A Phase Ib Study Evaluating the Safety and Tolerability of Vitamin C in Patients with Intermediate or high Risk Myelodysplastic Syndrome with TET2 Mutations

Protocol No.:	S17-00978
Test Product:	Vitamin C
Indication:	Intermediate or high Risk myelodysplastic syndrome patients with TET2 Mutations
Sponsor:	New York University Langone Medical Center Perlmutter Cancer Center
Development Phase:	Ib
Sites:	NYU Langone's Ambulatory Care Center University of Miami Health System
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Methodology:
This is an open label, Phase Ib study designed to evaluate the safety, toxicity and biological activity of high dose Vitamin C in bone marrow and peripheral blood when administered as therapy to patients with intermediate or high risk myelodysplastic syndrome according to the revised IPSS (international prognostic scoring system) criteria whose disease has a TET2 mutation.

Number of Patients:
We propose to treat up to 12 patients in Phase 1b and defer 34 patients in Phase IIa for a later clinical trial with intermediate or high risk MDS with a *TET2* mutation who have failed hypomethylating agent therapy. We will evaluate the safety and toxicity of continuous infusion high dose Vitamin C in MDS patients, confirm that we can achieve Vitamin C plasma concentrations needed to target *TET2* mutations, and monitor response and pharmacodynamics markers.

Diagnosis and Main Eligibility Criteria:
This study will enroll patients with intermediate or high risk myelodysplastic syndrome

Sponsor:
NYU Perlmutter Cancer Center
550 First Avenue, New York, NY 10016

Planned Study Period:
Q1 2018 to Q42022 (first patient first visit to last patient last visit)

Objectives:
Primary objectives are:
1. Evaluate the safety and toxicity of high dose Vitamin C
2. Estimate the proportion of MDS patients with TET2 mutations who exhibit a biological response defined as maintaining a vitamin C serum concentration of ≥1 mM over the treatment cycle.

Secondary objectives are:
1. Estimate the clinical efficacy, namely objective response rate (ORR), [including complete response (CR) and partial response (PR)], duration of response (DOR) and progression-free survival (PFS) as defined in the IWG (International Working Group) response criteria in myelodysplasia
2. Estimate the Maximum measure Vitamin C plasma concentration (Cmax)
3. Evaluate the pharmacokinetic profile (PK) of Vitamin C as hypomethylating or demethylating agent

Inclusion criteria:
1. Age ≥18 years
2. Histologically confirmed Myelodysplastic Syndrome with positive TET2 mutations (We will test all MDS patients for TET2 mutations using next generation sequencing and only patients with TET2 mutations will be included in our study)
3. Myeloblasts account for less than 20% of leukocytes on peripheral blood and bone marrow aspirate

4. Eastern Cooperative Oncology Group (ECOG) performance status ≤2 (Appendix 1)
5. Adequate organ function
    a) Platelets ≥20,000/μL
    b) Absolute neutrophil count ≥ 500/μL
    c) Bilirubin < 1.5 x institutional upper limit of normal (ULN) or < 3 x ULN in patients with Gilbert's disease or liver involvement
    d) Serum albumin ≥ 2.0 g/dL
    e) Aspartate aminotransferase (AST)/Alanine aminotransferase (ALT) ≤2.5 institutional ULN or, in the case of liver involvement by the primary disease AST/ALT ≤ 5 x ULN
    f) Creatinine≤1.5 x institutional ULN or estimated creatinine clearance of ≥45 mL/min by the Cockcroft-Gault equation or measured creatinine clearance >45 mL/min
6. Females of child bearing potential must have a negative serum pregnancy test with 7 days prior to first dose of treatment and use 2 methods of contraceptives while on treatment
7. Ability to understand and the willingness to sign a written informed consent document
8. Patients currently receiving or who previously received Hydroxyurea, Erythrocyte stimulating agents (ESA), or granulocyte colony stimulating factors (G-CSF) are allowed to participate in the study.

Exclusion criteria:
1. Concurrent hypomethylation agent usage; the last dose of treatment must be ≥4 weeks before the start of the Vitamin C infusion
2. Myeloblast count ≥20% in peripheral blood or bone marrow aspirate
3. Major surgery within 2 weeks prior to first dose of study drug
4. Allogeneic stem cell transplant
5. Any previous chemotherapy agent other than hypomethylating agents (e.g., Venetoclax)
6. Uncontrolled concurrent serious illness
7. Concurrent malignancy or history of a previous malignancy within 1 year prior to first dose of the current study, unless curatively resected basal, squamous cell carcinoma of the skin, breast ductal/lobular carcinoma in situ or cervical carcinoma in situ.
8. Active infections including hepatitis B carrier status, hepatitis C virus (HCV) infection (patients must have a negative Hep B and Hep C viral load at screening)
9. Known HIV-positive status
10. Any significant medical conditions, laboratory abnormality, or psychiatric illness that would exclude the subject from participation or interfere with study treatment, monitoring and compliance such as:
    a) Unstable angina pectoris, symptomatic congestive heart failure (NYHA III or IV), myocardial infarction ≤ 6 months prior to first study drug, clinically significant and uncontrolled cardiac arrhythmia (e.g. atrial fibrillation/flutter ventricular cardiovascular physiology is allowed), cerebrovascular accidents ≤ 6 months before study drug start
    b) Severely impaired lung function
11. Serious, systemic infection requiring treatment ≤7 days before the first dose of study drug
12. Any severe, uncontrolled disease or condition which in the investigator's opinion, may put the subject at significant risk, may confound the study results, or impact the subject's participation in the study
13. History of any renal calculi or hyperoxaluria or any other preexisting renal disorder
14. History of G6PD deficiency, hereditary spherocytosis or hemochromatosis
15. Patients on therapeutic or prophylactic anticoagulation will be excluded from enrollment on the protocol. However, patients can remain on the study if they develop a thrombosis that requires therapeutic anticoagulation during the course of protocol therapy
16. Uncontrolled hyponatremia, SIADH, hypokalemia, hyerpkalemia, hypomagnesemia or hypermagnesemia

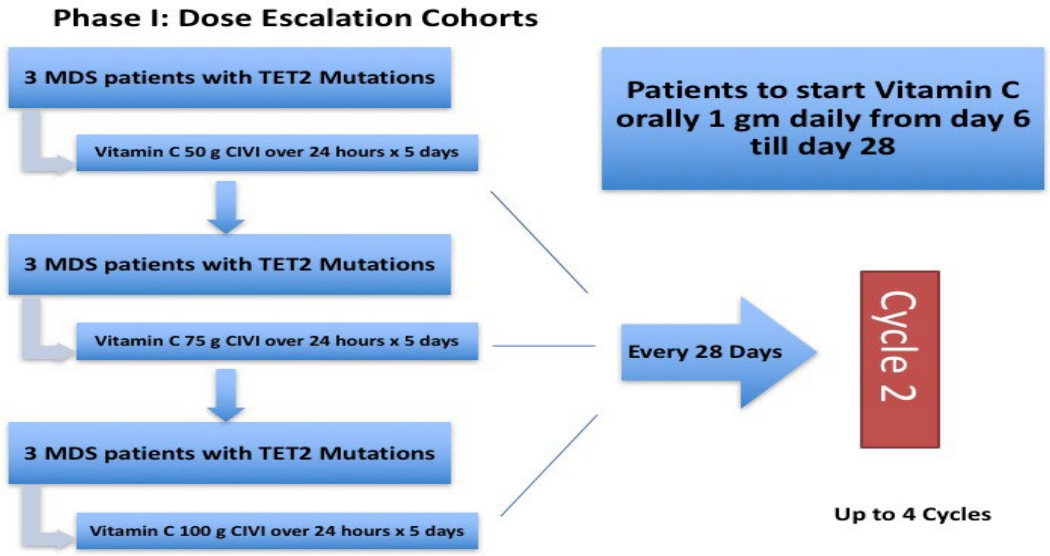
Study Design, Product, Dose modification, Mode of Administration and Sample Storage

Study Design, Test Product and Dose:

The gene that encodes L-gulonolactone oxidase (*GULO*), a key enzyme in Vitamin C biosynthesis, is dysfunctional in humans [2]. Consequently, we depend on constant dietary Vitamin C intake and digestive uptake, which occurs via two sodium-dependent Vitamin C Transporters (SVCTs). SVCT activity is homeostatically down-regulated in enterocytes, limiting the blood levels that can be achieved following oral administration of Vitamin C to a plasma concentration of approximately 70-80 μM [3]. This limitation can be circumvented by parenteral (intravenous, IV) injection. Notably, the early studies of Cameron and Pauling suggested anti-neoplastic effects of parenteral Vitamin C on various solid tumors [4], but these results were not reproduced in subsequent trials, including those by NIH investigators [5, 6]. ***However, the latter investigators only tested oral Vitamin C at up to 10 grams/d, and might not have achieved levels high enough for efficacy. Specifically, peak ascorbate levels were ~ 220-250 μM and declined rapidly after 30 minutes due to renal excretion. In addition, those studies, unlike the ones proposed here, were not biomarker-informed or –directed***. There have been limited studies of the effects of Vitamin C in AML/MDS patients. In one trial, Vitamin C at 1 gram/day was given IV in combination with arsenic trioxide to AML patients, and showed no additive efficacy [7]. Another study combined a very low dose of parenteral Vitamin C with decitabine in MDS and AML patients, with limited clinical evidence of efficacy [8]. In that study, the standard decitabine dose for MDS (20 mg/m^2/day intravenously for 5 days) was safely added to Vitamin C 1 g IV for 5 days in combination with arsenic trioxide. Vitamin C was also continued orally weekly between the cycles of decitabine and was well tolerated. Vitamin C did not add any benefit, but it was given at a low dose (1 gram), for a short period of time (less than 2 hours infusion), and to unselected MDS patients. *There has been no clinical study using high-dose Vitamin C (50 grams or more daily) as a hypomethylating agent to regulate levels of 5mC and 5hmC. Moreover, TET2 mutations have not been previously used as a potential biomarker for choosing Vitamin C therapy, as suggested by the extensive pre-clinical data described above.* We hypothesize that high doses of Vitamin C are necessary to achieve therapeutic levels and efficacy in MDS patients, and that response will be restricted to, or at least maximized in, patients with *TET2* mutations. Based on previous pharmacokinetic analyses, IV administration can produce blood levels at least 25 times those achieved by maximal oral dosing. Furthermore, such doses can be administered safely [9]. For example, high-dose intravenous Vitamin C was combined with gemcitabine and Erlotinib in a phase 1 study of pancreatic cancer without any major side effects [10]. Patients in that trial received different Vitamin C doses, with one group receiving up to 100 g intravenously 3 times/week per cycle without major adverse events.

We propose a Phase 1 component with 3 dose-escalation cohorts in a 3+3 design to determine the maximum tolerated dose (MTD) of Vitamin C. Vitamin C will be provided from McGuff based on a confidential agreement with them and McGuff provides the only FDA-approved injectable preparation of Vitamin C in the USA. For the dose- escalation cohorts, we suggest a starting Vitamin C dose for MDS patients of 50 grams, continuous intravenous infusion (CIVI) over 24 hours, given daily for 5 days for a total of 250 grams, followed by a 50% increase in dose for the next cohort to 75 grams/ day Vitamin C CIVI for 5 days for total of 375 grams for 5 days. The third cohort will have a 100% increase in the original dose with an additional 3 patients treated with a dose of 100 grams/ day CIVI for 5 days for total of 500 grams for 5 days (see below for schematic diagram). Patients will be maintained on 1 gram oral Vitamin C from the end of the CIVI until the beginning of the next cycle. One subject at a time will be enrolled into the first cohort until the first dose level cohort is completed and all patients treated successfully for one cycle. Two additional cohorts will

be treated similarly. If, as expected no patient experiences dose-limiting toxicity (DLT), then the last dose level cohort will be expanded to 6 subjects to confirm safety. After determining the recommended Phase 2 dose (RP2D) from the dose-escalation cohorts, we will proceed to a Phase 2 two-stage clinical trial at this dose however the phase 2 portion of our clinical trial have been deferred to a new clinical trial to conduct in the future and not as a part of this trial. The Vitamin C dose will not be increased above 100 grams/ day, even if, as expected, no DLTs are encountered, because higher dosages require larger volumes than the CADD pump can accommodate and potentially have more toxicity with limited to no additional benefit on Vitamin C pharmacokinetics and pharmacodynamics. Three patients in the first cohort (Vitamin C 50 grams CIVI) have been enrolled before the study redesign and tolerated treatment well without any side effects and should account for the completion of the first cohort.



Phase 1 Study Design
A 3+ 3 design will be used for the Phase 1 Dose Escalation Cohorts; 3 patients will receive Vitamin C as a continuous intravenous infusion (CIVI) of 50 grams (g) daily over 24 hours for 5 days for total of 250 grams over 5 days, then another 3 patients will receive 75 grams for total of 375 grams over 5 days, followed by a final cohort of 3 patients at 100 grams for total of 500 grams over 5 days. Each cycle will be 28-days, and patients can receive up to 4 cycles. Patients will be continued between each cycle on oral Vitamin C as 1 grams daily (from day 6 till 28).

We have incorporated plans to evaluate pharmacokinetics (PK) to identify the doses required to achieve our targeted dose. We performed extensive tests to confirm the safety and stability of diluting Vitamin C in normal saline, and its use in 24 hour infusions via CADD pumps. The rationale for starting the first cohort at a dose of 50 grams of Vitamin C is that when this dose is given in humans as an IV bolus, a peak concentration in plasma of at least 15-20 mM is achieved with a half-life around 30-45 minutes [11]. Accordingly, giving 50 grams daily Vitamin C as a continuous intravenous infusion (CIVI) should achieve a concentration of at least 1 mM continuously in the plasma of patients, and this is the expected concentration (based on our pre-clinical studies) required for demethylation. For further cohorts we expect to achieve at least a plasma concentration of 2 mM and 3 mM respectively for the 75 grams and the 100 grams Vitamin C doses cohorts and accordingly with higher Vitamin C plasma concentrations in *TET2* mutated MDS patients we expect to achieve higher demethylation effects and a better clinical response. Moreover, a very recent publication in BMC in May 2019 supported our PK assessments, and showed that the peak (Cmax) and the area under the curve (AUC) Vitamin C plasma concentrations increased

with higher doses. In that trial they used doses up to 1.5g/kg/ day Vitamin C as no MTD was seen, and they were able to achieve Vitamin C plasma concentrations up to 10-20 mM for 4 hours at 1.5g/kg dose with Cmax and AUC appearing to reach maximum values at this dose [12]. Also, Levine *et al*. showed earlier that patients receiving high doses of IV Vitamin C achieved plasma concentrations up to 14 mM, compared with a maximum plasma concentration of only 220 μM following high doses of oral Vitamin C [13]. Accordingly, given the above data we planned our study with 3 dosage escalations cohorts with Vitamin C 100 grams being the maximum planned dose. To measure plasma Vitamin C levels, we will use liquid chromatography, coupled with triple quadrupole mass spectrometry (LC-MS) on samples obtained from the peripheral blood of MDS patients [14]. The LC column is a Millipore ™ ZIC-pHILIC (2.1 x150 mm, 5 μm), coupled to Dionex Ultimate 3000 ™ system, and the column oven temperature will be set to 25° C for the gradient elution. MS analyses will be carried out by coupling the LC system to a Thermo Q Exactive HF ™ mass spectrometer, operating in heated electrospray ionization mode (HESI). The method duration will be 30 minutes with a polarity switching data-dependent top 5 methods for both positive and negative modes. Vitamin C measurements from both PCC and from the University of Miami site will be performed by Dr. Drew Jones, the director of the PCC Metabolomics Developing Shared Resource.

Dose modifications, Mode of Administration and Sample Storage:

For patients who present with a low absolute neutrophil count, no dose modifications will be required for cycle 2 and subsequent cycles. Vitamin C dosage for subsequent treatment will be at the discretion of the Principal Investigator.

Patients will require central access prior to starting the continuous infusion of vitamin C. Patients will require either an implantable device (e.g. Port-a-Cath) or a PICC line (peripherally inserted central catheter). Both procedures represent safe and effective methods of obtaining central access. The patient will sign a separate consent discussing the risks and benefits of catheter placement prior to the procedure. Peripheral inserted central catheter (PICC) placement has a minimal risk of thrombus formation and bleeding while Port-a-Cath placement has a minimal risk of thrombus formation and infection.

Bone marrow aspirates and peripheral blood samples will be stored in Dr. Neel's and Dr. Aifantis' Laboratory and measuring Vitamin C will be conducted at Dr. Drew Jones laboratory at NYU core facility and samples will be shipped directly for his facility. Samples collected at University of Miami will be kept and processed in the lab of Luisa Cimmino, PhD until all time points have been collected. University of Miami will send replicate samples to NYU. We plan for store the samples until all the studies required for this clinical trial are completed. The linking key between the samples and patient will only be kept with the PI of the study; any patient can ask to withdraw his/her sample from the study in writing to the PI.

Intended Duration of Treatment:
All patients will receive at least 1 cycle of treatment (4 weeks). Patients with clinical benefit (CR, PR, or SD) then will undergo a second 4-week cycle of treatment. Complete response (CR) is defined as myeloblasts <5% in bone marrow and peripheral blood showing neutrophils count >1000/μL, hemoglobin >11g/dL and platelets count>100,000/μL. Partial response (PR) is defined as any decrease in myeloblasts in the bone marrow or peripheral blood or improvement in peripheral blood count but not meeting CR criteria while stable disease (SD) shows no evidence of progression. Patients to receive a maximum of 16 weeks of treatment (4 cycles). If a patient progress after receiving a cycle of treatment then the patient will be withdrawn from the study.

We plan to follow up the patients that complete treatment for 6 months after finishing the last dose of treatment to continue to assess their response. After the end of treatment, each subject will be followed for 30 days for adverse event monitoring.

Assessments may continue for ongoing reportable adverse events. A visit is to be performed at 30 days after the last study drug is given plus/ minus 3 days.

After finishing last does of treatment, each subject will be followed for 6 months to continue to assess his or her response. Subjects to be contacted once a month by telephone to assess survival status and CBC records if available reviewed every 6 months after completing the study until they meet criteria for removal of study below.

- Withdrawal of permission to record at least survival data
- Lost to follow-up
- Death

If a subject withdraws permission to record at least survival data after coming off treatment, this must be documented along with the date the subject withdraws permission. Subjects will be considered lost to follow up if no medical records are available to be reviewed and two phone calls each to the subject and then the subject's next-of-kin (if the subject does not respond) are not returned over two consecutive 3 month periods.

Assessments:

Safety Assessments (primary endpoint)

(We will establish safety in all patients within and across dose levels and study phases)

- Determine the safety profile for each dose in Phase I

Biological effects (primary endpoint) within and across dose levels and study phases

- Estimate the serum bioavailability of Vitamin C in MDS patients with TET2 mutations
- Estimate the bioavailability of Vitamin C in isolated cell extracts from MDS patients to ensure it is increasing the intracellular concentrations as well

Activity Assessments (secondary endpoint) within and across dose levels and study Phases

- ORR (CR, PR)
- DOR
- PFS

Pharmacokinetic Assessments (secondary endpoint)

- Maximum measured Vitamin C plasma concentration (Cmax)
- Evaluate the pharmacokinetic profile (PK) of Vitamin C as hypomethylating or demethylating agent

Immunological Effects

- Phenotypic characterization of myeloblasts in bone marrow and peripheral blood

Correlative studies:

- To evaluate our therapeutic hypothesis, it is essential to study the effect of high dose Vitamin C on myeloblasts from MDS patients. In addition, a recent study of wild type and gulonolactone (L-) oxidase (*Gulo*) knockout mice

reported that parenteral Vitamin C increased Tregs in a sepsis model [15]. Vitamin C also plays an essential role in macrophage activation [16]. It is important to study the potential effects of high dose Vitamin C on these aspects of the MDS microenvironment, as they could modulate or enhance the cell-autonomous effects of Vitamin C on MDS blasts and might suggest future combination therapies. According we will evaluate the association of methylation, gene expression with response in Phase II.

Statistical Methods:
Statistical considerations:
Phase 1: At each dose level, safety data will be summarized by grade for each adverse experience by cycle and for each patient by worst grade recorded. The proportion of patients with each adverse experience will be reported, along with the proportion of patients who achieve a response. Responses will be defined as in the IWG (International Working Group) response criteria in myelodysplasia as: complete remission (CR), partial remission (PR), or hematologic improvement (HI) after completion of cycle 4 [17]. Overall response rate (PR+ CR+ HI) will be provided for each dose level and across dose levels for the Phase 1 portion of the trial (with exact 95% confidence intervals). The operating characteristics and the estimated probabilities of dose escalation are provided in Table 1. Table 1 lists the probability of escalating to the next dose, based on the true, but unknown DLT rate as the true DLT rate increases.

Table 1: Probability of Dose Escalation in Phase 1

True Toxicity DLT Rate	Probability of escalating to next
0.1	0.91
0.2	0.71
0.3	0.49
0.4	0.31

Initial Safety Evaluation
Adverse Events: In the phase I study, 3 patients per cohort will be treated unless a subject experiences a DLT, probably, possibly, or definitely related to treatment. DLT is defined as grade 3 or higher of any duration or as a Grade ≥2 adverse event (AE) that persists for ≥96 hours with the exception of Grade ≥ 2 AEs clearly related to the underlying MDS. Based on prior experience with Vitamin C, however, we do not anticipate any DLTs. In the unlikely event that ≥ 1 patients develop grade 2 or more toxicity the cohort will be expanded to enroll a total of 6 subjects at its current dose escalation cohort. If ≥ 2 patients experience DLT at that expanded cohort then MTD would be achieved. Upon completion of the dose-escalation phase, if no DLT is identified as expected, then the RP2D will be set at Vitamin C 100 grams CIVI over 5 days every 28 days, and that dose will be used for the Phase 2 trial. Given the established safety record of high-dose Vitamin C, it is unlikely that we will observe Grade 3 or greater toxicity. If, however, such an event where to occur, enrollment will be halted, and the study team will review all available safety data to determine if further accrual into that cohort is acceptable. After 3 patients in a cohort have been treated for at least 1 cycle of 28 days (the DLT period), we will perform a safety evaluation of continuous infusion high-dose Vitamin C. If two (2) or more grade II or greater toxicities (probably, possibly, or definitely related to treatment) that persist are observed, the dose will be considered too toxic, and we will select the preceding dose for the phase 2 trial. If only 3 patients were treated at the lower dose, we would expand to 6 patients at that dose. After 3 patients have completed at least one cycle at the lowest dose safely and without any toxicity, we will proceed with the dose-expansion cohort. Vitamin C at concentrations of up to 100 grams, delivered by IV infusion,

has been used in cancer patients, and either no or rare, very mild toxicities been reported. Reported toxicities were mainly central nervous system (CNS)-related, and consisted only of dizziness or headaches. Other non-CNS toxicities were mild (grades 1-2), and include thirst, nausea, vomiting, and mildly increased urinary frequency. There have been case reports of patients developing renal calculi due to high Vitamin C infusion, but most of these individuals had other risk factors for this disorder. Accordingly, any patient with history of any renal calculi or hyperoxaluria or any other preexisting renal disorder will be excluded from enrollment.

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I. Background:

A. Significance: Myelodysplastic syndromes (MDS) comprise a heterogeneous group of clonal stem cell disorders with an intrinsic tendency for transformation into acute myeloid leukemia (AML). The 2008 World Health Organization (WHO) classification system for hematologic neoplasms defines MDS as one of five major categories of malignant myeloid hematopoietic disorders [18], and includes refractory cytopenias with unilineage or multi-lineage dysplasia and refractory anemia with ring sideroblasts or excess blasts. Consequently, MDS is diagnosed based on peripheral blood cytopenia, peripheral blood and bone marrow (BM) dysplasia/blasts, and clonal cytogenetic abnormalities. With an incidence of 5 cases/ 100,000 individuals in the United States, there are ~13,000 new cases of MDS per year [19]. The incidence of MDS increases significantly in individuals above age 60, with 30-50 cases/100,000 per year in this population. In addition to age, risk factors include a history of exposure to chemotherapy or radiation and, to a lesser extent, tobacco use or occupational exposure to solvents or agricultural chemicals [20]. Up to 30% of high-risk MDS transforms into AML. Cytogenetic abnormalities are found at diagnosis in 20-70% of MDS patients, with the highest frequency occurring in patients with the refractory anemia with excessive blasts (RAEB) variant [21]. Survival of MDS patients depends on their R-IPSS (revised international prognostic scoring system) risk group classification. Low-risk patients have a median survival of 5.7 years, while those with intermediate risk have a median survival ranging from 1.2-3.5 years for high intermediate or low intermediate risk, respectively [22]. For patients in the high-risk group, median survival is only 5 months. *Clearly, new treatments are needed to improve survival for MDS patients, especially those at intermediate and high risk. This need is made even more urgent by the strong association of MDS with age, as disease burden will likely increase substantially in the next few decades owing to our aging population.*

MDS is part of the large spectrum of myeloid malignancies, which include AML and myeloproliferative neoplasms (MPNs) [23]. In the past, it was felt that these diseases were distinct biological entities, but we now know that they share significant genetic overlap. Over 50 recurrently mutated genes are involved in MDS pathogenesis, including those that encode proteins involved in pre-mRNA splicing, epigenetic regulation, and transcription. More recently, exome sequencing of peripheral blood samples from over 30,000 patients without known hematologic malignancies demonstrated recurrent somatic myeloid malignancy-associated mutations in up to 10% of patients over the age of 65 and in more than 20% of patients over the age of 90 [24, 25]. This phenomenon is now termed clonal hematopoiesis of indeterminate potential (CHIP) [26]. The most common recurrently mutated genes in MDS patients are DNA methyltransferase 3A (*DNMT3A*), *TET2*, additional sex combs-like 1 (*ASXL1*), *TP53* (which encodes p53), Janus kinase 2 (*JAK2*) and *SF3B1* [27]. Most of the initial mutations in MDS lead to increased HSC self-renewal, clonal expansion, and development of CHIP. As the mutant clone expands, it develops additional genetic or epigenetic defects that promote progression to frank hematological malignancy. These secondary, sub-clonal events tend to cause dysplasia, leading to MDS and eventually, secondary AML. Therefore, there are considerable similarities between MDS and AML.

B.TET2 and MDS: Ten-eleven translocation-2 (*TET2*) belongs to a small sub-family (comprising *TET1-3*) of α-ketoglutarate- and Fe2+-dependent dioxygenases (αKGDDs). The

60-80 αKGDDs (many only inferred by sequence similarity) in mammals catalyze a wide variety of hydroxylation and oxidative demethylation reactions by using αKG and O_2 as substrates and $Fe^{+2}/Fe^{+3}/Fe^{+4}$ shuttling for catalysis. TET proteins catalyze the sequential oxidation of 5-methyl cytosines (5mC) in DNA [28, 29] to 5-hydroxymethyl cytosine (5hmC), 5-formylcytosine (5-fC) and 5-carboxylcytosine (5caC), respectively. These modified nucleotides are key intermediates in DNA demethylation, which can occur actively, via base-excision repair (BER), or passively, following replication [30]. Either way, TET deficiency results in impaired 5mC oxidation and DNA hypermethylation [31]. Alterations in multiple genes that share the property of regulating DNA methylation [32] are causally associated with MDS pathogenesis. Accordingly, hematopoietic cells from MDS patients have globally altered methylation profiles [33], and DNA demethylating agents (azacitidine and decitabine) are the current mainstay of MDS therapy. Loss-of-function mutations in *TET2* are among the most frequent, occurring in ~ 30% of MDS patients [34]. Similar *TET2* mutations are found in other myeloid neoplasms, including ~50% of chronic myelomonocytic leukemia (CMML) and ~10% of sporadic acute myeloid leukemia (AML). *TET2* mutations are also seen at a high allele frequency in CD34-positive hematopoietic stem cells of patients with other myeloid malignancies [31, 35], as well as other hematopoietic neoplasms [33], suggesting that such mutations are early drivers of transformation in cells with multi-lineage potential. Frameshift, nonsense, or missense alternations in *TET2* are reported in MDS patients [31] and have the same effect on methylation [36]. As noted above, *TET2* mutations are also seen in the white blood cells (WBCs) of otherwise healthy adults with CHIP [37]. Hence, *TET2* mutations are associated with pre-leukemic lesions that can enable disease progression by altering the epigenetic landscape and/or by promotion of the acquisition of additional oncogenic lesions in aberrantly self-renewing stem cells. Therefore, restoring TET2 function could provide therapeutic benefit to patients with CHIP or MDS.

C.<u>Vitamin C and TETs:</u> Vitamin C (ascorbic acid/ascorbate) is an essential co-factor for TET family demethylases, as well as for most αKGDDs [38]. It interacts directly with the C-terminal catalytic domain (CD domain) of TET proteins [39], acting to reduce Fe^{+4} generated during the reaction cycle back to Fe^{+2}. Intriguingly, previous studies suggested that high-dose Vitamin C, acting via TET family members and possibly *Jumonji*-type histone demethylases, could dramatically enhance the function of embryonic stem cells (ESC) [40] and the generation of induced pluripotent cells (iPSCs). The use of Vitamin C in cancer has a sullied history, dating to claims by Cameron and Pauling, based largely on single arm studies or post-hoc analyses, that high dose Vitamin C has global anti-neoplastic and immune-stimulatory effects [41, 42]. Subsequently, controlled clinical trials of high dose *oral* Vitamin C showed no anti-cancer efficacy [5, 6]. *However, oral administration of Vitamin C at up to 10 grams/day does not produce the millimolar blood levels seen after parenteral administration, and recently, there has been renewed attention to the possible anti-neoplastic effects of high dose, intravenous (IV) Vitamin C [9, 43].*

<u>D. Summary and Potential Impact:</u> Finding a treatment with high efficacy and a lower toxicity profile than hypomethylating agents is essential for improving the treatment of MDS patients. Recently, our group found that genetic restoration of *Tet2* is sufficient to block aberrant self-renewal of pre-leukemic stem/progenitor cells and reverse *Tet2*-deficient disease in mouse models. We also found that, by enhancing the activity of residual TET2 and TET3, high dose Vitamin C can act as a “pharmacologic mimic” of *Tet2* restoration, reversing the cellular and molecular features of TET2 deficiency in mouse and human models [1]. Our proposed clinical trial of continuous infusion Vitamin C could lead to a new therapeutic strategy for MDS patients with *TET2* mutations, who represent ~30% of MDS patients overall. Our pre-clinical data also suggest salutary effects of Vitamin C on CMML and AML, and potential synergy with PARP inhibitors. Hence, our approach could have even broader impact in the future.

II. Vitamin C and its Utility as a Hypomethylating Agent:

Vitamin C (Ascorbic Acid/Ascorbate) first was used in the treatment of scurvy. Most animals have an ability to synthesize ascorbic acid (AA); however, humans depend upon dietary AA intake due to an inherited mutation in the gene that encodes L-gulonolactone oxidase (*GULO*), a key enzyme in AA biosynthesis [8]. DNA demethylation can be initiated by the oxidation of 5- methylcytosine (5mC) and the formation of 5-hydroxymethylcytosine (5hmC), which are catalyzed by the enzymatic activity of the TET1-3 family of dioxygenases. 5hmC is oxidized by TET proteins to form 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) [9]. AA is a cofactor of TET mediated oxidation of 5mC and accordingly has the capacity to regulate DNA methylation processes at the cellular level.

Studies demonstrate that AA directly interacts with the C-terminal catalytic domain (CD domain) of TET proteins [9]. AA significantly increases the level of all 5mC oxidation products (5fC and 5caC) leading to global loss of 5mC. The catalytic activity of TET dioxygenases for 5mC oxidation requires 2 cofactors: Iron (Fe^{2+}) and 2-oxoglutarate. AA enhances hydroxylase activity by reducing the inactive Iron (Fe^{3+}) to Fe^{2+} required for the activity for dioxygenases including prolyl- 4-hydroxylase. This strongly supports that Fe^{2+} and 2-oxoglutarate acts as a co-factor and co- substrate respectively to initiate the conversion of 5mC to 5hmC [10]. The epigenetic marker 5mC is largely erased by inhibiting or eliminating the activity of DNA methylation [11], a critical step for multiple biological processes like animal cloning, nuclear re-programing, development and highly locus-specific regulation of gene activities. AA also has been identified as the KSR component responsible for Dazl induction that occurs with the DNA methyltransferase (Dnmt) inhibitor 5-Azacytidine, suggesting that AA promotes DNA demethylation [12]. Finally, *TET1* CD domain significantly represses the expression of Cdh1 and Epcam only in the presence of AA suggesting that MET (mesenchymal-to-epithelial) might be a crucial step in controlling TET activity in the presence of AA [13]. Also as part of our preclinical laboratory work it was already shown in 2 different *TET2* knock in mutated mice with myeloid diseases that were created to be more sensitive to vitamin C treatment than the wild type (WT) mice. Accordingly AA can be utilized as a possible treatment for MDS patients with *TET2* mutations instead of the current available hypomethylating agents and the biggest advantage of AA would be a much better toxicity profile.

Most MDS patients will have been treated with hypomethylating agents (HMAs), the current standard of care, prior to trial entry. We will enroll patients on our trial if they have previously received HMAs, as long as the last dose was given at least 4 weeks before enrollment. No patient will be allowed to receive HMAs while on this study. In the open-label Phase 2 portion of the trial, two co-primary endpoints will be evaluated: the pharmacodynamic endpoint of demethylation and overall response rate. HMAs inhibit DNMTs to promote passive DNA demethylation and should not affect demethylation promoted by Vitamin C through its active conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) by TET enzymes. Furthermore, earlier studies have shown that patients who fail one hypomethylating agent can still respond to other hypomethylating agents as second line therapy. For example, in one study patients who failed or had loss of response to azacitidine had a 28% overall response rate to decitabine in the second line, and each HMA had different effects on global methylation [44]. Accordingly, we expect to achieve similar or even greater response frequencies in our MDS patient population, given the difference in expected demethylation mechanism between HMAs and Vitamin C. The design of our study to give Vitamin C for 5 consecutive days every 28-days is similar to how HMAs are given (decitabine is used for 5

consecutive days and azacitidine for 7 consecutive days in every 28-day cycle). As effects on DNA methylation, and presumably, their biological consequences, can last for weeks, we will start with Vitamin C CIVI for 5 days to achieve the highest demethylation effect and then maintain patients on Vitamin C as 1 gram daily afterwards to prolong the demethylation effect between cycles. Oral Vitamin C (1 gram daily) will start after the completion of Vitamin C 5 days CIVI and continue until the beginning of the next cycle (from day 6 until day 28 of the 28-day cycle).

III. Preclinical Data:

Hematopoietic stem/progenitors from mice with TET2 deficiency exhibit increased self-renewal capacity, as manifested by enhanced serial re-plating (*in vitro*) and a substantial advantage and myeloid bias in competitive bone marrow transplantation (BMT) assays (Figure 1) [1].

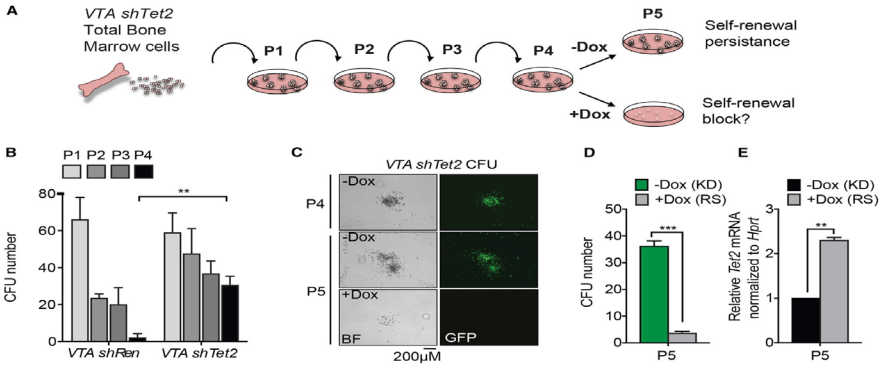


Figure 1: Tet2 Restoration Blocks Aberrant Hematopoietic Stem Cell Self-Renewal in Vitro and In Vivo
(A) Schematic of colony-forming assays performed with Tet2 knockdown cells.
(B) Total number of colony-forming units (CFU) generated by VTA shRen control or VTA shTet2 knockdown cells.
(C) Representative appearance of colonies generated after 4 passages (P4) with Tet2 knockdown re-plated ± Dox from P4–P5.
(D) Number of CFUs from passage 5 (P5) Tet2 knockdown (KD) and Tet2-restored (RS) cells.
(E) Tet2 mRNA levels normalized to Hprt in RS cells relative to KD from (D). Adapted from Cimmino *et al*. [1]

Several groups have developed mouse models of TET2 deficiency, either by generating *Tet2* knockout mice [45] or by inducibly expressing *Tet2* shRNA [46]. *Tet2* reversible knockdown mice were generated by our group; these mice express a doxycycline (Dox)-regulated, *Tet2*-specific miR30-based shRNA (*shTet2*) with linked green fluorescence protein (GFP) from the *Col1a1* locus (Figure 2) [1].

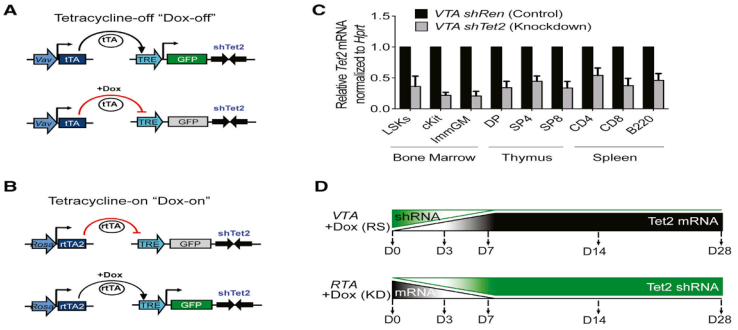


Figure 2: Generation of Inducible and Reversible Tet2 Knockdown Mice
(A) Schematic representation of *Vav-tTA*-driven *(VTA)* reversible *Tet2* knockdown mice.
(B) Schematic representation of *Rosa-rtTA*-driven (*RTA*) inducible *Tet2* knockdown mice.
(C) *Tet2* mRNA levels (normalized to *Hprt*) in bone marrow, thymus, and spleen cells from *VTA shTet2*mice, compared with cognate cells from *VTA shRen* control mice.
(D) Schematic representation of *Tet2* restoration (RS) versus knockdown (KD) in mice treated with doxycycline (Dox) food for 28 days (D0–D28). Adapted from Cimmino *et al*. [1]

Tet2-knockdown mice manifest progressive defects in hematopoiesis, characterized by myeloid lineage expansion, and a substantial fraction develop CMML-like disease, characterized by increased circulating WBCs, mainly monocytes and splenomegaly (Figure 3) [1].

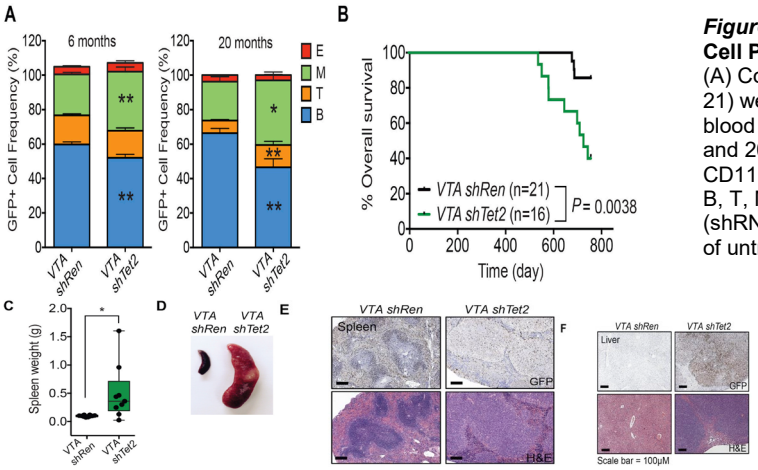


***Figure 3: Tet2*-Knockdown Mice Show Aberrant Stem Cell Phenotypes and Develop Myelo-erythroid Neoplasms**
(A) Cohorts of *Vav-tTA* (*VTA*) *shRen* (n = 16) and *VTA shTet2* mice (n = 21) were aged for 2 years in the absence of Dox treatment. Peripheral blood cell (PBC) lineages were monitored by flow cytometry at 6 months and 20 months. Cells were stained for B220 (B cells), CD3 (T cells) CD11b and Gr1 (Myeloid cells) and CD71 and Ter119 (erythroid cells). B, T, M, and E cell frequencies, respectively, were measured in GFP+ (shRNA)-expressing cells (70%–90% of PBCs). (B) Kaplan-Meier curve of untreated *VTA shRen* and *VTA shTet2* mice.

(C and D) Spleen weights (C) and shape (D) of sick *VTA shTet2* mice compared with age-matched control *VTA shRen* mice. (E and F) Histological analysis showing GFP/hematoxylin staining (upper panels) and H&E staining (lower panels) of spleen (E) and liver (F) sections from age-matched control *VTA shRen* and sick *VTA shTet2* mice. Adapted from Cimmino *et al.*[1]

Remarkably, by toggling *Tet2* expression on and off using our reversible shRNA system, we found that *Tet2*-deficient phenotypes (including CMML-like disease and even *Tet2*-mutant AML) and its biochemical effects (altered methylation) could be reversed by restoring *Tet2* expression (Figure 4) [1]. For example, as shown in Figures 4A-G, TET2 restoration promotes DNA demethylation, differentiation and the death of cKit+ cells, respectively [1].

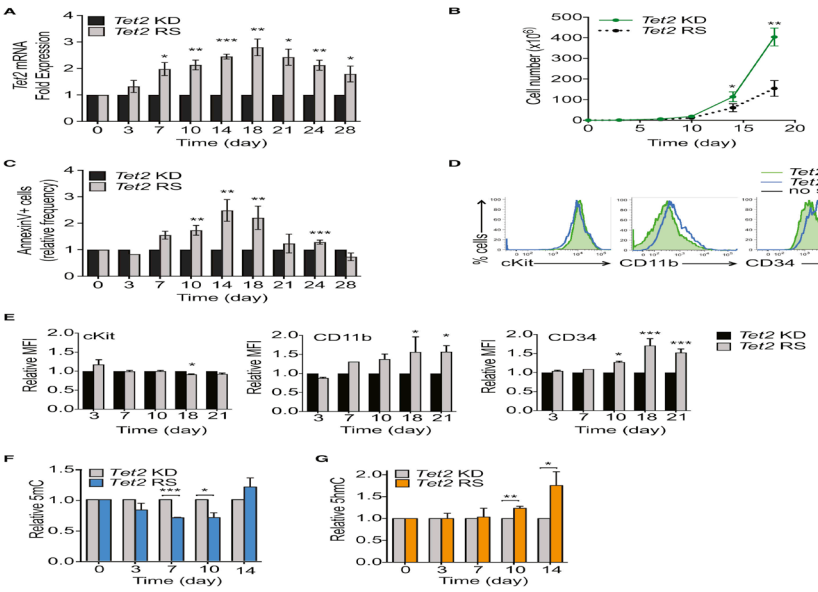


Figure 4: Tet2 Restoration Promotes DNA Demethylation, Differentiation, and Cell Death.
(A)Tet2 mRNA levels (normalized to *Hprt*).
(B) Proliferation.
(C) Apoptosis as assessed by Annexin V staining of *Tet2* RS versus sustained *Tet2* KD cells.
(D) Representative flow cytometry histograms of stem and progenitor (cKit and CD34) or differentiation (CD11b) marker expression upon *Tet2* KD or *Tet2* RS after 21 days in culture.
(E) Quantification of GFP by relative mean fluorescence intensity (MFI)
(F and G) Global changes in methylation were measured in genomic DNA isolated from cKit$^+$ cells during inducible *Tet2* RS, and compared to cells with sustained *Tet2* KD. Quantification was performed using ELISAs for 5mC (F) and 5hmC (G).
Adapted from Cimmino *et al.*[1]

These studies confirm the causal role of *TET2* mutations in myeloid neoplasia and, more importantly, show that the epigenetic changes evoked by, and pathogenic consequences of, decreased TET2 levels are potentially reversible. Consistent with this notion, although only a minority of MDS patients respond to hypomethylating agents (azacitidine and decitabine), *TET2*-mutant patients (especially those lacking additional *ASXL1* mutations) respond better to hypomethylating agents [47]. Unfortunately, even in this sub-group, the effects are not durable, and ultimately, disease progression ensues. Nearly all *TET2* mutations in patients with MDS or other hematologic neoplasms are heterozygous, leaving one normal copy of *TET2*, as well as a full complement of the other *TET2* family members, *TET1* and *TET3*. The pathogenic impact of *TET2* haploinsufficiency indicates that even small decreases in TET2 activity cause disease; conversely, the reversibility of myeloid neoplasia in *Tet2*-knockdown (*Tet2*-KD) mice argues that small increases in activity might be therapeutic. Capitalizing on the biochemical function and properties of TET2, our group found that high-dose Vitamin C could activate the remaining TET2 protein, as well as TET3 (Figure 5A-5D), block aberrant

hematopoietic self-renewal, and reverse the consequences of TET2 deficiency in mice (Figure 5E- 5G) [1].

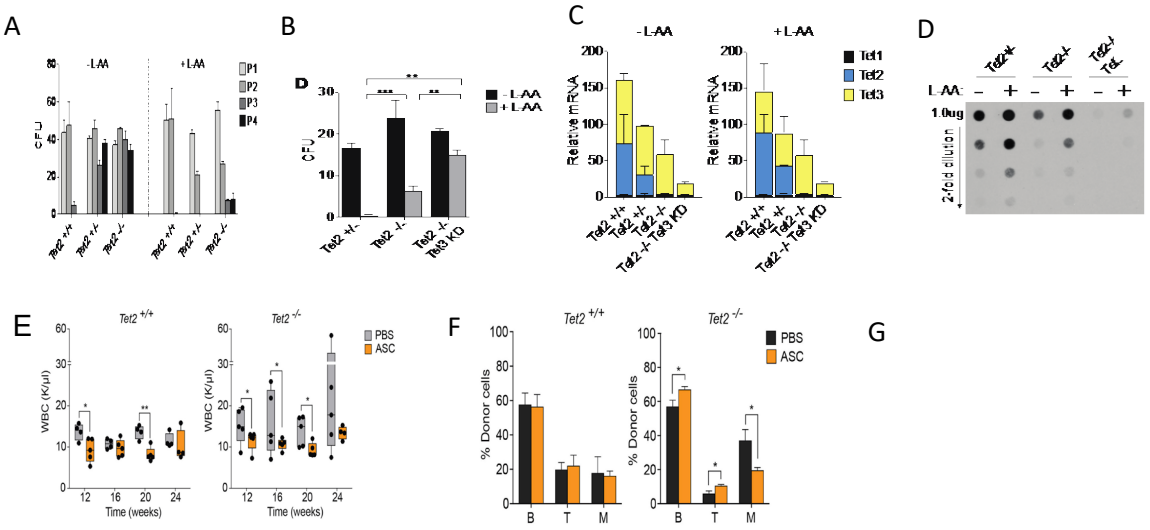
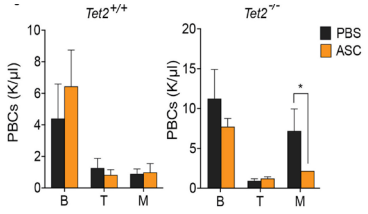


Figure 5: Vitamin C Treatment Mimics *Tet2* Restoration in HSCs and Blocks Myeloid Disease Progression.
(A) Colony-formation assays with Tet2+/+, Tet2+/_, and Tet2_/_ bone marrow cells treated with L-AA. Cells were re-plated for four passages (P1-P4).
(B) Tet2_/_ CFUs re-plated from passage 4 to 5 (P4–P5) and Tet2+/_, Tet2_/_, and Tet2/3 double-deficient colonies re-plated from P4 to P5.
(C) Relative Tet1, Tet2, and Tet3 mRNA levels in cKit+ cells, quantified by RT-PCR and normalized to Hprt
(D) DNA dot-blot for 5hmC in primary mouse cKit+ cells cultured for 6 days ± L-ascorbic acid (L-AA).
(E-G) Vitamin C treatment of mice reconstituted with *Tet2*$^{+/+}$ and *Tet2*$^{-/-}$ bone marrow. Mice were injected i.p. with PBS (control) or ascorbate (ASC, 4 g/kg), and WBC counts were monitored for 24 weeks post-transplant (PT) (E). Frequency (F) and number (G) of donor B and T lymphocytes and myeloid cells (M) in peripheral blood of recipients at 24 weeks PT treated with ASC or control (PBS). Adapted from Cimmino *et al*.[1]



Furthermore, although MDS patient-derived xenografts (PDXs) were not available, similar effects of high-dose Vitamin C were seen in human AML cultures (data not shown, but see Ref. [1]) and PDXs (Figure 6).

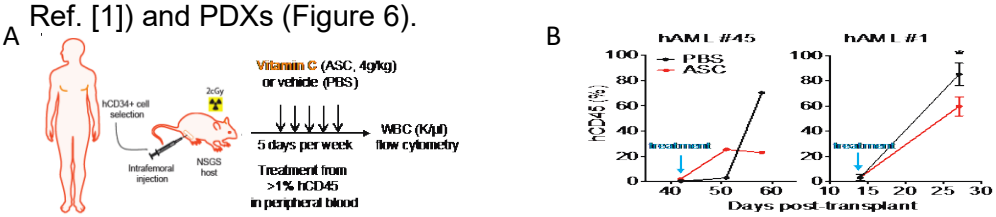


Figure 6: Vitamin C treatment of PDXs impairs human leukemia progression
(A) Schematic representation of primary human AML PDX generation and Vitamin C treatment.
(B) Disease progression in AML PDXs treated with Vitamin C (ASC) or vehicle (PBS) was monitored by flow cytometric analysis of hCD45+ cells in peripheral blood. Adapted from Cimmino *et al*.[1]

The ability of Vitamin C to block self-renewal and myeloid disease progression in mice motivated us to explore its effects on TET function and 5hmC generation in the HL60 and MOLM13 cell lines, representative of acute myeloblastic and acute monocytic leukemia, respectively. Consistent with our observations using mouse hematopoietic stem and progenitor cells (HSPCs), Vitamin C treatment for 72 hours (hr) caused increased 5hmC formation in both lines (Figure 7A) without altering *TET1-3* expression (Figure 7B). We performed 5hmC DNA immunoprecipitation (5hmeDIP) with genomic DNA from untreated HL60 cells (0 hr) and cells treated for 72 hr with Vitamin C. Nearly all differentially

hydroxymethylated peaks (29,492; 97%) exhibited gain of 5hmC upon Vitamin C treatment (Figure 7C). Increased 5hmC was enriched within gene bodies, compared with transcription start sites (TSS) or transcription end sites (TES) (Figure 7D) [1].

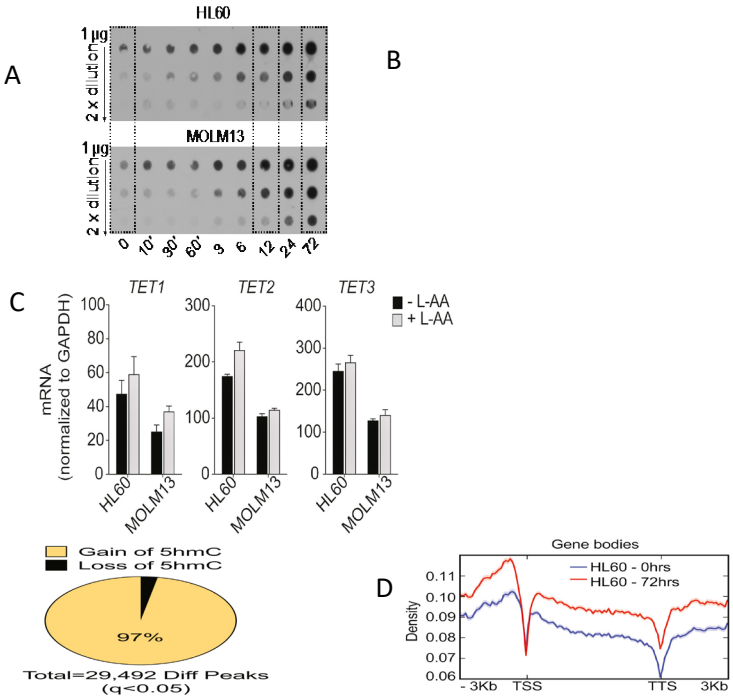


Figure 7: Vitamin C Treatment Increases TET Activity in Human AML and Drives DNA Hypomethylation.
(A) DNA dot blots for 5hmC in MOLM13 (acute monocytic leukemia cell line) and HL60 cells (acute myeloblastic leukemia cell line) treated for 72 hr with 250 mM L-ascorbic acid (L-AA).
(B) TET mRNA levels (assessed by qRT-PCR) in the human leukemia cell lines HL60 and MOLM13 treated for 3 days with L-AA (250 µM, 72 hr) or left untreated.
(C and D) Differentially hydroxymethylated peaks (Diff Peaks) with gain or loss of 5hmC (Diff Peaks, q < 0.05), assayed by 5hmeDIP-seq, in HL60 cells treated with 250 mM L-AA for 72 hr. Frequency and total number of significant Diff Peaks (C) and gene body distribution of the top 10,000 significant peaks following L-AA treatment (D), displayed as peak density ± 3 kb from the transcription start site (TSS) to the transcription

Our results comport with other published work. In a study published virtually simultaneously with ours, Vitamin C deficiency was found to phenocopy the effects of *Tet2* deficiency in promoting increased self-renewal and myeloid bias of HSPCs, as well as to cooperate with leukemogenic fusion proteins to generate AML in mice [48]. These data support the hypothesis that high-dose Vitamin C might have therapeutic efficacy in *TET2*-mutant human MDS. *Our exciting pre-clinical data provide the foundation for the clinical trial and correlative studies proposed herein. Restoring normal TET function in TET2-mutant MDS patients might be more specific than using global hypomethylating agents, and thereby have greater efficacy with less toxicity.*

IV. Objectives of the Study:

This is a phase Ib study to help us test the utility of Vitamin C as a treatment for patients with MDS. Many genes involved in pathways like Ras signaling, the p53 network and apoptosis are transcriptionally inactivated by the hypermethylation of specific CpG islands in cancer cells, making DNA methylation an effective therapy to target in MDS patients [14]. Multiple molecular subtypes of myeloid disease have been found to exhibit highly distinct DNA methylation profiles [15] and hypomethylating agents like decitabine and 5-Azacytidine have been used in the treatment of MDS patients, especially in elderly patients who are unable to tolerate intensive chemotherapy induction [16].

MDS patients with TET2 mutations will be enrolled in this study with the following objectives:

A. Primary objectives:

1. Evaluate the safety and toxicity of high dose Vitamin C
2. Estimate the proportion of MDS patients with TET2 mutations who exhibit a biological response defined as maintaining a vitamin C serum concentration of ≥1 mM over the treatment cycle within and across dose levels and study phases

B. Secondary objectives:

1. Estimate the clinical efficacy, namely objective response rate (ORR), [including complete response (CR) and partial response (PR)], duration of response (DOR) and progression-free survival (PFS) as defined in the IWG (International Working Group) response criteria in myelodysplasia within and across dose levels and study phases.
2. Estimate the Maximum measure Vitamin C plasma concentration (Cmax)
3. Evaluate the pharmacokinetic profile (PK) of Vitamin C as hypomethylating or demethylating agent

V: Dosing and Patient Selection:

Patients will receive up to 4 cycles of treatment, and responders will be allowed to continue treatment beyond the 4 cycles. MDS patients will be enrolled in the study if they have any of the following revised international prognostic scoring system (R-IPSS) risk scores: intermediate score (>3 and ≤ 4.5), high score (>4.5 and ≤6) or very high score (>6). MDS patients with very low score ≤1.5 or low score (>1.5 and ≤ 3) will be excluded, as they do not require treatment and have a better prognosis than patients with higher R-IPSS score. R-IPSS score will be calculated based on Table 2 specifics.

We propose a Phase 1 component with 3 dose-escalation cohorts in a 3+3 design to determine the maximum tolerated dose (MTD) of Vitamin C, followed by a Phase 2 dose-expansion cohort. Vitamin C will be provided from McGuff based on a confidential agreement with them and McGuff provides the only FDA-approved injectable preparation of Vitamin C in the USA. Such a trial using Vitamin C in MDS patients could lay the foundation for utilizing ascorbic acid as an anticancer agent and improve outcomes in this challenging group of patients.

Table 2: Revised International Prognostic Scoring System:

IPSS-R prognostic score values

Prognostic variable	**0**	**0.5**	**1**	**1.5**	**2**	**3**	**4**
Cytogenetics	Very good	—	Good	—	Intermediate	Poor	Very poor
BM blast, %	≤ 2	—	> 2%- < 5%	—	5%-10%	> 10%	—
Hemoglobin	≥ 10	—	8- < 10	< 8	—	—	—
Platelets	≥ 100	50-< 100	< 50	—	—	—	—
ANC	≥ 0.8	< 0.8	—	—	—	—	—

BM= bone marrow, ANC=absolute neutrophils count

VI. Safety Objectives:

Research Risks and Benefits

Risks associated to study participation include breach of confidentiality and risks associated with blood draw. Privacy procedures in place and good clinical practice guidelines are followed in the study to minimize risks associated with research procedures and participation. The risks associated with blood draw include weaknesses, redness, pain, bruising, bleeding or infection at the needle site. There may be no direct potential benefits to subjects participating in the sturdy, but the information obtained from this research may help others with this disease in the future. Intravenous vitamin C in high doses has been studied earlier with no major adverse events reported. However regardless we will be monitoring for any adverse event as follows.

1. Adverse Events

All adverse events (AEs) recorded during the study will be coded by MedDRA (Medical Dictionary for Regulatory Activities). AEs will be summarized by cohort, overall, primary system organ class, preferred terms and severity based on CTCAE version 5.0. Relationship to Vitamin C, AEs leading to discontinuation of study drug and SAEs will be summarized. No infusion-related AEs are expected; however if any do occur then they will be summarized by preferred term and severity. A list of AEs related to skin toxicity using pre-defined MedDRA preferred terms will be used to identify any AEs of special interest. All AEs will be listed.

2. Laboratory Abnormalities

Laboratory values will be converted into international system of units (SI) units, severity grades will be calculated using CTCAE version 4.0. For those parameters where there is no CTCAE grading then a defined classification into lower limit, normal and higher limit will be used. Shift tables for change from baseline will be presented as well as descriptive statistics mean, standard deviation, median, and range for absolute values by visit and change from baseline will be summarized. All lab values, abnormalities will be listed. The safety set will be used.

3. Vital Signs and Physical examination

Vital signs will be summarized by mean, standard deviation, median and range for absolute values by visit and change from baseline. The number of abnormal vital signs will be summarized by visit. All values will be listed. The safety set will be used.

Physical examination should include assessments of the following body parts/systems: heart, lungs, abdomen, extremities and a neurological exam.

4. Other Safety Parameters

All other safety parameters such as ECG will be listed for the safety set. Twelve-lead ECG measurements will be conducted in triplicate at protocol specified time point (see Table 3) keeping the leads in place and patient supine during recordings. Additional ECG measurements should be performed as clinically indicated. Electronic copies of the tracings will be submitted to the sponsor's designee for central assessment.

5. Interim Analysis

The NYUCI Data Safety Monitoring Committee (DSMC) will review the safety data

after 6 patients are enrolled and evaluated to assure the safety of the participating patients based on the rules described in Section VII.

A formal interim analysis is planned at the completion of the first stage of the Simon 2-stage design (see Statistical Considerations).

6. Clinical Laboratory Tests

The following laboratory assessments will be performed by local laboratories at the scheduled time points (See Table 3) during the course of the study:

- Weekly CBC with differential count to include the following tests: white blood cell count with five part differential (neutrophils, lymphocytes, monocytes, eosinophils, and basophils), platelet count, hemoglobin and hematocrit. Also, CBC to include peripheral myeloblast count (if present).
- Weekly chemistry panel to include the following tests: sodium, potassium, bicarbonate, chloride, glucose, magnesium, calcium, phosphorus, albumin, alkaline phosphatase, ALT, AST, blood urea, nitrogen, calcium creatinine, LDH, phosphorus, total bilirubin, and uric acid
- Weekly urinalysis with microscopy to assess for hematuria since vitamin C may cause oxalate nephropathy.
- Weekly serum anion gap as vitamin C can be associated with elevated anion gap acidosis. If anion gap is elevated (>11mEq/L) then we will hold the study drug for one week and recheck anion gap. If still elevated (>11mEq/L), we will withdraw the patient from the study, If not elevated (≤11mEq/L) we will resume vitamin C treatment.
- A serum or urine β-HCG pregnancy test for females of childbearing potential to be done within one week of starting the trial and before starting a new cycle.
- Creatinine clearance-the same method of assessment should be used at each time point.
- PT/PTT/INR

7. ECOG Performance Status

ECOG performance status will be evaluated at protocol specific time points (See Table 3)

VII. Subject Identification, Recruitment and Consent

A. Method of Subject Identification and Recruitment

Patients will be recruited from physicians at the NYU Langone's Ambulatory Care Center (ACC) in New York, USA, and at University of Miami, Health System. Consenting, screening, and treatment will take place at the NYU ACC or at University of Miami, Health System under the supervision of the PI or Co-Investigators. Prospective subjects will receive detailed information about this study: its investigational nature, required study procedures, alternative treatments, risks and potential benefits of the study. They will also receive the informed consent document to read. All questions are answered by the PI, Co investigators and qualified research personnel.

The patients who are eligible for this research study will come directly from the study investigators' clinical patient population. Thus, the investigators are very familiar with their patients' disease status and potential eligibility given the protocol's inclusion and exclusion

criteria. All efforts will be made to actively recruit and retain women and members of minority groups in this study. The inclusion and exclusion criteria in this study should not have a negative effect on the enrollment of these populations. Target enrollment for this study is up to 12 patients. The target accrual goal is 6 patients per year. Patients will be recruited from the outpatient clinics in the NYU ambulatory cancer center as well as University of Miami Cancer Center for any patients undergoing workup or treatment for Myelodysplastic Syndrome (MDS).

The investigator will approach eligible potential subjects and explain the study in a private room, including the reasons why subjects will be eligible, risks, benefits, and the regimes to be evaluated. It is NYULMC policy that Nurses and Fellows cannot obtain consent to greater than minimal risk trials, unless Fellows are listed as co-investigators. Consent will be obtained in a private room by the PI or a Co-investigator at the time of the subject's visit prior to any study assessments/procedures.

The subjects will be given a chance to ask questions to the person consenting him/her and will be able to take the consent home to discuss it with family/friends prior to signing. If the subject agrees s/he will sign the consent form either at the first contact (if the investigator/delegate is convinced that the subject understands) or at the time of a return visit after having had time to study the consent in more depth. Study procedures will not begin until after the consent form has been properly obtained. The subject is entitled to decide not to participate in the trial, without affecting their right to other medical care, and may discontinue participation in the trial at any time without penalty or loss of benefits to which they are entitled.

For non-English speaking patients, institutional translation services will be utilized. All procedures for consenting non-English speaking patients will be in accordance with NYULMC PCC CTO guidelines and policy.

For patients who cannot read a witness, not related to the research study will be present. The consent will be read to the patient. The patient will also be allowed to ask any questions s/he may have. The investigator will ask the patient questions to ensure s/he understands the study. If the investigator determines the subject understands the study, the patient will mark an X where his/her name would go and the witness will sign the consent form.

B. Documentation of Consent

The Principal Investigator or IRB approved Co-investigator will be responsible for documentation in the medical record that consent has been obtained from all participants. A signed copy of the consent form will be given to each participant. Original consent forms will be stored in the subject's medical chart.

C. Multi-Site Surveillance

As the lead investigator in a multi-site trial, the Overall Principal Investigator is responsible for organizing and conducting monthly teleconferences with all participating sites. The PI will also be responsible for including data from all of the participating sites within the overall trial's yearly Data and Safety Monitoring report to the DSMC to include minutes from monthly PI teleconferences. Each participating site will be responsible for submitting the results and recommendations from the DSMC's yearly review to their IRB of record at the time of continuing review.

D. Patient Informed Consent at Additional Sites

The Principal Investigator (PI) at each participating site will identify the physician members of the study team who will be obtaining consent and signing the consent form for therapeutic protocols.

The Investigator must ensure that each participant, or his/her legally acceptable representative, is fully informed about the nature and objectives of the study and possible risks associated with participation. All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and dated by the participant or the participant's legally authorized representative (if applicable), and by the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

All parties will ensure protection of participant personal data and will not include participant names on any sponsor forms, reports, publications, or in any other disclosures, except where required by laws. In case of data transfer, NYULMC Perlmutter Cancer Center (PCC) will maintain high standards of confidentiality and protection of participant personal data.

The informed consent form must be in compliance with ICH/GCP, local regulatory requirements, and legal requirements. The informed consent form used in this study, and any changes made during the course of the study, must be prospectively approved by both the IRB and NYULMC before use.

E. Method of Assigning Subject Identification

All potential study patients will be screened and eligibility will be determined prior to enrollment. Each patient must sign and date an informed consent form before undergoing any study specific procedure unless a procedure is being performed as part of the patient's standard of care. Once a patient has signed consent, the PI, Co-investigator will:

1. Obtain written informed consent and privacy authorization prior to initiating any protocol- required procedure that is not considered standard of care
2. Review eligibility criteria
3. Review medical chart for past medical/surgical history
4. Record medications and prior treatment regiments
6. Documentation of MDS
7. Assess and record ECOG Performance Status (Appendix 1)
8. Submit registration to NYULMC Perlmutter Cancer Center (PCC) Clinical Trials Office (CTO)
9. Receive registration confirmation from the Research Coordinator at NYULMC PCC CTO

F. Registration

Each patient must sign and date an informed consent form before undergoing any study specific procedure unless a procedure is being performed as part of the patient's standard of care. Enrollment in the study requires that all inclusion and exclusion criteria have been met. Enrolment occurs upon confirmation of registration from the NYULMC PCC Clinical Trials Office. The following materials must be submitted to the Research Coordinator for registration:

1. Complete signed and dated informed consent form
2. Complete signed and dated eligibility checklist
3. All supporting documentation verifying each criterion has been met
4. Planned date of bone marrow aspirate and biopsy if applicable

Registration will occur within 48 hours of research coordinator receipt of all of the above documents. A written confirmation of enrollment including a unique study identification number assigned by the research coordinator will be distinguished to the study team upon registration. Pretreatment evaluation will therefore be as directed by standard clinical practice. Eligible subjects will be entered on study by the study coordinator.

Once eligibility is verified, a unique patient study number will be issued within 24 hours of receiving all required registration material. The patient will not be identified by name. This is the point, at which, the patient is considered accrued on study.

Patient Registration at Other Participating Institutions

The Principal Investigator (PI) at each participating site will identify the physician members of the study team who will be obtaining consent and signing the consent form for therapeutic protocols. It is NYULMC policy that Nurses and Fellows cannot obtain consent to greater than minimal risk trials, unless Fellows are listed as co-investigators.

Enrollment at additional sites can occur once each site's IRB has approved this protocol, a copy of each site's IRB approval, Citi training certificates, Medical Licenses and signed CVs

are provided to NYULMC Perlmutter Cancer Center (PCC) Clinical Trials Office. Once, all required documents are provided to NYULMC PCC Clinical Trials Office an activation notification will be sent to the PI and research coordinator at that site.

Central registration for this study will take place at NYULMC PCC CTO.

Each patient must sign and date an informed consent form before undergoing any study procedures unless a procedure is being performed as part of the patient's standard of care. Once a patient has signed consent, each site must notify the NYULMC PCC Clinical Trial Office within 24 hours.

Enrollment in the study requires that all inclusion and exclusion criteria have been met. Enrollment occurs upon confirmation of registration from the NYULMC PCC Clinical Trials Office and forward a copy of the signed consent to NYULMC PCC Clinical Trials Office within 24 hours.

The following materials must be submitted to the Research Coordinator at NYULMC via email for registration:

1. Complete signed and dated informed consent form
2. Complete signed and dated informed consent checklist
3. Complete signed and dated eligibility checklist
4. All supporting documentation verifying each criterion has been met
5. Planned date of bone marrow aspirate and biopsy if applicable

Registration will occur within 48 hours of research coordinators receipt of all of the above documents. Once eligibility is verified, a unique subject study number will be issued within 24 hours of receiving all required registration material. This number is unique to the participant and must be written on all data and correspondence for the participant. The NYULMC PCC Clinical Trials Office will return a signed eligibility confirmation worksheet with the subject's unique study number. The subject will not be identified by name; this is the point, at which, the patient is considered on the study.

Except in very unusual circumstances, each participating institution will order the study agent directly from the supplier. Each site is responsible for reporting all unexpected problems involving risks to participants or others to NYULMC PCC Clinical Trials Office and to their IRB as per site institutional policy.

-Costs to the Subject.
No additional cost beyond the required standard of care procedures will be incurred.

-Payment for Participation
Neither reimbursements nor payments will be given for the participants in this study

VIII. Data Analysis and Safety Monitoring

At the NYU PCC, all investigator-initiated protocols are subject to a standardized data and safety monitoring plan, which includes scientific peer review and IRB review for therapeutic protocols. This investigator-initiated study will be monitored by the Data and Safety Monitoring Committee (DSMC) of the New York University (NYU) Perlmutter Cancer Center (PCC). The DSMC operates based on the National Cancer Institute approved Charter. It is an existing and multidisciplinary committee (consisting of clinical investigators/oncologists, biostatisticians, nurses, and research administration staff knowledgeable of research methodology and design and in proper conduct of clinical trials) that is responsible for monitoring safety, conduct and compliance in accordance with protocol data monitoring plans for clinical trials conducted in the NYULMC Perlmutter Cancer Center that are not monitored by another institution or agency. The DSMC reports to the Director of the NYULMC PCC.

Per the NYU PCC Institutional Data Safety and Monitoring Plan, this phase Ib trial will be monitored by DSMC quarterly (from the date the first patient is enrolled), after the first 6 patients are enrolled and evaluated for safety, at times of pre-specified response assessment, and at the completion of the study prior to study closure. This review includes accrual data, subject demographics and adverse events. Accrual to the next dose within a cohort will be held until real-time review of the toxicity from the prior cohort has occurred to assure no defined DLTs have occurred prior to proceeding to the next level or expanding the current cohort. Principal Investigators are required to attend the review of their studies. Additional reviews can be scheduled based on SAE reports, investigator identified issues, external information, etc.

This study will be monitored according to the monitoring plan detailed below. The review of AEs and trial conduct for this trial occurs at several levels:

(1) Principal Investigator: Adverse events are evaluated monthly by the principal investigator in conjunction with the research nurses, data manager and research team.

(2) DSMC, quarterly

(3) Institutional Review Board (IRB): An annual report to the IRB is submitted by the trial PI for continuation of the protocol. It includes a summary of all AEs, total enrollment with

demographics, protocol violations, and current status of subjects as well as available research data.

(4) In addition, the quality assurance unit will monitor this trial extensively, including real-time review of all eCRFs to ensure completeness and compliance with the protocol, accuracy and consistency of the data; and adherence to ICH Good Clinical Practice guidelines. Also, a first subject audit will be conducted within four weeks of enrollment.

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable Institution compliance and quality assurance offices. The investigator will contact the PCC CTO immediately if contacted by a regulatory agency about an inspection at the center.

A. Safety monitoring and reporting

- Adverse Events

All adverse events (AEs) per the description below will be captured in the Adverse Event Form.

Unanticipated Problems Involving Risk to Subjects or Others
Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc.)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

Adverse Event
An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

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

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

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

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

Adverse Event Reporting Period
The study period during which adverse events must be reported is normally defined as the

period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

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

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

Post-study Adverse Event
All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

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

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

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

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

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the

study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

1. Recording of Adverse Events

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

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

2. Reporting of Serious Adverse Events and Unanticipated Problems

Investigators and the protocol sponsor must conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others (see definitions, Section 8.1).

Serious adverse event reporting will begin in conjunction with the date of informed consent. Any SAEs occurring prior to study drug administration that the investigator believes may have been caused by a protocol procedure must be reported immediately to the Sponsor or its designee and recorded on the case report form.

All fatal or life-threatening adverse events must be immediately reported to the Sponsor by telephone or e-mail. Within 24 hours of the event, the Serious Adverse Event (SAE) Form supplied by NYULMC must be faxed to the Sponsor, who must then inform the NYULMC IRB, PCC CTO, and DSMC within 24 hours of the event whether full information regarding the event is known or not. Additional follow-up by the investigator will be required if complete information is not known. De-identified source documentation of all examinations, diagnostic procedures, etc. which were completed with respect to the event should be included with the SAE form. Care should be taken to ensure that the patient's identity is protected and the patient's identifiers (as assigned at the time of study enrollment) are properly mentioned on any copy of source document provided to the Sponsor. For laboratory results, include the laboratory normal ranges.

Other Reportable events:

- **Deviations from the study protocol**
  Deviations from the protocol must receive both Sponsor and the investigator's IRB approval before they are initiated. Any protocol deviations initiated without Sponsor and the investigator's IRB approval that may affect the scientific soundness of the study, or In case of accidental or intentional overdose of study drug, even if asymptomatic or not fulfilling a seriousness criterion, the overdose is to be reported to the Sponsor immediately (within 1 working day) using the AE and SAE forms supplied by NYULMC. Overdose of study drug will be defined as at least 2 times the intended dose of study drug within the intended therapeutic window.

All serious adverse events (SAEs) will be evaluated by the DSMC If meeting the requirements for expedited reporting, the Sponsor will report the adverse event to all regulatory authorities with jurisdiction over ongoing trials with the study drug and to all other investigators involved in clinical trials with the study drug. The investigator is responsible for reporting all SAEs to the appropriate IRB, DSMC, and FDA. **For Narrative Reports of Safety Events**

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

In the event the investigator is informed of an SAE that occurs after 30 days after the last dose of study treatment, only those SAEs or other AEs of concern deemed by the investigator to be related to study treatment will be reported to the sponsor. The investigator should make every effort to obtain follow-up information on the outcome of a treatment-related SAE until the event is considered chronic and/or stable.

Additionally, an FDA Form 3500A (MEDWATCH Form) must be completed by the investigator and faxed to the study sponsor within 24 hours. The investigator shall maintain a copy of the MEDWATCH Form on file at the study site or can be obtained from the FDA website: http://www.fda.gov/safety/medwatch/howtoreport/downloadforms/default.htm

Contacts:

NYUPCCsafetyreports@nyulangone.org
AND
Mohammad Maher Abdul Hay, MD
New York University School of Medicine 240
East 38th Street, 19th Floor
New York, NY 10016
Email:
Maher.Abdulhay@nyulangone.org
Phone: 646-501-4818

- Anything that affect the rights, safety, or welfare of study subjects, must be reported to the Sponsor and to the investigator's IRB as soon as a possible, but ***no later than 5 working days*** of the protocol deviation.
- **Withdrawal of IRB approval**
  An investigator shall report to the sponsor a withdrawal of approval by the investigator's reviewing IRB as soon as a possible, but ***no later than 5 working days*** of the IRB notification of withdrawal of approval.

3. Investigator reporting: notifying the IRB

Federal regulations require timely reporting by investigators to their local IRB of unanticipated problems posing risks to subjects or others. The IRB requirements reflect the current guidance documents released by the Office of Human Research Protection (OHRP), and the Food and Drug Administration (FDA) and are respectively entitled "Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events" and "Guidance for Clinical Investigators, Sponsors, and IRBs: Adverse Event Reporting-Improving Human Subject Protection." The following describes the NYULH IRB reporting requirements, though Investigators at participating sites are responsible for meeting the specific requirements of their IRB of record.

The NYU IRB address is:

NYULH IRB
1 Park Avenue, 6th Floor
New York, NY 10016

Report promptly, but no later than 5 working days:
Researchers are required to submit reports of the following problems promptly but no later than 5 working days from the time the investigator becomes aware of the event:

- ***Unanticipated problems including adverse events* that are unexpected and related**
  - *Unexpected: An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.*
  - *Related to the research procedures: An event is related to the research procedures if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.*
  - *Harmful: either caused harm to subjects or others, or placed them at increased risk*

Other Reportable events:
The following events also require prompt reporting to the IRB, though ***no later than 5 working days***:

- ***Complaint of a research subject*** when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- ***Protocol deviations or violations*** (includes intentional and accidental/unintentional deviations from the IRB approved protocol) for any of the following situations:
  - *one or more participants were placed at increased risk of harm*
  - *the event has the potential to occur again*
  - *the deviation was necessary to protect a subject from immediate harm*

- ***Breach of confidentiality***
- ***Incarceration of a participant*** when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- ***New Information indicating a change to the risks or potential benefits*** of the research, in terms of severity or frequency. (e.g. analysis indicates lower-than-expected response rate or a more severe or frequent side effect; Other research finds arm of study has no therapeutic value; FDA labeling change or withdrawal from market)

Reporting Process
The reportable events noted above will be reported to the IRB using the form: "Reportable Event Form" or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

4. Sponsor reporting: Notifying the FDA

The study sponsor is required to report certain study events in an expedited fashion to the FDA. These written notifications of adverse events are referred to as IND safety reports. The following describes the safety reporting requirements by timeline for reporting and associated type of event:

- ***Within 7 calendar days*** *(via telephone or facsimile report)*
  Any study event that is:
  – associated with the use of the study drug
  – unexpected,
  – fatal or life-threatening

- ***Within 15 calendar days*** *(via written report)*
  Any study event that is:
  – associated with the use of the study drug,
  – unexpected, and
  – serious, but not fatal or life-threatening
  -or-
  – a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

  Any finding from tests in laboratory animals that:
  – suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Additional reporting requirements
Sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

Reporting Process
Adverse events may be submitted on FDA Form 3500A (MEDWATCH Form, obtained from the FDA website: http://www.fda.gov/safety/medwatch/howtoreport/downloadforms/default.htm), or in a narrative format. If supplied as in a narrative format, the minimum information to be supplied is

noted above at the beginning of section 8.3. The contact information for submitting IND safety reports is noted below:

Email:
NYUPCCsafetyreports@nyulangone.org
Tel: 212-263-4427

5. Sponsor reporting: Notifying participating investigators

It is the responsibility of the study sponsor to notify all participating investigators of any adverse event that meets the FDA 15-day reporting requirement criteria as note above. The same materials and timeline used to report to the FDA are used for notifying participating investigators.

B. Medical Monitoring

It is the responsibility of the Overall Principal Investigator to oversee the safety of the study at all participating sites. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan. The Data Safety and Monitoring Committee (DSMC) will review the study at least annually. Medical monitoring will include a regular assessment of the number and type of serious adverse events.

When necessary (due to logistical constraints), Sub-sites will be monitored remotely by a designated Quality Assurance Specialist. Sites will be informed of this remote monitoring process on a site by site basis. Sub-sites will be monitored by the Quality Assurance Specialist on both a regulatory level, as well as a clinical data/source documentation review level.

C. Data Collection and Storage.

- Data Labeling.

Once a subject is enrolled on this trial s/he will be assigned a unique study ID number. We will code the data collected from the patients with this unique study ID. Samples will be labeled with a unique ID number and disease type. The link between the patients and the numbers will only be kept with Dr. Abdul Hay, the Principal Investigator. The reason for such a link is to identify specific data and samples to discard if a patient decided to withdraw from the study. Numbers will be assigned randomly to maintain confidentiality.

- Data Storage and Access

This study will store data in an electronic database capture system that will be created to record the data for this trial Research coordinators will input clinical trial data into the database. This database is password protected and only the PI, assigned research coordinator, and CTO quality assurance specialists will have access to the database. The electronic database capture system is the primary data collection instrument for the study. All data required for this study must be inputted into the system. All missing data must be explained. The quality assurance specialists will monitor this trial every 4-6 weeks for data entry accuracy.

Information collected in the database includes patients' information, which will constitute a medical records number, date of birth, gender, ethnicity, complete blood count and percentage of blast.

The study team will maintain clinical and laboratory data in a designed manner to ensure subject confidentiality. All study personnel have passed human subject protection courses. If applicable, blood and tissue samples sent to collaborators outside of NYU will only be labeled with an identification number; no patient identifiers will be used. Systems used for electronic data capture are compliant with FDA regulations in 21 CFR Part 11 and applicable local regulatory agency guidelines. All documents are kept in strictly confidential password protected files and are only made accessible for review of sponsors, monitors and authorized representatives of regulatory agencies as described in the informed consent document.

Samples will be labeled with a unique subject ID. The PI and Co-Investigators are the only ones to have access to the samples and data. Only the PI will have the linking keys. It is the investigator's responsibility to retain study essential documents for at least 3 years after formal closure of the study. Also Tsivia Hochman from the statistics department will have access to the data collected to help with the analysis of the study and the outcome.

- Case Report Forms
All the data requested in the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write alit Assurance Specialist". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

-Records Retention
The CTOs Retention for records retention will be followed. It is the investigator was not asked, write alit Assurance Specialist".

-Subject Data Withdrawal Subjects can withdraw from the study at any time. Request for withdrawal should be in writing to the PI, Dr. Abdul Hay. Once a request is received, then the data collected from the subject will be completely discarded. Any unused specimens will be destroyed as well.

Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, government regulatory bodies, and Institution compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable Institution compliance and quality assurance offices. The investigator will contact the PCC CTO immediately if contacted by a regulatory agency about an inspection at the center.

Statistical Evaluation:

1. Statistical Methods

This is a 2 center study with a Phase ib component. This study will enroll up to 12 patients in Phase 1b and 34 patients in Phase IIa has been deferred for a later study. The patients enrolled in Phase Ib will be evaluated for safety. The proportion of patients with biological responses will be based on the data collected from the phase 1b. There will be demographic and baseline characteristics documented, biological activity measurements, safety observations, efficacy observations, PK and potential biomarker observations. The clinical database lock will occur after all the data are reconciled (I.E. "cleaned") for all patients. A single clinical study report will be generated for this study. The Statistical Analysis Plan (SAP) will be finalized and signed before the database lock.

2. Sample Size and Power

Phase 1: At each dose level, safety data will be summarized by grade for each adverse experience by cycle and for each patient by worst grade recorded. The proportion of patients with each adverse experience will be reported, along with the proportion of patients who achieve a response. Responses will be defined as in the IWG (International Working Group) response criteria in myelodysplasia as: complete remission (CR), partial remission (PR), or hematologic improvement (HI) after completion of cycle 4 [17]. Overall response rate (PR+ CR+ HI) will be provided for each dose level and across dose levels for the Phase 1 portion of the trial (with exact 95% confidence intervals).

Once our phase 1 study is completed to establish safety and confirm we are achieving steady level of Vitamin C on ≥1 mM in > 75% of our patients we will aim to proceed with a Phase 2 study. The phase 2 study will be a separate clinical trial. The co-primary endpoints of the Phase 2 study will be the pharmacokinetics endpoint, achievement of a steady level of Vitamin C of ≥1 mM in 75% of patients expected to be associated with demethylation, and the overall response (PR, CR or HI) by the completion of 4 cycles of treatment. With 18 patients planned in the first stage of the 2-stage Phase 2 portion of the study (12 from our phase 1 study), we can estimate this feasibility rate with an exact 95% Clopper Pearson confidence interval of 49.4% to 92.0% if the observed rate is 75%. With the combined 24 patients (6 from RP2D in Phase 1 and 18 from Phase 2), we can detect a difference from the null hypothesis of +/-25% in the percentage of patients achieving Vitamin C levels of ≥1 mM with alpha of 0.047 and power of greater than 80.4%. We expect to achieve higher pharmacodynamic effects on demethylation with higher Vitamin C doses. At the same time, the co-primary endpoint of response (CR+PR+HI) by 4 months will be evaluated in the Phase 2 Simon 2-stage optimal design to test the null hypothesis that the rate is ≤10%, versus the alternative that the rate is ≥30%, with alpha of 0.031 and power of 81.3%. The first stage will include the 18 patients enrolled into the Phase 2 cohort and evaluated for Vitamin C levels at the same 4-cycle time point. If the Vitamin C levels are adequate based on the assessment in these patients, and there are 4 or more responses in these first 18 patients combined from phase 1 and 2 study, the trial would proceed to the full accrual of 34 patients in the combined stages of the Simon design, and the Vitamin C regimen would be considered for further study if a total of 7 or more responses are observed in the total cohort. There will be no statistical adjustment for the simultaneous testing of the two co-primary endpoints. Because the expected response rate in this population is unknown, we define a response rate (CR + PR + HI) ≤10% as futile and ≥30% promising. Overall response rates (CR+PR+HI) will be summarized by dose cohort in the Phase 1 and Phase 2 portions of the study using exact 95% confidence intervals.

Patient and disease characteristics will be summarized descriptively for each dose cohort of the trial by using summary statistics and graphical displays for continuous variables and frequency distributions and contingency tables for qualitative variables. Times to best response will be measured from start of treatment. Duration of responses will be measured from initial response to relapse.

3. Study Population

The safety set consists of all patients that have received at least one dose of Vitamin C. If a patient drops out before completing at least one cycle of treatment that patient will be replaced with another patient to complete at least one cycle. Data from all patients entered will be included in the estimate of biological activity. Patients who are not evaluable are considered non responders in the primary analysis.

4. Demographics, Baseline Characteristics and Medical History

Demographics, baseline characteristics and medical history will be summarized for the first 6 safety patients and for the entire cohort. Similarly, summaries will be provided by completion status. For continuous variables, descriptive statistics will be presented including: mean, standard deviation, median and range. For categorical variables frequency distributions with percentages will be presented. All patients will have baseline measurements and at the end of the study we will compare patients that completed the study versus those that only got partial treatment and didn't complete the study.

5. Treatment, Compliance, Disposition and Protocol Deviations

As part of compliance we will summarize the actual number of weeks of vitamin C received per patient. The reason for discontinuation of a study treatment including study completion as per protocol will be presented. Patients with major protocol deviations as defined by the study team prior to database lock will be summarized. The safety set will be used. All data will be listed.

6. Ethical Considerations:

This study is to be conducted in accordance with applicable US government regulations and international standards of Good Clinical Practice, and applicable institutional research policies and procedures. This protocol and any amendments will be submitted to the NYU Institutional Review Board (IRB) in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable

surrogate, and the investigator-designated research professional obtaining the consent.

The consenting process and documentation will follow Standard Operating Procedures (Obtaining Informed Consent for Clinical Trials) of the NYULMC PCC CTO and University of Miami Cancer Center.

7. Publication Plan

The study PI holds the primary responsibility for publication of the results of the study.

8. Schedule of Assessments: (Table 3)

Table 3: Schedule of Assessments

Week	**Screening**	**1**	**1**	**1**	**1**	**1**	**2**	**3**	**4***	**EoS/PD**	**FU**
Day	-28 to 0	1	2	3	4	5	8	15	22	Last dose +1w	Last Dose +30 days
Vitamin C pump Administration [a]		x	x	x	x	x					
Informed consent	x										
Eligibility	x										
Demographics	x										
Medical/surgical history	x										
ECOG performance status	x	x					x	x		x	
Pregnancy test	x	x									
Bone marrow aspirate and biopsy[§]	x								See Below	x	
Physical examination [c]	x	x					x	x		x	
ECG (rest 12-lead) [d]	x										
Vital signs [e]	x	x					x	x		x	
Clinical chemistry	x	x					x	x		x	
Hematology	x	x					x	x		x	
G6PD levels [f]	x										
Hepatitis Profile	x										
Coagulation	x										
Urinalysis	x	x					x	x		x	
Vitamin C Serum levels [g,h]		x	x	x	x	x					
Correlative Studies[i]		x				x	x	x		x	
Circulating Myeloblasts	x	x					x	x		x	
Concomitant medication	x	x					x	x		x	
Adverse event		x					x	x		x	x

ECOG=Eastern Cooperative Oncology Group; ECG=electrocardiogram; EoS=end of study; PD= progression of disease. PK=pharmacokinetics; SD=stable disease; PR=partial response; CR=complete response

Additional assessments may be conducted as clinically indicated

Assessments made on drug administration days are to be conducted prior to the start of study medication infusion, unless specified otherwise

*Subjects with clinical benefit (SD, PR, CR) will receive a second cycle starting at week 5 (Maximum treatment: 4 cycles).

§ Restaging to be done after cycle 2 and cycle 4 or end of study

a. Vitamin C to be administered by a pump as CIVI/ 24 hours with the cassette for the CADD pump replaced every 24 hours.

b. A histological confirmation of MDS must be established before enrolling patients for treatment. However, if a previous bone marrow biopsy is available that was performed within 4 weeks before screening without any worsening in peripheral blood counts a repeat bone marrow aspirate and biopsy can be deferred before enrolling the patient in the study.

c. Physical examination will include recording the patient's weight and height at screening and weight at the end of study visit. Symptom-directed physical examinations at other time points are to be done as needed.

d. Further ECGs will be conducted as needed.

e. Vital signs collected will include temperature, systolic blood pressure, diastolic blood pressure, heart rate and respiratory rate.

f. If G6PD deficiency is detected the patient will be excluded from the trial.

g. Blood samples for full PK to be taken prior to pump exchange on weekly bases with the exception of week one of cycle 1.

h. Blood needs to be centrifuged and serum to be frozen.

i. Day 5 PBMC collected at NYULH site ONLY

Appendices

Appendix 1: ECOG Performance Status Scale

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

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Appendix 2. Summary of Revisions

Changes made from protocol version 1.1 (Dated March 28, 2018) to version 1.2 (Dated July 09th, 2018)

Section	Changes
Inclusion criteria	- Revised the structure of inclusion criterion #8 to clarify that inclusion criteria #9,10,11 are all subsets of inclusion criterion #8. The protocol should read as below: #8Patients are currently receiving or have previously received one of the following therapies: a. Hypomethylating agent, OR b. Hydroxyurea, OR c. Erythrocyte stimulating agents (ESA) OR granulocyte colony stimulating factors (G-CSF), OR d. Allogenic stem cell transplant
Test Product, Dose, and Mode of Administration	Added language specifying that a low ANC will not require a dose modification for cycle 2 and onward, and that subsequent treatment dosage will be at the discretion of the PI.
IX. Statistical evaluation **1.** Statistical Methods	- Revised the first sentence of this section to clarify that the trial includes 2 participating sites in addition to NYU the coordinating center.
General	- Administrative changes were made throughout the protocol

Changes made from protocol version 1.2 (Dated July 09th, 2018) to version 1.3 (Dated August 28, 2018)

Section	Changes
V. Schedule of Assessments: (Table 3)	**i. Correlative Studies: Day 5 PBMC collected at NYULH site ONLY**

Changes made from protocol version 1.3 (Dated August 28, 2018) to version 1.4 (Dated March 1, 2019)

Section	Changes
Title Page	**Sites** • **Removal of Princess Margaret Cancer Center, University Health Network** **Co-Investigators** • **Removal of Mark Minden, MD** • **Removal of Simon Kavanagh, MD**
Test Product, Dose and Mode of Administration	**Addition of: Samples collected at University of Miami kept and processed at Luisa Cimmino, PhD lab at University of Miami**
Intended Duration of Treatment:	**Clarification of Follow Up Schedule.** • **Subject followed for 30 days for adverse event monitoring.** • **A visit is to be performed at 30 days after the last study drug is given plus/ minus 3 days.** • **After finishing last does of treatment, each subject will be followed for 6 months to continue to assess their response.** • **Subjects to be contacted once a month by telephone to assess survival status** • **CBC records if available reviewed every 6 months after completing the study**

Schedule of Assessments	• **Criteria for removal of study added** **Addition of Follow Up (Last Dose +30days)**

Changes made from protocol version 1.4 (Dated March 1, 2019) to version 2.0 dtd 2020 Oct 01

Protocol Summary/ Synopsis	• Increasing target accrual from 18 to 30 patients. • Extending planned study period from Q3 2019 to Q4 2022. • Updated inclusion and exclusion criteria
Study Design, Product, Dose modification, Mode of Administration and Sample Storage	• Scientific rational added to support the study's hypothesis • Changing the study from 1 cohort to a 3 cohort dose-escalation study to determine the maximum tolerated dose of Vitamin C
Assessments	• Updated safety assessments to include 30 subjects • Updating correlative studies based on recent research findings
Statistical Methods Initial Safety Evaluation	• Sections updated to reflect the new dose-escalation cohorts
I. Background	• Updating language regarding the scientific rational for the study
II. Vitamin C and its Utility as a Hypomethylating Agent	• Information added regarding prior HMA therapy and trial eligibility
III. Preclinical Data	• Section added to support scientific rationale for the study
V. Dosing and Patient Selection	• Section updated to include language regarding the new dose-escalation cohorts
Safety Monitoring and Reporting	• Language was rearranged in this section for clarity.
VIII. Statistical Evaluation	• Updated to include the new target accrual of 30 subjects along with the new dose-escalation cohorts
References	• Updated to reflect correct references within the protocol

Changes made from protocol version 2.0 (Dated October 1, 2020) to version 2.1 (dated March 17, 2021)

Protocol Summary/ Synopsis	• Clarification added regarding number of proposed patients to enroll
Study Design, Product, Dose modification, Mode of Administration and Sample Storage	• Clarification added regarding PK evaluation • Sentence added to state that the first 3 patients enrolled in the study prior to its redesign tolerated treatment well and shall count as the first cohort.
Assessments	• Clarification added to include all dose levels and all study phases
Statistical Methods Initial Safety Evaluation	• Removal of "The Simon stage 2 patients would be enrolled with separate funding from the PCC." • Updated definition of DLT to include any event grade 3 or higher
IV. Objectives of the study	• Clarification added to include all dose levels and all study phases
VI. Safety Objectives	• Added "A formal interim analysis is planned at the completion of the first stage of the Simon 2-stage design"
VII. Subject Identification, Recruitment and Consent	• Clarified target enrollment and accrual numbers.
VIII. Data Analysis and Safety Monitoring	• Clarification added to state that this is a 2 center with phase Ib and Phase II components.

References

1. Cimmino, L., et al., *Restoration of TET2 Function Blocks Aberrant Self-Renewal and Leukemia Progression.* Cell, 2017. **170**(6): p. 1079-1095 e20.
2. Yin, R., et al., *Ascorbic acid enhances Tet-mediated 5-methylcytosine oxidation and promotes DNA demethylation in mammals.* J Am Chem Soc, 2013. **135**(28): p. 10396-403.
3. Cimmino, L., B.G. Neel, and I. Aifantis, *Vitamin C in Stem Cell Reprogramming and Cancer.* Trends Cell Biol, 2018.
4. Cameron, E. and A. Campbell, *The orthomolecular treatment of cancer. II. Clinical trial of*

high-dose ascorbic acid supplements in advanced human cancer. Chem Biol Interact, 1974. **9**(4): p. 285-315.
5. Creagan, E.T., et al., *Failure of high-dose vitamin C (ascorbic acid) therapy to benefit patients with advanced cancer. A controlled trial.* N Engl J Med, 1979. **301**(13): p. 687-90.
6. Moertel, C.G., et al., *High-dose vitamin C versus placebo in the treatment of patients with advanced cancer who have had no prior chemotherapy. A randomized double-blind comparison.* N Engl J Med, 1985. **312**(3): p. 137-41.
7. Aldoss, I., et al., *Adding ascorbic acid to arsenic trioxide produces limited benefit in patients with acute myeloid leukemia excluding acute promyelocytic leukemia.* Ann Hematol, 2014. **93**(11): p. 1839-43.
8. Welch, J.S., et al., *Combination decitabine, arsenic trioxide, and ascorbic acid for the treatment of myelodysplastic syndrome and acute myeloid leukemia: a phase I study.* Am J Hematol, 2011. **86**(9): p. 796-800.
9. Padayatty, S.J., et al., *Vitamin C pharmacokinetics: implications for oral and intravenous use.* Ann Intern Med, 2004. **140**(7): p. 533-7.
10. Monti, D.A., 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): p. e29794.
11. Duconge, J., et al., *Pharmacokinetics of vitamin C: insights into the oral and intravenous administration of ascorbate.* P R Health Sci J, 2008. **27**(1): p. 7-19.
12. Wang, F., et al., *Phase I study of high-dose ascorbic acid with mFOLFOX6 or FOLFIRI in patients with metastatic colorectal cancer or gastric cancer.* BMC Cancer, 2019. **19**(1): p. 460.
13. Padayatty, S.J., et al., *Intravenously administered vitamin C as cancer therapy: three cases.* CMAJ, 2006. **174**(7): p. 937-42.
14. Szultka, M., et al., *Determination of ascorbic acid and its degradation products by high-performance liquid chromatography-triple quadrupole mass spectrometry.* Electrophoresis, 2014. **35**(4): p. 585-92.
15. Gao, Y.L., et al., *The Parenteral Vitamin C Improves Sepsis and Sepsis-Induced Multiple Organ Dysfunction Syndrome via Preventing Cellular Immunosuppression.* Mediators Inflamm, 2017. **2017**: p. 4024672.
16. Carr, A.C. and S. Maggini, *Vitamin C and Immune Function.* Nutrients, 2017. **9**(11).
17. Cheson, B.D., et al., *Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia.* Blood, 2006. **108**(2): p. 419-25.
18. Vardiman, J.W., et al., *The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes.* Blood, 2009. **114**(5): p. 937-51.
19. Ma, X., *Epidemiology of myelodysplastic syndromes.* Am J Med, 2012. **125**(7 Suppl): p. S2-5.
20. Nisse, C., et al., *Exposure to occupational and environmental factors in myelodysplastic syndromes. Preliminary results of a case-control study.* Leukemia, 1995. **9**(4): p. 693-9.
21. Pozdnyakova, O., et al., *Cytogenetic abnormalities in a series of 1,029 patients with primary myelodysplastic syndromes: a report from the US with a focus on some undefined single chromosomal abnormalities.* Cancer, 2008. **113**(12): p. 3331-40.
22. Greenberg, P.L., et al., *Revised international prognostic scoring system for myelodysplastic syndromes.* Blood, 2012. **120**(12): p. 2454-65.
23. Montalban-Bravo, G. and G. Garcia-Manero, *Myelodysplastic syndromes: 2018 update on diagnosis, risk-stratification and management.* Am J Hematol, 2018. **93**(1): p. 129-147.
24. Jaiswal, S., et al., *Age-related clonal hematopoiesis associated with adverse outcomes.* N Engl J Med, 2014. **371**(26): p. 2488-98.

25. Xie, M., et al., *Age-related mutations associated with clonal hematopoietic expansion and malignancies.* Nat Med, 2014. **20**(12): p. 1472-8.
26. Steensma, D.P., et al., *Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes.* Blood, 2015. **126**(1): p. 9-16.
27. Genovese, G., et al., *Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence.* N Engl J Med, 2014. **371**(26): p. 2477-87.
28. Minor, E.A., et al., *Ascorbate induces ten-eleven translocation (Tet) methylcytosine dioxygenase-mediated generation of 5-hydroxymethylcytosine.* J Biol Chem, 2013. **288**(19): p. 13669-74.
29. Pastor, W.A., L. Aravind, and A. Rao, *TETonic shift: biological roles of TET proteins in DNA demethylation and transcription.* Nat Rev Mol Cell Biol, 2013. **14**(6): p. 341-56.
30. Shen, L., et al., *Mechanism and function of oxidative reversal of DNA and RNA methylation.* Annu Rev Biochem, 2014. **83**: p. 585-614.
31. Delhommeau, F., et al., *Mutation in TET2 in myeloid cancers.* N Engl J Med, 2009. **360**(22): p. 2289-301.
32. Jiang, Y., et al., *Aberrant DNA methylation is a dominant mechanism in MDS progression to AML.* Blood, 2009. **113**(6): p. 1315-25.
33. Guillamot, M., L. Cimmino, and I. Aifantis, *The Impact of DNA Methylation in Hematopoietic Malignancies.* Trends Cancer, 2016. **2**(2): p. 70-83.
34. Langemeijer, S.M., et al., *Acquired mutations in TET2 are common in myelodysplastic syndromes.* Nat Genet, 2009. **41**(7): p. 838-42.
35. Papaemmanuil, E., et al., *Genomic Classification and Prognosis in Acute Myeloid Leukemia.* N Engl J Med, 2016. **374**(23): p. 2209-2221.
36. Patnaik, M.M., et al., *Number and type of TET2 mutations in chronic myelomonocytic leukemia and their clinical relevance.* Blood Cancer J, 2016. **6**(9): p. e472.
37. Sperling, A.S., C.J. Gibson, and B.L. Ebert, *The genetics of myelodysplastic syndrome: from clonal haematopoiesis to secondary leukaemia.* Nat Rev Cancer, 2017. **17**(1): p. 5-19.
38. Markolovic, S., S.E. Wilkins, and C.J. Schofield, *Protein Hydroxylation Catalyzed by 2-Oxoglutarate-dependent Oxygenases.* J Biol Chem, 2015. **290**(34): p. 20712-22.
39. Jackson, M., et al., *Severe global DNA hypomethylation blocks differentiation and induces histone hyperacetylation in embryonic stem cells.* Mol Cell Biol, 2004. **24**(20): p. 8862-71.
40. Blaschke, K., et al., *Vitamin C induces Tet-dependent DNA demethylation and a blastocyst-like state in ES cells.* Nature, 2013. **500**(7461): p. 222-6.
41. Cameron, E. and L. Pauling, *Supplemental ascorbate in the supportive treatment of cancer: Prolongation of survival times in terminal human cancer.* Proc Natl Acad Sci U S A, 1976. **73**(10): p. 3685-9.
42. Cameron, E. and L. Pauling, *Supplemental ascorbate in the supportive treatment of cancer: reevaluation of prolongation of survival times in terminal human cancer.* Proc Natl Acad Sci U S A, 1978. **75**(9): p. 4538-42.
43. Schoenfeld, J.D., et al., *O2(-) and H2O2-Mediated Disruption of Fe Metabolism Causes the Differential Susceptibility of NSCLC and GBM Cancer Cells to Pharmacological Ascorbate.* Cancer Cell, 2017. **31**(4): p. 487-500 e8.
44. Borthakur, G., et al., *Activity of decitabine in patients with myelodysplastic syndrome previously treated with azacitidine.* Leuk Lymphoma, 2008. **49**(4): p. 690-5.
45. An, J., et al., *Acute loss of TET function results in aggressive myeloid cancer in mice.* Nat Commun, 2015. **6**: p. 10071.
46. Vasilatou, D., et al., *Epigenetic alterations and microRNAs: new players in the pathogenesis of myelodysplastic syndromes.* Epigenetics, 2013. **8**(6): p. 561-70.
47. Bejar, R., et al., *TET2 mutations predict response to hypomethylating agents in myelodysplastic syndrome patients.* Blood, 2014. **124**(17): p. 2705-12.

48. Agathocleous, M., et al., *Ascorbate regulates haematopoietic stem cell function and leukaemogenesis.* Nature, 2017. **549**(7673): p. 476-481.