehensive Cancer Network : JNCCN 2011;9:57-63.
3. Garcia-Manero G. Prognosis of myelodysplastic syndromes. Hematology / the Education Program of the American Society of Hematology American Society of Hematology Education Program 2010;2010:330-7.
4. Bejar R, Stevenson K, Abdel-Wahab O, et al. Clinical effect of point mutations in myelodysplastic syndromes. The New England journal of medicine. 2011; 364(26): 2496-506.
5. Haferlach T, Nagata Y, Grossmann V, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia. 2014; 28(2): 241-7.
6. Mullighan CG: TET2 mutations in myelodysplasia and myeloid malignancies. Nat Genet 41:766-767, 2009
7. Genovese G, Kahler AK, Handsaker RE, et al: Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med 371:2477-87, 2014
8. Jaiswal S, Fontanillas P, Flannick J, et al: Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med 371:2488-98, 2014
9. Solary E, Bernard OA, Tefferi A, et al: The Ten-Eleven Translocation-2 (TET2) gene in hematopoiesis and hematopoietic diseases. Leukemia 28:485-96, 2014
10. Yin R, Mao SQ, Zhao B, et al: Ascorbic acid enhances Tet-mediated 5-methylcytosine oxidation and promotes DNA demethylation in mammals. J Am Chem Soc 135:10396-403, 2013
11. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, et al. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic

syndromes: a randomised, open-label, phase III study. The Lancet Oncology 2009;10:223-32.

12. Itzykson R, Kosmider O, Cluzeau T, et al: Impact of TET2 mutations on response rate to azacitidine in myelodysplastic syndromes and low blast count acute myeloid leukemias. Leukemia 25:1147-52, 2011
13. Bejar R, Lord A, Stevenson K, et al. TET2 mutations predict response to hypomethylating agents in myelodysplastic syndrome patients. Blood 2014;124:2705-12.
14. Liu M, Ohtani H, Zhou W, et al. Vitamin C increases viral mimicry induced by 5-aza-2′-deoxycytidine. *Proceedings of the National Academy of Sciences* 2016; **113**(37): 10238-44.
15. Bejanyan N, Tiu RV, Raza A, et al. A phase 2 trial of combination therapy with thalidomide, arsenic trioxide, dexamethasone, and ascorbic acid (TADA) in patients with overlap myelodysplastic/myeloproliferative neoplasms (MDS/MPN) or primary myelofibrosis (PMF). *Cancer* 2012; **118**(16): 3968-76.
16. Levine M, Conry-Cantilena C, Wang Y, et al. Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. *Proceedings of the National Academy of Sciences of the United States of America* 1996; **93**(8): 3704-9.
17. Cameron E, Pauling L. Supplemental ascorbate in the supportive treatment of cancer: Prolongation of survival times in terminal human cancer. *Proceedings of the National Academy of Sciences of the United States of America* 1976; **73**(10): 3685-9.
18. Padayatty SJ, Riordan HD, Hewitt SM, Katz A, Hoffer LJ, Levine M. Intravenously administered vitamin C as cancer therapy: three cases. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne* 2006; **174**(7): 937-42.
19. Monti DA, Mitchell E, Bazzan AJ, et al. Phase I evaluation of intravenous ascorbic acid in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer. *PloS one* 2012; **7**(1): e29794.
20. Welsh JL, Wagner BA, van't Erve TJ, et al. Pharmacological ascorbate with gemcitabine for the control of metastatic and node-positive pancreatic cancer (PACMAN): results from a phase I clinical trial. *Cancer chemotherapy and pharmacology* 2013; **71**(3): 765-75.

21. Ma Y, Chapman J, Levine M, Polireddy K, Drisko J, Chen Q. High-dose parenteral ascorbate enhanced chemosensitivity of ovarian cancer and reduced toxicity of chemotherapy. *Science translational medicine* 2014; **6**(222): 222ra18.
22. Chen Q, Espey MG, Krishna MC, et al: Pharmacologic ascorbic acid concentrations selectively kill cancer cells: action as a pro-drug to deliver hydrogen peroxide to tissues. Proc Natl Acad Sci U S A 102:13604-9, 2005.

APPENDIX I

PERFORMANCE STATUS CRITERIA

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

APPENDIX II

CYCLE 1 SUBJECT PILL DIARY

Subject Name _____Protocol #_____ Subject Study ID _____
Cycle #: _____ Month #: _____

INSTRUCTIONS FOR THE SUBJECT:

1. You will take 1 tablet of 1000 mg *of* Ascorbic Acid (Vitamin C) pills each day. ***Take the tablets with food***
2. Record the date, the number of tablets you took, and what time you took them.
3. If you have any comments please record them in the "Comments" column below.
4. Please bring your pill bottle and this form to your physician when you come for your next appointment.
5. Please sign your name at the bottom of the diary.

Date	Day	Time	# of 1000 mg Ascorbic Acid (Vitamin C) pills and time taken	Comments
	-3			
	-2			
	-1			
	1			
	2			
	3			
	4			
	5			
	6			
	7			
	8			
	9			
	10			
	11			
	12			
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	14			
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	26			
	27			
	28			

Subject's Signature: _____ Date: _____

APPENDIX III

CYCLE 2 + SUBJECT PILL DIARY

Subject Name _____Protocol #_____ Subject Study ID _____
Cycle #: _____ Month #: _____

INSTRUCTIONS FOR THE SUBJECT:

1. You will take 1 tablet of 1000 mg *of* Ascorbic Acid (Vitamin C) pills each day. ***Take the tablets with food***
2. Record the date, the number of tablets you took, and what time you took them.
3. If you have any comments please record them in the "Comments" column below.
4. Please bring your pill bottle and this form to your physician when you come for your next appointment.
5. Please sign your name at the bottom of the diary.

Date	Day	Time	# of 1000 mg Ascorbic Acid (Vitamin C) pills and time taken	Comments
	1			
	2			
	3			
	4			
	5			
	6			
	7			
	8			
	9			
	10			
	11			
	12			
	13			
	14			
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	17			
	18			
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	23			
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	25			
	26			
	27			
	28			

Subject's Signature: _____ Date: _____

APPENDIX IV: STUDY DESIGN TIME POINT DEFINTIONS

Study Time Points	Definitions
Screening	Screening will occur from the time of the consent until day -4
Baseline/Enrollment	Days -17[b] to Day -3[c]
Treatment Period	Day 1[c] to C6D28 or patient meets end of treatment criteria before completing 6 cycles
Extended Treatment Period (ETP)	The extended treatment period is defined as C7D1 to the patient meets EOT criteria
Study Treatment	The treatment period and if applicable the extended treatment period
End of Treatment (EOT)	The day patient meets the defined EOT criteria (Table 1) and discontinues study treatment.
Follow up	Patient will be followed bimonthly (60 days +/- 7 days) for a duration of 1 year after study
End of Study (EOS)	The time the patient completes 1 year of survival follow up and/or patient's death, whichever were to occur first.
A: Day 1 is defined as first dose of Azacitidine B: Day -17 is defined as 14 days prior to first dose of study Drug (Day -3) C: Day -3 is defined as day of Study Drug	

APPENDIX V: RESPONSE CRITERIA FOR EXTENDED TREATMENT

Response Criteria for extended treatment eligibility

- complete remission, partial remission, or hematologic improvement (CR/PR/HI) per IWG 2006 criteria for patients with MDS
- (CR/PR/marrow CR) per IWG 2003 criteria for patients with AML
- If the response is stable disease and the investigator determines the patient is gaining clinical benefit, patient may continue on treatment until progression.

APPENDIX VI: CLINICAL LABORATORY PARAMETERS

Hematology	Serum Chemistry	Vitals	Physical Exam	Bone Marrow Biopsy & Aspiration
Red blood cell count	Sodium	Temp	Height(only at C1D1)	Marrow blast percent
Hemoglobin	Potassium	Pulse	Weight	Marrow Cellularity
Hematocrit	Chloride	Resp		Auer Rods
White blood cell count	Bicarbonate	Blood Pressure		Dysplasia
Neutrophil	BUN	SpO2*		Cytogenetics
Platelets	Glucose (non-fasting)			
Blasts percent	Albumin			
MCV	AST			
MCHC	ALT			
Eosinophils	Serum creatinine			
Basophils	Protein			
Monocytes	Calcium			
Lymphocyte	Bilirubin			
	Alkaline phosphate			

- This test should only occur at Screening visit

APPENDIX VII: WHO classification of myeloid neoplasms and acute leukemia Arber et al, 2016

WHO myeloid neoplasm and acute leukemia classification
Myeloproliferative neoplasms (MPN)
Chronic myeloid leukemia (CML), *BCR-ABL1*$^{+}$
Chronic neutrophilic leukemia (CNL)
Polycythemia vera (PV)
Primary myelofibrosis (PMF)
PMF, prefibrotic/early stage
PMF, overt fibrotic stage
Essential thrombocythemia (ET)
Chronic eosinophilic leukemia, not otherwise specified (NOS)
MPN, unclassifiable
Mastocytosis
Myeloid/lymphoid neoplasms with eosinophilia and rearrangement of *PDGFRA*, *PDGFRB*, or *FGFR1*, or with *PCM1-JAK2*
Myeloid/lymphoid neoplasms with *PDGFRA* rearrangement
Myeloid/lymphoid neoplasms with *PDGFRB* rearrangement
Myeloid/lymphoid neoplasms with *FGFR1* rearrangement
Provisional entity: Myeloid/lymphoid neoplasms with PCM1-JAK2
Myelodysplastic/myeloproliferative neoplasms (MDS/MPN)
Chronic myelomonocytic leukemia (CMML)
Atypical chronic myeloid leukemia (aCML), *BCR-ABL1*$^{-}$
Juvenile myelomonocytic leukemia (JMML)
MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T)

WHO myeloid neoplasm and acute leukemia classification
MDS/MPN, unclassifiable
Myelodysplastic syndromes (MDS)
MDS with single lineage dysplasia
MDS with ring sideroblasts (MDS-RS)
MDS-RS and single lineage dysplasia
MDS-RS and multilineage dysplasia
MDS with multilineage dysplasia
MDS with excess blasts
MDS with isolated del(5q)
MDS, unclassifiable
Provisional entity: Refractory cytopenia of childhood
Myeloid neoplasms with germ line predisposition
Acute myeloid leukemia (AML) and related neoplasms
AML with recurrent genetic abnormalities
AML with t(8;21)(q22;q22.1);*RUNX1-RUNX1T1*
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);*CBFB-MYH11*
APL with *PML-RARA*
AML with t(9;11)(p21.3;q23.3);*MLLT3-KMT2A*
AML with t(6;9)(p23;q34.1);*DEK-NUP214*
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2*, *MECOM*
AML (megakaryoblastic) with t(1;22)(p13.3;q13.3);*RBM15-MKL1*
Provisional entity: AML with BCR-ABL1
AML with mutated *NPM1*
AML with biallelic mutations of *CEBPA*

WHO myeloid neoplasm and acute leukemia classification
Provisional entity: AML with mutated RUNX1
AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms
AML, NOS
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Pure erythroid leukemia
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma
Myeloid proliferations related to Down syndrome
Transient abnormal myelopoiesis (TAM)
Myeloid leukemia associated with Down syndrome
Blastic plasmacytoid dendritic cell neoplasm

APPENDIX VIII: PROTOCOL AMENDMENT SUMMARIES

Amendment Number/Version Number/Date: Amendment 1, protocol version 2, 11/20/2017	
Section #/page	Description of change (s)
Preparation for Intravenous Administration, pg 32	Corrected Chloride Injection or Lactated Ringer's Injection to Chloride Infusion or Lactated Ringer's Infusion.
Investigational Agents, pg 32	Added option to deliver Azacitidine to patients subcutaneously due to the national IV bag shortage.
Investigational Agents, pg 33	Corrected the dosages amount for the ascorbic acid as well as the amount of tablets.
Screen test procedures	Removed Vitamin C level testing
CLINICAL LABORATORY PARAMETERS, pg 53	Clarified that SpO2 testing should only occur at screening visit
Appendix IV, pg 54	Clarified days to align with study calendar
Study Calendar	Provided a 3 day window for each day 1 visit requiring a physician visit

Amendment Number/Version Number/Date: Amendment 2, protocol version 3, 05/24/2018	
Section #/page	Description of change (s)
Clinical Trials.gov number, pg 1	Added Clinical Trials.gov Number
Protocol Date and Protocol version number	Updated the protocol date and version number throughout the entire protocol.
Eligibility Criteria, pg 20	Updated the eligibility criteria from heterozygous TET2 mutations, to include all TET2 mutations
Background of Study Disease, pg 10-11	Updated rationale as to why the eligibility criteria is changing from heterozygous TET2 mutations, to include all TET2 mutations
Study Calendar, pg 43/44	Adding Cycle 1 Day 14 has a +/- 1 day window to account if the day falls on a holiday or weekend.