A Phase 1b/2 Study to Evaluate the Safety and Efficacy of APR-246 in Combination with Azacitidine for the Treatment of TP53 Mutant Myeloid Neoplasms

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TITLE: A Phase 1b/2 Study to Evaluate the Safety and Efficacy of APR-246 in Combination with Azacitidine for the Treatment of *TP53* Mutant Myeloid Neoplasms.

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Protocol Synopsis

Title: A Phase 1b/2 Study to Evaluate the Safety and Efficacy of APR-246 in Combination with Azacitidine for the Treatment of *TP53* Mutant Myeloid Neoplasms.

Study Objectives	Primary Objective (Phase1b): 1. To determine the safe and recommended Phase 2 dose (RP2D) of APR-246 in combination with azacitidine as determined by dose-limiting toxicities (DLTs) Primary Objective (Phase 2): 2. To determine the proportion of patients with *TP53* mutant myeloid neoplasms who are in complete remission as measured by the international working group criteria (IWG 2006) Secondary Objectives: 3. To determine the duration of response 4. To determine the proportion of patients with *TP53* mutant myeloid neoplasms alive at 8 months 5. To determine overall best response rates as measured by the international working group criteria (IWG 2006) 6. To determine if mutant *TP53* variant allele frequency (VAF) or p53 protein expression predicts response to APR-246 with azacitidine 7. To determine if p53 protein expression correlates with *TP53* VAF 8. To determine if APR-246 with azacitidine treatment leads to mutant *TP53* clonal suppression 9. To determine if *TP53* clonal suppression correlates with outcomes. 10. To determine if APR-246 treatment upregulates p53 target genes 11. To determine if APR-246 induces reactive oxygen species (ROS) production 12. To determine the rate and time to acute myeloid leukemia (AML) transformation 13. To determine the median overall survival (OS) 14. To determine the median leukemia free survival (LFS) 15. To determine whether recurrent genetic mutations are predictive of response
Study Endpoints	Primary 1. Phase 1b: DLTs occurring in the first 2 cycles (8 weeks), as defined below, graded according to the NCI CTCAE, Version 4.03 2. Phase 2: CR rate by IWG 2006 criteria (appendix B) Secondary 3. Duration of response defined as the time between achieving response and progression of

	disease 4. OS at 8 months 5. Proportion of subjects achieving hematological improvement (HI), partial response (PR), complete response (CR), and/or marrow CR (mCR) by the IWG 2006 criteria (appendix B) 6. Proportion of subjects who obtain a response as defined by IWG 2006 criteria with a baseline *TP53* VAF ≥ 20% versus < 20% or high versus low nuclear p53 protein expression 7. Correlation of *TP53* VAF with nuclear p53 protein expression as determined by immunohistochemistry (IHC). 8. Proportion of subjects who achieve ≥ 50% reduction in *TP53* VAF 9. Progression free survival (PFS) and OS in clonal responders (≥ 50% reduction in *TP53* VAF) versus non-responders 10. Profile expression of genes related to p53-mediated signal transduction before and after APR-246 treatment 11. Profile ROS production before and after APR-246 treatment 12. AML transformation according to World Health Organization (WHO) criteria 13. OS 14. LFS 15. Determine recurrent gene mutations in *ASXL1, BCOR, BRAF, CALR, CBL, CEBPA, CSF3R, DNMT3A, ETV6, EZH2, GATA2, GNAS, IDH1, IDH2, JAK2, KIT, KRAS, MPL, NF1, NPM1, NRAS, PDGFRA, PDGFRB, PHF6, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SMC1A, SMC3, SRSF2, STAG2, STAT3, STAT5b, TET2, TP53, U2AF1, WT1,* and *ZRSR2* at study entry and serially throughout treatment to assess changes in somatic mutation landscape
Eligibility Criteria	**<u>Inclusion Criteria</u>**: 1. Patient has signed the Informed Consent (ICF) and is able to comply with protocol requirements. 2. Patient has adequate organ function as defined by the following laboratory values: a. Serum creatinine ≤ 2 x upper limit of normal (ULN) b. Total serum bilirubin < 1.5 x ULN or total bilirubin ≤ 3.0 x ULN with direct bilirubin within normal range in patients with well documented Gilbert's Syndrome or hemolysis or who required regular blood transfusions c. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) < 2.5 x ULN 3. Age ≥18 years at the time of signing the informed consent form 4. Documented diagnosis of myelodysplastic syndrome (MDS), MDS/ myeloproliferative neoplasm (MPN), chronic myelomonocytic leukemia (CMML) by WHO criteria or AML with 20-30% myeloblasts (refractory anemia with excess blasts in transformation

(RAEB-T) by French-American-British (FAB) criteria).

5. Documentation of a *TP53* gene mutation by next-generation sequencing (NGS) based on central or local evaluation.
6. Revised International Prognostic Scoring System (IPSS-R) criteria for Intermediate, High-risk or Very High-risk.
7. An Eastern Cooperative Oncology Group (ECOG) performance status score of 0, 1, or 2 is required.
8. If of childbearing potential, negative pre-treatment urine or serum pregnancy test.
9. If of childbearing potential (males and females), willing to use an effective form of contraception such as latex condom, hormonal birth control, intrauterine device or double barrier method during chemotherapy treatment and for at least six months thereafter.

Exclusion Criteria:

1. Patient has a known history of HIV or active hepatitis B or active hepatitis C infection (testing not mandatory).
2. Patient has any of the following cardiac abnormalities (as determined by treating MD):
    a. symptomatic congestive heart failure
    b. myocardial infarction ≤ 6 months prior to enrollment
    c. unstable angina pectoris
    d. serious uncontrolled cardiac arrhythmia
    e. QTc ≥ 470 msec
3. Concomitant malignancies or previous malignancies with less than a 1-year disease free interval at the time of signing consent. Patients with adequately resected basal or squamous cell carcinoma of the skin, or adequately resected carcinoma in situ (e.g. cervix) may enroll irrespective of the time of diagnosis.
4. Prior exposure to azacitidine, decitabine or investigational hypomethylating agent
5. Use of cytotoxic chemotherapeutic agents, or experimental agents (agents that are not commercially available) for the treatment of MDS, MDS/MPN, CMML or AML within 14 days of the first day of study drug treatment.
6. No concurrent use of erythroid stimulating agents, G-CSF, GM-CSF is allowed during study except in cases of febrile neutropenia where G-CSF can be used for short term. Growth factors must be stopped 14 days prior to study.
7. Patients with history of allogeneic stem cell transplantation.
8. Pregnant women are excluded from this study because APR-246 has not been studied in pregnant subjects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with APR-246, breastfeeding should be discontinued if the mother is treated with APR-246.

Baseline Assessment (within 4 weeks of starting treatment)	1. Medical history including: a. disease characteristics such as WHO subtype, IPSS/R-IPSS score, prior treatments b. ECOG performance status c. Concurrent medication review 2. Routine physical examination 3. EKG 4. Bone marrow examination, including cytomorphology, cytogenetic assessment,and flow cytometry analysis 5. Documentation of a *TP53* gene mutation by NGS based on central or local evaluation 6. Laboratory assessments: • CBC with differential (including platelet count) • Clinical chemistries including BUN, creatinine, sodium, potassium, alkaline phosphatase, ALT, AST, total bilirubin and albumin • Urine or serum pregnancy test for females of childbearing potential will be performed between Screening and first day of treatment on study, prior to first dose of study medication. 7. Review and record any red blood cell and platelet transfusions for the prior 8 weeks.
Treatment plan	Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 11. Appropriate dose modifications for APR-246 are described in Section 10. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's disease. APR-246 will be supplied by Aprea. APR-246 will be administered as a 6 hour intravenous infusion daily on days 1-4 for each 28 day cycle. Azacitidine will be given at the standard dose of 75mg/m^2 over 7 days (7 consecutive days, 4-10; or 2+5 (i.e., days 4-5 and 8-12) as a subcutaneous injection or intravenous infusion. A maximum of 51 subjects will be treated. See study calendar for assessment on study.
Definition of DLT	Patients will be evaluated for DLTs during the lead-in phase and first cycle of combination therapy, i.e., 6 weeks for purpose of deciding the dose for next cohort (see Table 1) but DLTs will continue to be evaluated and reported through all cycles on study. DLT is defined as follows based on the CTCAE v 4.03: -Treatment related non-hematological CTCAE grade 3-4 toxicity that lead to dose modification or withdrawal. -Absolute neutrophil count (ANC) not recovering to >500/μL by day 42 of a cycle in the absence of active leukemia/myelodysplasia will be considered a DLT. -Grade 3 metabolic/electrolyte abnormalities that are not clinically significant, and are adequately controlled within 72 hours are not to be considered DLT. -Grade 3 nausea/vomiting/diarrhea or CNS toxicity that does not resolve within 28 days despite

	treatment interruption and maximum medical therapy will be considered a DLT.
Dose delay / modifications	Dose delays/modifications are allowed as described in section 10.
Duration of Therapy	Subjects will be treated for a total of 6 cycles. For subjects responding or who have stable disease following cycle 6, treatment may continue until one of the following criteria applies: • Inter-current illness that prevents further administration of treatment, • Unacceptable adverse event(s), • Patient decides to withdraw from the study, or • General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator. • Evidence of disease progression by the IWG 2006 criteria (see Appendix B, not applicable at bone marrow evaluation at day -10 of lead in phase). Subjects who wish not to continue treatment at time of disease assessment at end of cycle 6 will complete their end of treatment visit upon completion of cycle 6.
Duration of Follow-Up	Subjects will be followed as per calendar on treatment for 6 cycles (Figure 2). After 6 cycles, subjects who continue on study will be followed monthly. Off treatment data on AML transformation and overall survival will be updated every 6 months or until death, whichever occurs first. Subjects removed from the study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. <u>Criteria for Removal from Study</u> Study drug treatment can continue for subjects receiving clinical benefit, unless: one or more withdrawal criteria are met, or at the subject's discretion, or if the study is terminated. 1. Subject Completion A subject will be considered to have completed the study if the subject meets at least 1 of the following criteria: - The subject has progressive disease. - The subject died during the study. - The subject experienced a treatment related AE that led to withdrawal from the study. - The subject starts new treatment for their underlying disease (MDS/CMML/AML). 2. Subject Withdrawal from Study: A subject may voluntarily withdraw from study medication or withdraw consent from the study at any time. The investigator may also, at his or her discretion, discontinue a subject from participating in the study at any time. The investigator and/or designated staff will record the date and the reason for subject withdrawal from the study. 3. Subject Withdrawal from Study Medication: If the subject is permanently withdrawn from treatment with study medication, but does not withdraw consent, the investigator must make every effort to have the subject complete all

	withdrawal assessments at the time of withdrawal, and complete all scheduled follow-up visits. **<u>Treatment with study medication must be discontinued if (Withdrawal Criteria)</u>**: Evidence of Disease progression according to IWG 2006 criteria (see Appendix B, not applicable at bone marrow evaluation at day -10 of lead in phase). A subject becomes pregnant. A subject is significantly non-compliant with the requirements of the protocol; A subject has an adverse experience that would, in the investigator's judgment, make continued participation in the study an unacceptable risk A subject withdrawals consent
Follow up on study	See calendar Section 13

Statistics

Study Design:

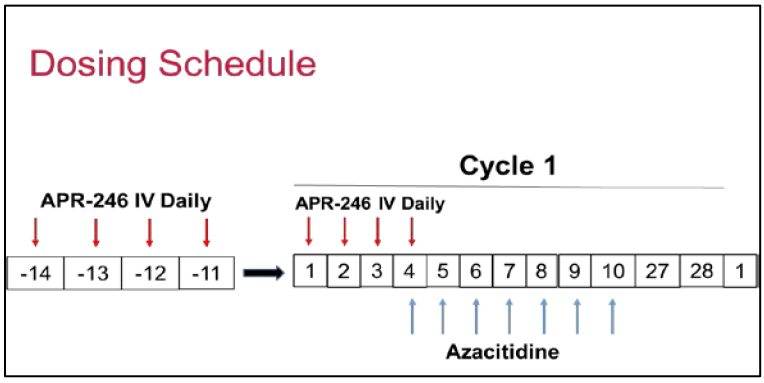
This study will be a multi-institution, open-label, phase Ib/II clinical trial conducted in 2 parts: a Phase 1b part followed by a Simon's two-stage Phase 2 design. The study will assess the safety and efficacy of APR-246 in combination with azacitidine for the treatment of *TP53* mutant myeloid neoplasms. All patients will have pre-screening NGS on peripheral blood (PB) or bone marrow (BM) samples to determine *TP53* mutational status and therefore eligibility to participate in this study. A PB sample will be obtained prior to treatment for NGS by the central laboratory to evaluate baseline *TP53* VAF and for serial analysis.

Evaluable: Patients will be evaluable for inclusion in the Phase 1b and Phase 2 portions of the study if they receive at least one dose of protocol therapy.

Phase 1b Part:

The initial Phase 1b part will evaluate APR-246 administered with azacitidine (see Figure 1). Cohorts of at least 3 evaluable patients will be enrolled using a modified 3 + 3 design.

Figure 1



Patients will receive intravenous infusions of APR-246 as a lead-in phase on days -14 to -11 starting at Dose Level 1 (see Table 1) prior to starting cycle #1 of combination therapy with azacitidine (see Figure 1). Combination therapy will consist of APR-246 on days 1-4 and azacitidine on days 4-10 (or days 4-5 and 8-12) of a 28 day cycle. Patients will be followed for at least 6 weeks (lead in phase + cycle 1) before the safety of each cohort can be fully assessed and decisions made for dose escalation in the next cohort. The MTD is defined as the dose level below which DLT is manifested in ≥33% of the patients or at dose level 3 if DLT is manifested in <33% of the patients.

Table 1. Dose Levels for Treatment Part 1: APR-246+Azacitidine

Dose Level	APR-246 (mg/kg lean body weight (LBW))	Azacitidine (mg/m^2)
1 (Starting dose)	50	75
2	75	75
3	100	75

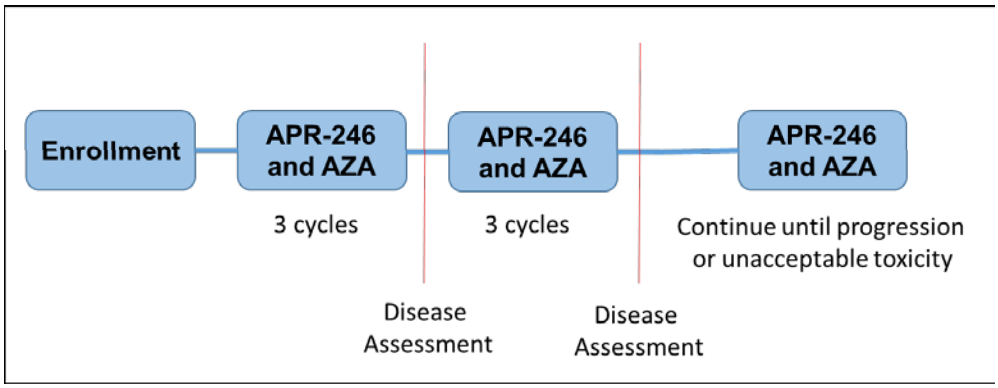
Phase 1b, patients (n = 6 to approximately 18) will receive APR-246 in combination with azacitidine. Cohorts of at least 3 evaluable patients will be enrolled using a modified 3+3 design. If no DLT is observed in a cohort, the subsequent patient group will be enrolled at the next planned dose level. If a DLT is observed in 1 of 3 patients in a cohort at any dose level, then up to 3 additional patients will be enrolled at that dose level. If only 1 DLT occurs in the 6 patients treated at that dose level, the subsequent patient group will be enrolled at the next planned dose level. If 2 of 6 patients in a cohort have a DLT, dose escalation ceases and the MTD is the previous dose level. If only 3 patients were treated at the MTD, an additional 3 patients will be included in the cohort and if only 0 or 1 DLT occur in the 6 patients treated at that dose level, the MTD will be defined and we will proceed to Phase 2.

Phase 2: Simon's two-stage minimax design:
Following completion of the Dose Finding Phase, we will conduct a dose expansion, whereby patients will be treated with APR-246 administered at the MTD with azacitidine on a 28 day cycle utilizing the same dosing schedule as in Phase 1b with the exception of no lead in phase (i.e. patients will start at day 1 of combination therapy with APR-246 on days 1-4 and azacitidine on days 4-10 (or days 4-5 and 8-12) of a 28 day cycle, see Figure 1). If dose level 3 (i.e. 100mg/kg LBW) is the RP2D based on the phase 1b part of the trial, the dosing will be changed to an equivalent fixed dose regimen of 4500mg/patient (see below). All patients who complete at least one treatment cycle of APR-246 and azacitidine and who have at least one post-treatment clinical response assessment will be considered eligible for the efficacy-evaluable population and will be summarized for all efficacy variables. Patients who fail to complete one treatment cycle will also be considered efficacy-evaluable if they show clear evidence of clinically significant disease progression. Patients who are not efficacy-evaluable will be replaced. A Simon's two-stage minimax design will be applied, as follows:

Stage 1: Enroll a total of 24 evaluable patients at the MTD (including patients who were enrolled during the Phase 1 part of the study). If less than 6 of 24 patients achieve CR, then the study will be terminated early with the conclusion that the regimen does not warrant further investigation. If 6 or more patients achieve CR, then enrollment will be permitted to continue to Stage 2.

Stage 2: Enroll 21 more evaluable patients for a total of 45. If less than 14 of 45 patients achieve CR, then there is insufficient evidence to support continued study of this treatment. If at least 14 of 45 patients achieve CR, then there is sufficient evidence to support further study of APR-246 in combination with azacitidine in Phase 3. Up to 39 additional evaluable patients will be treated at the level of the MTD in the Phase 2 portion of the trial. The maximum total accrual is 51 patients. Disease assessments will occur following 3 cycles of therapy (Figure 3).

Figure 3. Phase 2 Schema



This rule has the following operating characteristics: 90% power, with alpha=0.05 under the null hypothesis that the proportion of patients achieving CR is ≤ 20% versus the alternative hypothesis that it is ≥ 40%. This design has a probability of early termination equal to 0.66 if the true CR rate is 20%.

Number of Patients Planned:

The number of patients enrolled will depend on the number of dose levels evaluated before reaching DLT. Three to 6 evaluable patients will be entered per dose level. It is anticipated that up to 18 evaluable patients can be treated in the Phase 1b portion of the study. Up to 39 additional evaluable patients will be treated at the level of the MTD in the Phase 2 portion of the trial. The maximum total accrual is 51 patients.

Analyses to address study objectives

To address the primary objective for Phase 1b, we will apply the 3+3 design above.

To address the primary objective for Phase 2, we will apply the Simon's two-stage minimax design above.

<u>Analysis of the following secondary endpoints will be of exploratory nature</u>:

Duration of response defined as the time between achieving response and progression of disease.

OS at 8 months

Proportion of subjects achieving hematological improvement (HI), partial response, complete response (CR), and/or marrow CR (mCR) by the IWG 2006 criteria (see appendix B)

Proportion of subjects who obtain a response as defined by IWG 2006 criteria with a baseline *TP53* VAF ≥ 20% versus < 20% or high versus low nuclear p53 protein expression

Correlation of *TP53* VAF with nuclear p53 protein expression as determined by IHC

Proportion of subjects who achieve ≥ 50% reduction in *TP53* VAF

Progression free survival (PFS) and OS in clonal responders (≥ 50% reduction in *TP53* VAF) versus non-responders

Profile expression of genes related to p53-mediated signal transduction before and after APR-246 treatment

Profile ROS production before and after APR-246 treatment

AML transformation according to World Health Organization (WHO) criteria

OS

LFS

Determine recurrent gene mutations in *ASXL1, BCOR, BRAF, CALR, CBL, CEBPA, CSF3R, DNMT3A, ETV6, EZH2, GATA2, GNAS, IDH1, IDH2, JAK2, KIT, KRAS, MPL, NF1, NPM1, NRAS, PDGFRA, PDGFRB, PHF6, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SMC1A, SMC3, SRSF2, STAG2, STAT3, STAT5b, TET2, TP53, U2AF1, WT1,* and *ZRSR2* at study entry and serially throughout treatment to assess changes in somatic mutation landscape

1. OBJECTIVES

1.1. Primary Objective (Phase1b):

1.1.1. To determine the safe and recommended Phase 2 dose (RP2D) of APR-246 in combination with azacitidine as determined by dose-limiting toxicities (DLTs)

1.2. Primary Objective (Phase 2):

1.2.1. To determine the proportion of patients with *TP53* mutant myeloid neoplasms who are in complete remission as measured by the international working group criteria (IWG 2006)

1.3. Secondary Objectives:

1.3.1. To determine the duration of response

1.3.2. To determine the proportion of patients with *TP53* mutant myeloid neoplasms alive at 8 months

1.3.3. To determine overall best response rates as measured by the international working group criteria (IWG 2006)

1.3.4. To determine if mutant TP53 variant allele frequency (VAF) or p53 protein expression predicts response to APR-246 with azacitidine

1.3.5. To determine if p53 protein expression correlates with TP53 VAF

1.3.6. To determine if APR-246 with azacitidine treatment leads to mutant TP53 clonal suppression

1.3.7. To determine if TP53 clonal suppression correlates with outcomes.

1.3.8. To determine if APR-246 treatment upregulates p53 target genes

1.3.9. To determine if APR-246 induces reactive oxygen species (ROS) production

1.3.10. To determine the rate and time to acute myeloid leukemia (AML) transformation

1.3.11. To determine the median overall survival (OS)

1.3.12. To determine whether recurrent genetic mutations are predictive of response

2. BACKGROUND

2.1. Myelodysplastic Syndromes

Myelodysplastic syndromes (MDS) are a heterogeneous group of neoplasms arising from a clonal hematopoietic precursor[1,2]. MDS is characterized clinically by cytopenias and a tendency to transform to acute myeloid leukemia (AML)[3]. Prognostic scoring systems have been

developed to help stratify patients based on predicted survival and progression to AML[3-5]. In practice, treatment decisions are made by grouping patients with MDS into lower and higher risk subgroups as defined by clinical prognostic scoring systems. Specifically, the International Prognostic Scoring System (IPSS) and its more modern version, the revised-IPSS (RIPSS), represent the most widely used clinical prognostic tools[3,4]. Those patients that fall in the higher risk group have an expected survival of less than 12 months if left untreated and progress to AML greater than 30% of the time.

2.2. *TP53* Mutations in MDS and AML

The advent of next generation sequencing (NGS) has provided a rapid and efficient platform for genome studies that has revolutionized the diagnostic, prognostic, and therapeutic realms of myeloid malignancies[6]. Current NGS myeloid panels incorporate 20-50 genes and can identify mutations in the vast majority of patients[7,8]. In MDS, mutations of *ASXL1*, *ETV6*, *EZH2*, *RUNX1* and *TP53* were found to be independently associated with decreased survival[9]. Of these, recent investigations have suggested that the mutational status of *TP53* is the most important negative prognostic factor in MDS patients. To date, the only disease modifying agents in MDS include lenalidomide in patients with isolated deletion of 5q (del(5q)) and azacitidine (a DNA methyltransferase (DNMT) inhibitor/hypomethylating agent (HMA)) in MDS patients with higher risk disease (i.e., intermediate 2/high risk by IPSS and high/very-high by RIPSS). Lenalidomide was approved based on the MDS-003 trial with 67% of del (5q) patients achieving transfusion independence and 45% of patients with complete cytogenetic response[10]. However, primary resistance to lenalidomide has been directly linked to *TP53* mutation which occurs in 20% of patients[11]. Whereas partial cytogenetic responses occur in up to 73% of patients, *TP53* mutated del(5q) patients only have cytogenetic responses in 0-11% of cases[12-14]. For higher risk MDS patients, azacitidine represents the standard of care based on the AZA-001 trial that demonstrated a survival advantage for azacitidine when compared with induction chemotherapy, low dose cytarabine, or best supportive care (24.5 months versus 15 months, $p<0.0001$)[15]. A recent study in MDS showed strong association of *TP53* mutation with poor outcome in azacitidine treated patients[16]. In addition, Bejar and colleagues recently confirmed decreased OS in *TP53* mutated MDS patients treated with HMA without effect on response rates[17]. Lastly, mutations of *TP53* strongly predict for lack of benefit to allogeneic bone marrow transplantation,

which represents the only curative option for patients with MDS[18]. This study highlights the prognostic importance of *TP53* mutations as patients with complex karyotype without *TP53* mutation had similar survival to patients with normal karyotype.

We have recently analyzed our own experience on molecularly profiled patients at Moffitt Cancer Center and identified that the clonal burden, or variant allele frequency (VAF), had significant impact on clinical phenotype and more importantly further stratified prognosis over binary mutational analysis alone and/or clinical prognostic models[19]. Specifically, MDS patients with a *TP53* VAF > 40% had a median overall survival (OS) of 124 days versus an OS that was not reached in patients with VAF < 20% (hazard ratio (HR), 3.52; $P = 0.01$) with validation in an independent cohort (HR, 4.94, $P = 0.01$). Overall, *TP53* mutant MDS and AML patients have a median OS ranging from 5-9 months with 8 months representing the best estimate when restricting to MDS and oligoblastic AML (20-30% blasts)[9,19-22]. Together, these data highlight the dismal outcomes of *TP53* mutant patients and the dire need for the development of novel therapeutic strategies, particularly in this patient population.

2.3. APR-246

Restoration of wild type function from mutant or otherwise dysfunctional/unfolded p53 has been suggested to be an efficient strategy for selective elimination of tumor cells[23]. A molecule that reactivates mutant p53 should be less effective in cells with wild type p53 because the wild type protein is found at low levels in the absence of stress. In contrast, mutant p53 often accumulates at high levels and is already "pre-activated" by posttranslational modifications in tumor cells due to constitutive stress signaling, i.e., oncogene activation, DNA damage, and hypoxia. This has been commonly observed in MDS with *TP53* mutation and we have recently shown that *TP53* VAF directly correlates with p53 expression in analysis of bone marrow from *TP53* mutant patients[24]. Therefore, mutant p53 reactivation should trigger robust apoptosis in tumor cells but not in normal cells[25].

Wiman and colleagues identified APR-017 (also called PRIMA-1) in a cellular screen for compounds that induce apoptosis preferentially in human tumor cells expressing exogenous mutant p53[26]. The effect of APR-017 was more pronounced in AML cells with homozygous *TP53* deletions than in wild type *TP53* cells, while commonly used anti-leukemic drugs showed

the opposite pattern, being less pronounced in AML cells with abnormal chromosome 17[27]. The optimization program of the lead compound APR-017 resulted in the identification of the structural analogue APR-246, Aprea's candidate drug in clinical development[28]. APR-246 has more drug-like properties in comparison to APR-017, e.g., being more potent and showing better cellular permeability[29].

2.4. Mechanism of action of APR-246

APR-246 (like the parent compound APR-017) is a prodrug, which under physiological conditions is spontaneously converted to methylene quinuclidinone (MQ), a Michael acceptor that binds covalently to cysteines in mutant p53[30]. Transfer of MQ-modified mutant p53 protein into tumor cells lacking p53 induced massive apoptosis, indicating that covalent binding of MQ per se is sufficient to activate mutant p53 and induce a p53 dependent biological response. These and other experiments directly looking at the conformational state of p53 protein have confirmed that binding of MQ pushes unfolded mutant or wild type p53 towards a functional wild type conformation. MQ is a 'soft' electrophile, hence strongly preferring 'soft' nucleophiles such as thiols. Once formed from APR-246, MQ could potentially bind to cysteines in many proteins in the cell. However, various factors may limit the number of protein targets, including accessibility of the thiol, nucleophilicity of the sulfur, and structural flexibility of the targeted protein domain.

Thioredoxin reductase 1 (TrxR1) was identified as a target of MQ, potentially contributing to the anti-cancer effect[31]. MQ converts this enzyme from a reductase to an oxidase that can produce reactive oxygen species (ROS). This may contribute to APR-246-induced cell death and probably accounts, at least in part, for the observed effects of APR-246 in *TP53* null cancer cells. Further *in vitro* experiments have shown that MQ readily reacts with glutathione, a central molecule in the cellular redox system[28,32]. Thus, APR-246 via MQ has effects that lead to impairment of the tumor cells capacity to handle oxidative stress, including depletion of glutathione [13]. In tumor cells, the redox system is pushed to the limit of its capacity, while coping with enhanced oxidative stress compared with normal cells, making the redox system an Achilles' heel of tumor cells. This set of properties constitutes synthetically lethal targets which may be utilized to selectively kill tumor cells. The dual activity of APR-246 restoring wt p53 function of mutant p53 and increasing oxidative stress is illustrated in **Figure 1**.

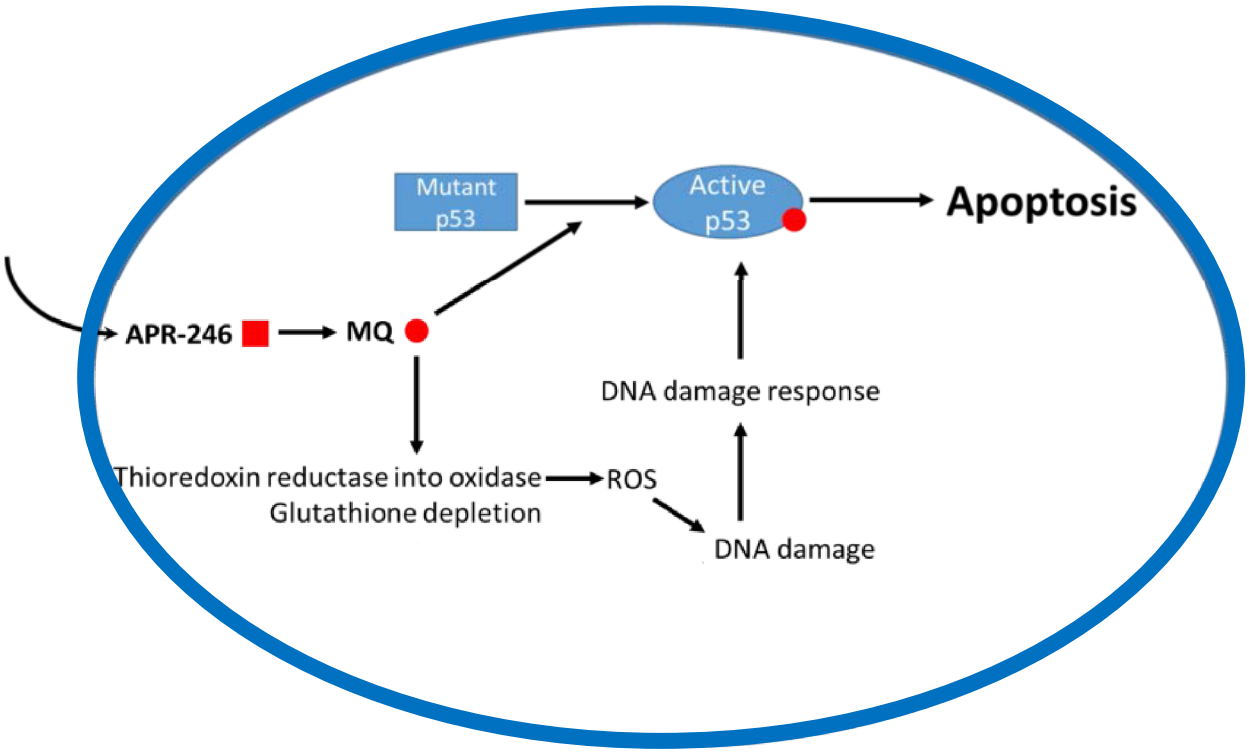


Figure 1: Mechanism of action of APR-246 leading to induction of apoptosis.

2.5. APR-246 in Solid and Hematologic Malignancies

This first in human study was a multicenter, open label, non-comparative, Phase I/II dose escalating study of APR-246 infusions in patients with refractory hematological malignancies or prostate cancer (APR-246-01)[33]. The study assessed the safety, tolerability, pharmacokinetics and pharmacodynamics of multiple escalating doses of APR-246. An extension of this clinical trial has been conducted with the main objective to evaluate safety at an optimal dose in a more homogenous patient population (see below). The patients received APR-246 on 4 consecutive days as a 2-hour daily intravenous (IV) infusion. Dosing was conducted with three patients at each dose level. The first dose level was 2 mg/kg and was followed by 3, 10, 30, 60 and 90 mg/kg. The treatment phase was followed by a 17 days follow-up phase to reveal any late adverse effects. The HFD was defined as the dose expected to result in maximal plasma concentration (Cmax) close to but not exceeding 110 µg/mL in any single patient without showing signs of dose limiting toxicity (DLT), or the dose level below the dose where DLT occurred, whichever came first. In total 22 patients, 4 (18.2 %) female and 18 (81.8 %) male with an average age of 67.0 years were enrolled into the study. The indications for the 22 included

patients were acute myeloid leukemia (AML) (7 patients), prostate carcinoma (7 patients), non-Hodgkin´s lymphoma (NHL) (4 patients), chronic lymphocytic leukemia (CLL) (3 patients) and multiple myeloma (MM) (1 patient). Eighteen (18) patients completed the study, i.e., completed the day 21 visit and 4 patients were prematurely discontinued.

In regards to activity, the apoptosis pattern in patients with hematological malignancies showed cell cycle changes compatible with cell cycle arrest in all patients with non T-cell malignancy[33]. Also, other apoptosis markers were affected such as increased staining for Annexin-V, up regulation of BAX (Bcl-2 associated X protein), NOXA and PUMA expression and up regulation of DcR2 (decoy receptor 2, a marker considered to be associated with senescence). The one responding patient in the initial phase 1 clinical trial was the only *TP53* mutant AML patient. Specifically, this patient had a reduction of blast cell count from 46% to 26% and showed activation of p53 targets supporting in vivo activity.

2.5.1. Phase I/II Clinical Study (APR-246-01, dose de-escalation)

This was a multi-center, open label, non-comparative, phase I, dose de-escalating study of APR-246 infusions in patients with refractory hematologic malignancies. In this study (Amendment No. 6) a longer infusion time was applied resulting in an increased exposure of APR-246[28]. In Amendment No. 6, the objective was to increase the knowledge of the optimal way to administer APR-246 and study the safety and tolerability as well as the pharmacokinetic (PK) profile of APR-246. Ten patients with acute myeloid leukemia (AML) or chronic lymphocytic leukemia (CLL) were planned for enrolment. The treatment schedule was 4 consecutive days with APR-246 with the modified dosing regimen of a start boosting infusion of 50 mg/kg during 45 min, followed by an 85 mg/kg infusion for 5.15 hours. If toxicity was shown, the dose was to be decreased to Dose level 2 (45 mg/kg for 45 min [infusion rate 60 mg/kg/h], followed by 60 mg/kg [infusion rate 10 mg/kg/h] for 5.15 hours), on the discretion of the investigator and/or the Study Board. Out of the 2 AML patients with *TP53* mutation, both had a blast reduction, one constituting a response according to the response criterion[28]. On the lower dose of 67.5mg/kg, there were no DLTs or SAEs. Overall in this study there were 6/10 patients treated at 67.5 mg/kg or higher with no or only mild side effects supporting the 67.5mg/kg dose as the HFD and recommended for further development.

The pharmacokinetics was studied at selected time points during the treatment and the data

showed that no accumulation of APR-246 was observed after four daily doses or after 3 cycles. Plasma clearance and volume of distribution were comparable between Day 1 and Day 4, suggesting a time-independent kinetics. The PK parameters obtained in the present study, were also in line with the previous single dose study (2-90 mg/kg, 2 h infusion) and linear kinetics could be concluded for APR-246 up to 135 mg/kg. Several patients tolerated the mid-dose, 105 mg/kg treatment, where the Cmax was in the range of 45-85 µg/mL with an exposure up to 860 µg*h/mL (AUC0-24h), whereas one patient withdrew having the highest exposure (1060 µg*h/mL) in the whole study. The results of the extension study of APR-246 in patients with AML and CLL suggest that the dose regimen with HFD of 67.5 mg/kg given as a 6-hour bi-phasic infusion was suitable and that APR-246 was well tolerated with limited number of AEs reported. The pharmacokinetic data suggested time-independent and dose-independent kinetics. In the patients, plasma concentrations consistently above those associated with effects in both ex vivo and in vivo pre-clinical models have been achieved. Four patients showed signs of response and 2 of them were responders according to the definition in the protocol. A response was observed in 4 of the 5 patients carrying mutant *TP53*. The effect of APR-246 on tumor burden and apoptosis markers indicates beneficial effects. Given the critical unmet need for treatment of *TP53* mutant MDS and AML patients, we propose this phase Ib/II study.

2.5.2. APR-246 in advanced ovarian cancer

A 2-part study has been designed (APR-407) in women with advanced high grade serous ovarian cancer (HGSCO) given APR-246 in combination with carboplatin and pegylated liposomal doxorubicin (PLD). This is based on the fact that >90% of HGSOC are *TP53* mutant and *in vitro* and *in vivo* data suggesting synergism with platinum agents[28]. The APR-407 Phase Ib portion is nearly completed in the European Union and the recommended Phase II dose of 67.5 mg/kg (corresponding to 100 mg/kg lean body mass (LBM)) has been confirmed based upon assessment of cumulative safety data from the Phase Ib. Currently, additional patients are being enrolled in Phase Ib expansion cohort to collect additional safety and cardiac QTc and PK data at the Phase II dose (100 mg/kg LBM); the target is to have matched ECG and PK data from 20 patients at the Phase II dose. The APR-407 Phase II portion will open to accrual in the EU after regulatory clearance, and in the US pending IND activation. A summary of the adverse events seen in this protocol are detailed below.

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[redacted]

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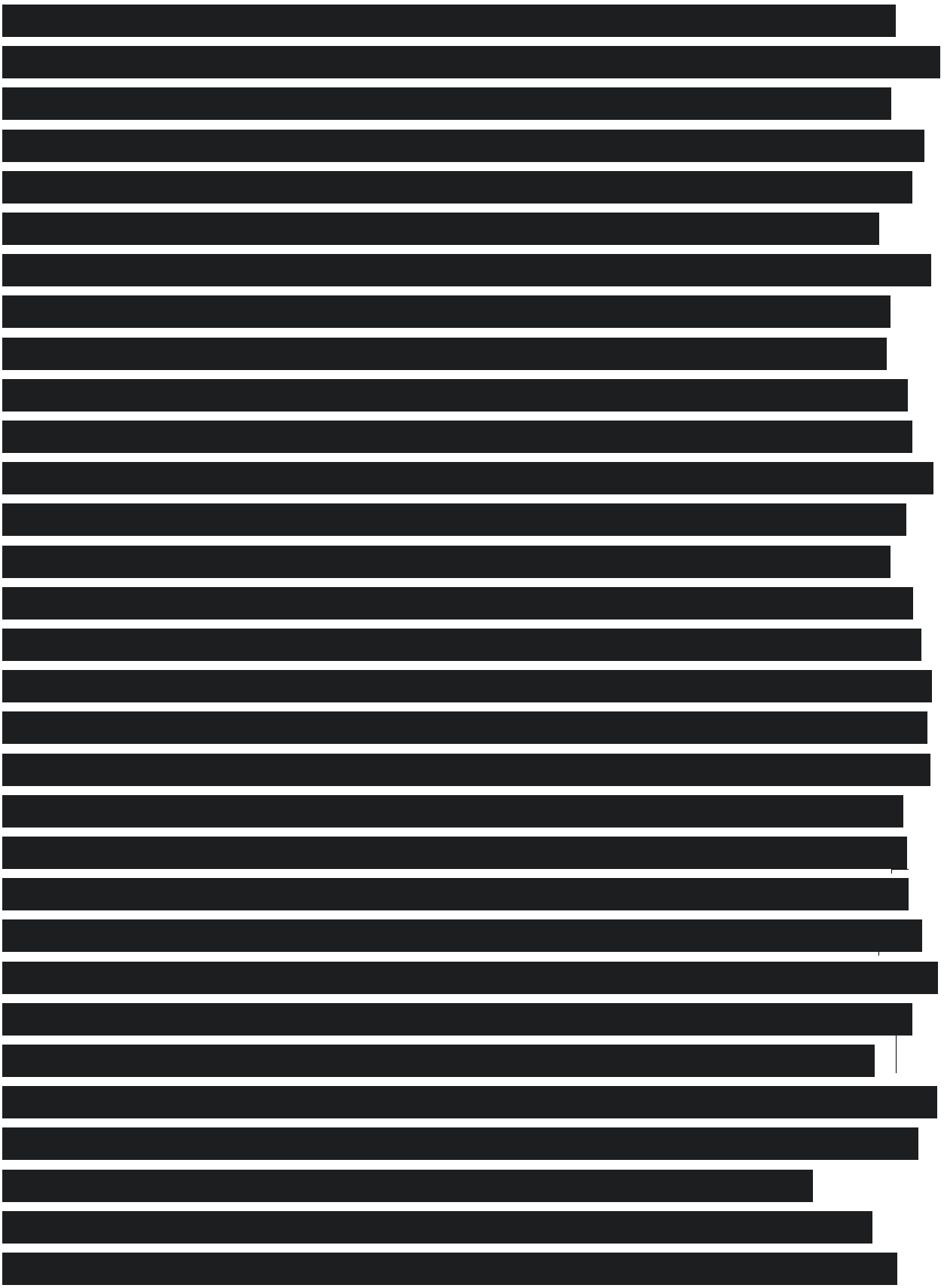

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[redacted]

[redacted]

[redacted]

3. STUDY ENDPOINTS

3.1. Primary

3.1.1. Phase 1b: DLTs occurring in the first 2 cycles (8 weeks), as defined below, graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), Version 4.03

3.1.2. Phase 2: CR rate by IWG 2006 criteria (appendix B)

3.2. Secondary

3.2.1. Duration of response defined as the time between achieving response and progression of

disease.

3.2.2. OS at 8 months

3.2.3. Proportion of subjects achieving hematological improvement (HI), partial response (PR), complete response (CR), and/or marrow CR (mCR) by the IWG 2006 criteria (see appendix B)

3.2.4. Proportion of subjects who obtain a response as defined by IWG 2006 criteria with a baseline *TP53* VAF ≥ 20% versus < 20%

3.2.5. Proportion of subjects who achieve ≥ 50% reduction in *TP53* VAF.

3.2.6. Progression free survival (PFS) and OS in clonal responders (≥ 50% reduction in *TP53* VAF) versus non-responders

3.2.7. To determine expression of genes related to p53-mediated signal transduction before and after APR-246 treatment

3.2.8. To determine ROS production before and after APR-246 treatment

3.2.9. AML transformation according to World Health Organization (WHO) criteria

3.2.10. OS

3.2.11. LFS

3.2.12. Determine recurrent gene mutations in *ASXL1, BCOR, BRAF, CALR, CBL, CEBPA, CSF3R, DNMT3A, ETV6, EZH2, GATA2, GNAS, IDH1, IDH2, JAK2, KIT, KRAS, MPL, NF1, NPM1, NRAS, PDGFRA, PDGFRB, PHF6, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SMC1A, SMC3, SRSF2, STAG2, STAT3, STAT5b, TET2, TP53, U2AF1, WT1,* and *ZRSR2* at study entry and serially throughout treatment to assess changes in somatic mutation landscape

4. PATIENT SELECTION

4.1. Eligibility criteria

Inclusion Criteria:

1. Patient has signed the Informed Consent (ICF) and is able to comply with protocol requirements
2. Patient has adequate organ function as defined by the following laboratory values:
   a. Serum creatinine ≤ 2 x upper limit of normal (ULN)
   b. Total serum bilirubin < 1.5 x ULN or total bilirubin ≤ 3.0 x ULN with direct bilirubin within normal range in patients with well documented Gilbert's Syndrome or

hemolysis or who required regular blood transfusions

c. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) < 2.5 x ULN

3. Age ≥18 years at the time of signing the informed consent form
4. Documented diagnosis of myelodysplastic syndrome (MDS), MDS/ myeloproliferative neoplasm (MPN), chronic myelomonocytic leukemia (CMML) by WHO criteria or AML with 20-30% myeloblasts (refractory anemia with excess blasts in transformation (RAEB-T) by French-American-British (FAB) criteria)
5. Documentation of a *TP53* gene mutation by NGS based on central or local evaluation
6. Revised International Prognostic Scoring System (IPSS-R) criteria for Intermediate, High-risk or Very High-risk.
7. An Eastern Cooperative Oncology Group (ECOG) performance status score of 0, 1, or 2 is required.
8. If of childbearing potential, negative pre-treatment urine or serum pregnancy test.
9. If of childbearing potential (males and females), willing to use an effective form of contraception such as latex condom, hormonal birth control, intrauterine device or double barrier method during chemotherapy treatment and for at least six months thereafter.

Exclusion Criteria

1. Patient has a known history of HIV or active hepatitis B or active hepatitis C infection (testing not mandatory).
2. Patient has any of the following cardiac abnormalities (as determined by treating MD):
   a. Symptomatic congestive heart failure
   b. Myocardial infarction ≤ 6 months prior to enrollment
   c. Unstable angina pectoris
   d. Serious uncontrolled cardiac arrhythmia
   e. QTc ≥ 470 msec
3. Concomitant malignancies or previous malignancies with less than a 1-year disease free interval at the time of signing consent. Patients with adequately resected basal or squamous cell carcinoma of the skin, or adequately resected carcinoma in situ (e.g. cervix) may enroll irrespective of the time of diagnosis.
4. Prior exposure to azacitidine, decitabine or investigational hypomethylating agent.
5. Use of cytotoxic chemotherapeutic agents, or experimental agents (agents that are not

commercially available) for the treatment of MDS, MDS/MPN, CMML or AML within 14 days of the first day of study drug treatment.

6. No concurrent use of erythroid stimulating agents, G-CSF, GM-CSF is allowed during study except in cases of febrile neutropenia where G-CSF can be used for short term. Growth factors must be stopped 14 days prior to study.
7. Patients with history of allogeneic stem cell transplantation.
8. Pregnant women are excluded from this study because APR-246 has not been studied in pregnant subjects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with APR-246, breastfeeding should be discontinued if the mother is treated with APR-246.

4.2 Inclusion of Women and Minorities

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

5. STUDY DESIGN

This study will be a multi-institution, open-label, phase Ib/II clinical trial conducted in 2 parts: a Phase 1b part followed by a Simon's two-stage Phase 2 design. The study will assess the safety and efficacy of APR-246 in combination with azacitidine for the treatment of *TP53* mutant myeloid neoplasms. All patients will have pre-screening NGS on peripheral blood (PB) or bone marrow (BM) samples to determine *TP53* mutational status and therefore eligibility to participate in this study. A PB sample will be obtained prior to treatment for NGS by the central laboratory to evaluate baseline *TP53* VAF and for serial analysis.

6. TREATMENT PLAN

a. APR-246 administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 11. Appropriate dose modifications for APR-246 are described in Section 10. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's MDS or MDS/MPN. APR-246 will be supplied by Aprea.

Phase 1b Part:

The initial Phase 1b part will evaluate APR-246 administered with azacitidine. Cohorts of at least 3 evaluable patients will be enrolled using a modified 3 + 3 design. Patients will receive IV infusions of APR-246 as a lead-in phase on days -14 to -11 starting at Dose Level 1 (see Table 5) prior to starting cycle #1 of combination therapy with azacitidine (see Figure 2). In all cycles, APR-246 will be administered as a 6-hour infusion daily for four consecutive days (study days -14 to -11 of the lead in phase and day 1 to 4 of each combination cycle). **The entire dose should be given even if the infusion needs to be extended beyond a total time of six hours (e.g., due to slightly larger start volume in prefilled infusion bags)**.

- Dose Level 1 (Starting dose): APR-246 21.0 mg/kg LBW (for first 45mins) + 29 mg/kg LBW (for 5hrs 15mins) on Days 1 to 4
- Dose Level 2: APR-246 30.0 mg/kg LBW (for first 45mins) + 45.0 mg/kg LBW (for 5hrs 15mins) on Days 1 to 4
- Dose Level 3: APR-246 37.0 mg/kg LBW (for first 45mins) + 63.0 mg/kg LBW (for 5hrs 15mins) on Days 1 to 4

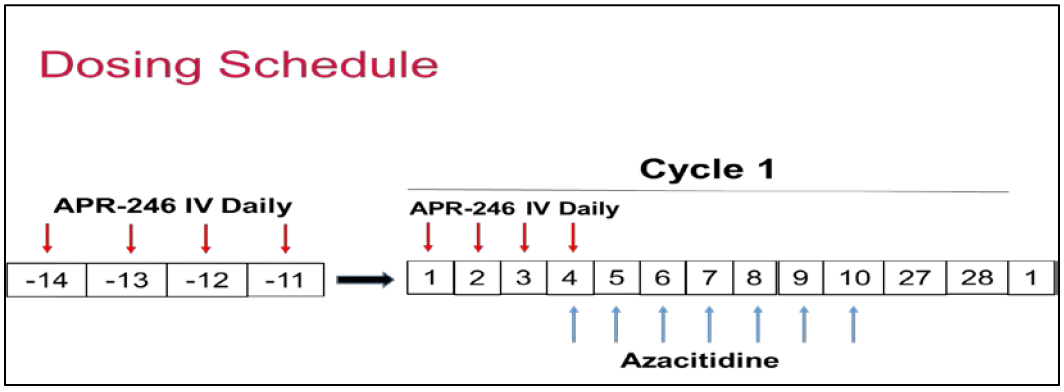

Figure 2. Dosing schedule for Phase 1b/2 investigation of APR-246 in combination with azacitidine

Combination therapy will consist of APR-246 on days 1-4 and azacitidine on days 4-10 of a 28 day cycle. Azacitidine will be administered subcutaneously (SC) or IV (SC preferred) at 75mg/m^2 for 7 days (either 7 consecutive days (days 4-10) or 2+5 (i.e., days 4-5 and 8-12)). Azacitidine will be administered on day 4 at the same time as the final infusion of APR-246. Azacitidine administration will occur following the completion of APR-246 infusion. Patients

will be followed for at least 6 weeks (lead-in phase + cycle 1) before the safety of each cohort can be fully assessed and decisions made for dose escalation in the next cohort. The MTD is defined as the dose level below which DLT is manifested in ≥33% of the patients or at dose level 1 if DLT is manifested in <33% of the patients. Phase 1b patients (n = 6 to approximately 18) will receive APR-246 in combination with azacitidine. Cohorts of at least 3 evaluable patients will be enrolled using a modified 3+3 design. If no DLT is observed in a cohort, the subsequent patient group will be enrolled at the next planned dose level. If a DLT is observed in 1 of 3 patients in a cohort at any dose level, then up to 3 additional patients will be enrolled at that dose level. If only 1 DLT occurs in the 6 patients treated at that dose level, the subsequent patient group will be enrolled at the next planned dose level. If 2 of 6 patients in a cohort have a DLT, dose escalation ceases and the MTD is the previous dose level. If only 3 patients were treated at the MTD, an additional 3 patients will be included in the cohort and if only 0 or 1 DLT occur in the 6 patients treated at that dose level, the MTD will be defined and we will proceed to Phase 2.

Table 5. Dose Levels for Treatment Part 1: APR-246+Azacitidine

Dose Level	APR-246 (mg/kg LBW)	Azacitidine (mg/m^2)
1 (Starting dose)	50	75
2	75	75
3	100	75

Phase 2 Part: Simon's two-stage minimax design

Following completion of the Dose Finding Phase, we will conduct a dose expansion, whereby patients will be treated with APR-246 administered at the MTD with azacitidine on a 28 day cycle utilizing the same dosing schedule as in Phase 1b with the exception of no lead in phase (i.e. patients will start at day 1 of combination therapy with APR-246 on days 1-4 and azacitidine on days 4-10 (or days 4-5 and 8-12) of a 28 day cycle, see Figure 2). If dose level 3 (i.e. 100mg/kg LBW) is the RP2D based on the phase 1b part of the trial, the dosing will be changed to an equivalent fixed dose regimen of 4500mg/patient (i.e. APR-246 administration in 2 consecutive steps as a loading dose (1500mg for first 45 minutes) and a maintenance dose

(3000mg for 5 hours 15minutes)). If dose reductions occur during the phase 2 part of the trial, administration of the APR-246 will also be given as a loading dose of 45 minutes and a maintenance dose of 5 hours and 15 minutes (see below).

Dose Modification	APR-246 Dose
Phase 2 Starting Dose Level (DL)	APR-246 4.5 g/patient/day 1.5 g/patient (for first 45 minutes) + 3.0 g/patient (for 5 hours 15 minutes)
First dose reduction DL-1	APR-246 4.0 g/patient/day 1.33 g/patient (for first 45 minutes) + 2.67 g/patient (for 5 hours 15 minutes)
Second dose DL-2*	APR-246 3.5 g/patient/day 1.16 g/patient (for first 45 minutes) + 2.34 g/patient (for 5 hours 15 minutes)

A Simon's two-stage minimax design will be applied, as follows:

Stage 1: Enroll a total of 24 evaluable patients at the MTD (including patients who were enrolled during the Phase 1 part of the study). If less than 6 of 24 patients achieve CR, then the study will be terminated early with the conclusion that the regimen does not warrant further investigation. If 6 or more patients achieve CR, then enrollment will be permitted to continue to Stage 2.

Stage 2: Enroll 21 more evaluable patients for a total of 45. If less than 14 of 45 patients achieve CR, then there is insufficient evidence to support continued study of this treatment. If 14 of 45 patients achieve CR, then there is sufficient evidence to support further study of APR-246 in combination with azacitidine in Phase 3. Up to 39 additional evaluable patients will be treated at the level of the MTD in the Phase 2 portion of the trial. The maximum total accrual is 51 patients. Disease assessments will occur following 3 cycles of therapy (Figure 3).

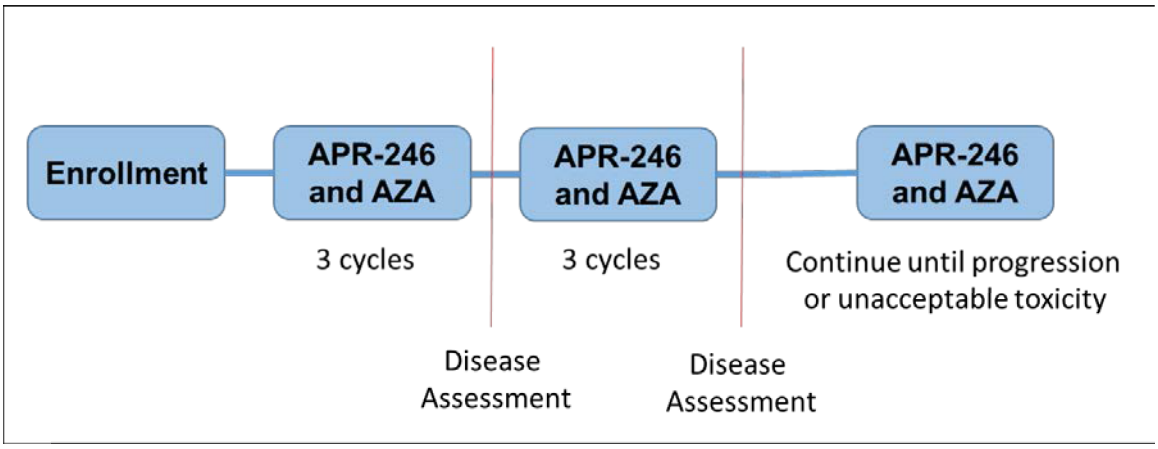

Figure 3. Phase 2 Schema

b. General Concomitant Medication and Supportive Care Guidelines

In general, the use of any concomitant medications/therapies deemed necessary for the care of the patient are allowed, with specific exceptions below. Prophylactic anti-emetic therapy is permitted where indicated.

Any disease progression that requires other specific anti-tumor therapy will be cause for discontinuation from the trial.

c. **Permitted Medications**

i. Growth Factors

Erythropoiesis-stimulating agents (ESAs) are not allowed for anemia during the study. G-CSF is allowed during study only in cases of febrile neutropenia where G-CSF can be used for short term.

ii. Anticoagulant Therapy

Subjects who are taking warfarin (or equivalent) may participate in this study; however, it is recommended that prothrombin time (PT-INR) and PTT be monitored carefully at least once per week for the first month, then monthly if the INR is stable. Subcutaneous heparin, low molecular weight heparin and oral anticoagulants are permitted.

iii. QT prolonging Agents

[redacted]

[redacted] For most such drugs there are alternative medicines that could be considered. For prophylaxis for nausea/vomiting, palonosetron is the preferred agent over ondansetron. If fungal prophylaxis/treatment is indicated, isavuconazonium is preferred. Additionally, the following medications are contraindicated while on APR-246:

- anti-arrhythmics class IA (e.g. quinidine, hydroquinidine, disopyramide)
- anti-arrhythmics class III (e.g. amiodarone, sotalol, dofetilide, ibutilide)
- antipsychotics (e.g. phenothiazines, pimozide, sertindole, haloperidol, sultopride)
- tricyclic antidepressants
- some antimicrobials (e.g. saquinavir, sparfloxacin, intravenous erythromycin, pentamidine, antimalarials particularly halofantrine)
- some antihistamines (e.g. terfenadine, astemizole, mizolastine)
- other medicines (e.g. cisapride, intravenous vincamine, bepridil and diphemanil).

iv. Blood Products

The use of blood products to include packed red blood cells (PRBCs) and platelet transfusions are permitted and to be given at the discretion of the treating physician. Recommended guidelines for transfusion include a platelet threshold of 10,000/L for platelet transfusion and a hemoglobin threshold of 8.0g/dL for PRBC transfusion or as clinically deemed by the discretion of treating physician.

d. Prohibited Medications

i. Other anticancer therapy

Anticancer therapy (chemotherapy, endocrine, biologic or radiation therapy, and surgery) other than the study treatments must not be given to patients while the patient is enrolled in the treatment portion of the trial. If such agents are required for a patient then, the patient must be permanently discontinued from the treatment portion of the study. Exception: for breast cancer or prostate patients on adjuvant hormonal therapy (e.g., anastrozole/tamoxifen or leuprolide) who have been disease free for at least 1 year.

ii. Other investigational therapies

Other investigational therapies must not be used while the patient is on the study.

7. Duration of Therapy

Subjects will be treated for a total of 6 cycles. For subjects who have stable disease or better, treatment may continue until one of the following criteria applies:

e. Inter-current illness that prevents further administration of treatment,

f. Unacceptable adverse event(s),

g. Patient decides to withdraw from the study, or

h. General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

e. Evidence of disease progression by the IWG 2006 criteria, not applicable at bone marrow evaluation at day -10 of lead in phase.

Subjects who wish not to continue treatment will complete their end of study visit upon completion of cycle 6.

8. Duration of Follow-Up

Subjects will be followed as per calendar on treatment for 6 cycles. After 6 cycles, patients who continue on treatment will be followed monthly. Off treatment data on AML transformation and overall survival will be updated every 6 months or until death, whichever occurs first. Subjects removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

9. Criteria for Removal from Study

a. Subject Completion

A subject will be considered to have completed the study if the subject meets at least 1 of the following criteria:

- The subject has progressive disease.
- The subject died during the study.
- The subject experience a treatment related AE that led to withdrawal from the study.
- The subject starts new treatment for their underlying disease (MDS/CMML/AML).

b. Subject Withdrawal from Study

A subject may voluntarily withdraw from study medication or withdraw consent from the study at any time. The investigator may also, at his or her discretion, discontinue a subject from participating in the study at any time. The investigator and/or designated staff will record the date and the reason for subject withdrawal from the study.

c. Subject Withdrawal from Study Medication

If the subject is permanently withdrawn from treatment with study medication, but does not withdraw consent, the investigator must make every effort to have the subject complete all withdrawal assessments at the time of withdrawal, and complete all scheduled follow-up visits. Treatment with study medication must be discontinued if:

- The subject withdraws consent.
- Further participation would be injurious to the subject's health or well-being in the investigator's medical judgment.
- The study is terminated.
- The subject becomes pregnant.
- Evidence of disease progression according to IWG 2006 criteria (see Appendix B, not applicable at bone marrow evaluation at day -10 of lead in phase).
- A subject is significantly non-compliant with the requirements of the protocol.
- A subject has an adverse experience that would, in the investigator's judgment, make continued participation in the study an unacceptable risk.

d. Study safety-related stopping rules

In the following conditions, the study may be temporarily or permanently stopped, pending review by the Steering Committee and the Sponsor:

1. Patient death due to APR-246. If there is Grade 5 toxicity definitely or probably attributed to study treatment within 28 days of APR-246 administration or if there are subsequent deaths that are definitely or probably related to study treatment.

10. DOSING DELAYS/MODIFICATIONS

1. Dose modification and dose delay

For patients who do not tolerate the protocol-specified dosing schedule, dose adjustments are permitted in order to allow the patient to continue the study treatment. All dose modifications, interruptions or discontinuations must be based on the worst preceding toxicity as graded by the

NCI Clinical Toxicity Criteria (NCI-CTCAE version 4.03). Once a dose has been reduced during a treatment cycle, re-escalation will not be permitted during any subsequent cycle (will exception of CNS events, see 10.2). If the administration of APR-246 is interrupted for reasons other than toxicity, then treatment with the respective study drug may be resumed at the same dose. The same provision applies if the patient experienced an unacceptable toxicity not specifically described in Table 8 provided that this toxicity resolved to ≤ CTCAE grade 1, unless otherwise specified.

2. Treatment of adverse events of the CNS related to APR-246

If a patient reports any clinical adverse event of any grade during the administration period of APR-246 that could be considered to originate from the central nervous system (e.g., dizziness, vertigo, nausea) then the following medications may be used (Table 6). Prochlorperazine is not required to be taken at any point but is per discretion of the treating physician.

Table 6. Adjuvant Medications for use with APR-246

Indication	**Supportive Measure**
Central Nervous System (CNS) symptoms (Treatment and Prevention)	Prochlorperazine 10 mg orally tid as needed Start Day -1 prior to APR-246 administration
Persistent CNS symptoms	Diphenhydramine 50 mg IV (or similar anti-histamine)

Prochlorperazine 10 mg orally tid (three times daily) to continue until end of Day 4 of the cycle. When used prophylactically for future treatment, start the day prior to Day 1 administration of APR-246. If during the infusion the patient continues to remain symptomatic, intravenous administration of diphenhydramine 50 mg (or similar anti-histamine) should be considered. If prophylactic prochlorperazine results in ≤ grade 1 CNS toxicity on subsequent doses, re-escalation to previous dose level can occur with the next cycle at the discretion of the study investigator.

3. Treatment interruption and treatment discontinuation

Patients requiring a dose delay of > 28 days must be permanently discontinued from study drug. Following 4 cycles of therapy for responding patients (i.e. CR, PR, HI, or marrow CR), treatment with APR-246 and azacitidine can be delayed per the study investigator for up to 14 days to allow for count recovery or at investigator discretion post discussion with Moffitt PI. Non-hematologic grade 4 treatment related adverse events will lead to permanent discontinuation, irrespective of recovery time, unless otherwise specified. Exceptions would include nausea/vomiting/diarrhea which can be controlled by medications and/or asymptomatic electrolyte imbalances which can be corrected. In addition, in most instances, patients that experience a prolonged treatment interruption because of an adverse event and/or a grade 3 adverse event will decrease the dose of study drug after their recovery (see specific tables for dose adjustment guidelines). Furthermore, patients requiring >1 dose reduction for APR-246 will be permanently discontinued from study drug. Patients who permanently discontinue all study drugs should have follow-up within 30 days after discontinuation of all study treatment or resolution of the AE to ≤ grade 1, whichever occurs first, that includes all study assessments appropriate to monitor the event.

4. DLT Definition

Patients will be evaluated for DLTs during the lead-in phase and first cycle of combination therapy, i.e., 6 weeks for purpose of deciding the dose for next cohort (see Table 1) but DLTs will continue to be evaluated and reported through all cycles on study. DLT is defined as follows based on the CTCAE v 4.03:

-Treatment related non-hematological CTCAE grade 3-4 toxicity that lead to dose modification or withdrawal.

-Absolute neutrophil count (ANC) not recovering to >500/μL by day 42 of a cycle in the absence of active leukemia/myelodysplasia will be considered a DLT.

-Grade 3 metabolic/electrolyte abnormalities that are not clinically significant, and are adequately controlled within 72 hours are not to be considered DLT.

-Grade 3 nausea/vomiting/diarrhea or CNS toxicity that does not resolve within 28 days despite treatment interruption and maximum medical therapy will be considered a DLT.

5.Criteria for APR-246 dose modifications (Phase 2)

For patients who do not tolerate APR-246 initial dosing schedule, dose adjustment is permitted in order to allow the patient to continue on study drug. In general, doses should not be reduced

for grade 1 toxicities or grade 2 toxicities with resolution on dose interruption (see Table 8), but treatment to control symptoms should be provided as appropriate. The starting dose level of APR-246 will be based on the recommended phase 2 dose (RP2D) upon completion of the phase 1 component of the trial (see Table 5). If dose level 3 (i.e. 100mg/kg LBW) is the RP2D based on the phase 1b part of the trial, the dosing will be changed to an equivalent fixed dose regimen of 4500mg/patient. For each patient, a maximum of 2 dose reductions of APR-246 (as outlined in Table 7) will be allowed. Patients requiring an additional dose reduction will be discontinued from treatment with APR-246. Patients whose treatment is interrupted or permanently discontinued due to an adverse event or clinically significant laboratory value must be followed up within 30 days, or resolution of the AE to ≤ grade 1, whichever occurs first, that includes all study assessments appropriate to monitor the event.

Table 7 Dose reduction steps for APR-246 (Phase 2 only)

APR-246 dose levels and dose reduction*	
Starting dose level	RP2D days 1-4 of each cycle
Dose level – 1	RP2D-500mg fixed dose days 1-4 of each cycle**
Dose level – 2	RP2D-1000mg fixed dose days 1-4 of each cycle**

*Dose reduction should be based on the worst preceding toxicity.

** = If a dose reduction below dose level –2 is required, the patient should be permanently discontinued from APR-246

Guidelines for dose modification and dose interruption for toxicities suspected to be related to APR-246 are described in Table 8.

Table 8 APR-246 – recommended dose modifications and criteria for treatment interruption and re-initiation with treatment-related adverse events

These changes must be recorded on the Dosage Administration Record CRF.

Worst toxicity (CTCAE 4.03 Grade)**	**Dose Modifications for APR-246**
HEMATOLOGICAL (only applicable for patients with normal baseline absolute neutrophil count (ANC) and platelets)	
Neutropenia (ANC)	
Severe Febrile neutropenia	Omit dose until resolved, then ↓ 1 dose level

Worst toxicity (CTCAE 4.03 Grade)**	**Dose Modifications for APR-246**
(ANC < 0.5 x 10^9/L, temperature of ≥ 38 °C and ICU admission)	
Thrombocytopenia	
Grade 4 (PLT < 25 x 10^9/L) **AND** major bleeding event	Permanently discontinue patient from APR-246
RENAL	
Serum creatinine	
Grade 1 (< 2 x ULN)	Maintain dose level
Grade 2 (2 – 3 x ULN)	Omit dose until resolved to ≤ grade 1, then: If resolved in ≤ 7 days, then maintain dose level If resolved in > 7 days, then ↓ 1 dose level
Grade 3 (> 3.0 – 6.0 x ULN)	If resolved in ≤ 7 days, then ↓ 1 dose level If resolved in > 7 days, discontinue patient from APR-246
Grade 4 (> 6.0 x ULN)	Permanently discontinue patient from APR-246
HEPATIC	
Bilirubin (*for patients with Gilbert Syndrome, these dose modifications apply to changes in direct bilirubin only) will be fractionated if elevated	
Grade 1 (> ULN - 1.5 x ULN)	Maintain dose level with LFTs* monitored as per protocol
Grade 2 (> 1.5 - 3.0 x ULN) with ALT or AST ≤ 3.0 x ULN	Omit dose until resolved to ≤ Grade 1, then: If resolved in ≤ 7 days, then maintain dose level If resolved in > 7 days, then ↓ 1 dose level
Grade 3 (> 3.0 - 10.0 x ULN) with ALT or AST ≤ 3.0 x ULN	Omit dose until resolved to ≤ Grade 1, then: If resolved in ≤ 7 days, ↓ 1 dose level If resolved in > 7 days, discontinue patient from APR-246
Grade 4 (> 10.0 x ULN)	Permanently discontinue patient from APR-246
AST or ALT	
Grade 1 (> ULN – 3.0 x ULN)	Maintain dose level with LFTs* monitored per protocol
Grade 2 (> 3.0 - 5.0 x ULN) without total bilirubin elevation to > 2.0 x ULN	Omit dose until resolved to ≤ Grade 1, then If resolved in ≤ 7 days, then maintain dose level If resolved in > 7 days, then ↓ 1 dose level
Grade 3 (> 5.0 - 20.0 x ULN) without total bilirubin elevation to > 2.0 x ULN	Omit dose until resolved to ≤ Grade 1, then If resolved in ≤ 7 days, then maintain dose level If resolved in > 7 days, then ↓ 1 dose level

Worst toxicity (CTCAE 4.03 Grade)**	**Dose Modifications for APR-246**
Grade 4 (> 20.0 x ULN) without bilirubin elevation to > 2.0 x ULN	Permanently discontinue patient from APR-246
*(LFTs include albumin, ALT, AST, total bilirubin (fractionated if total bilirubin > 2.0 x ULN), alkaline phosphatase.	
CNS – dizziness, dyskinesia and ataxia	
Grade 1	Maintain dose level
Grade 2	If resolved with medical therapy (e.g., prochlorperazine or antihistamines), continue same dose level If not resolved despite treatment interruption and maximal medical therapy, stop infusion and ↓ 1 dose level for subsequent dose
Grade 3	If resolved with medical therapy (e.g., prochlorperazine or antihistamines), continue same dose level If not resolved despite treatment interruption and maximal medical therapy, stop infusion and ↓ 1 dose level for subsequent dose
Grade 4	Permanently discontinue patient from APR-246.
Infusion Related Reaction	
Grade 1	Maintain dose level.
Grade 2	Maintain dose level; Symptomatic management (e.g., antihistamines, corticosteroids, narcotics, IV fluids)
Grade 3	If resolved in < 4 hours with treatment interruption and medical therapy (e.g., antihistamines, corticosteroids, narcotics, IV fluids), continue same dose level and rate. If not resolved in < 4 hours despite treatment interruption and maximal ~~m~~edical therapy, stop infusion and ↓ 1 dose level for subsequent dose
Grade 4	Permanently discontinue patient from APR-246.
Nausea/Vomiting/Diarrhea	
Grade 1	Maintain dose level
Grade 2	If resolved, then maintain dose level If not resolved despite maximal medical therapy, then ↓ 1 dose level
Grade 3	If resolved, then maintain dose level If not resolved despite maximal medical therapy, then ↓ 1 dose level
Grade 4	Permanently discontinue patient from APR-246

Worst toxicity (CTCAE 4.03 Grade)**	Dose Modifications for APR-246
QTc Prolongation	
Grade 1	Maintain dose level
Grade 2	Correct electrolytes and discontinue concomitant medications that cause QTc prolongation. If resolved, then maintain dose level. If not resolved, then ↓ 1 dose level
Grade 3	Omit dose until resolved to ≤ Grade 1, If resolved, then ↓ 1 dose level If not resolved despite correction of electrolytes and discontinuation of concomitant medications that cause QTc prolongation, permanently discontinue maximal medical therapy
Grade 4	Permanently discontinue patient from APR-246
** Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. These changes must be recorded on the Dosage Administration Record eCRF.	

11. ADVERSE EVENTS: REPORTING REQUIREMENTS

All SAEs should be reported to the principal investigator at H. Lee Moffitt Cancer Center as well as the clinical research coordinator immediately. All SAEs must also be reported within 24 hours of being known to Aprea. These toxicities will be reviewed by the principal investigator and appropriate measures taken in terms of delaying or reducing or omitting the next cycle of therapy. Any SAE occurring from the time of administration of the first dose of study drug until 30 days after the final dose of study drug must be promptly reported to Aprea via fax on a redacted copy of the Oncore database SAE form. Any SAE occurring after this time that is thought to be possibly related to prior treatment with study drug should also be reported and recorded on the AE log. SAEs must be followed until resolution or deemed irreversible. All relevant follow-up information must be promptly reported and recorded in the Oncore database.

a. Definition of an AE

Any untoward medical occurrence in a subject or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. Note: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated)

temporally associated with the use of a medicinal product. Events meeting the definition of an AE include:

- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after investigational product administration even though it may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational product or a concomitant medication (overdose per se will not be reported as an AE/SAE). "Lack of efficacy" or "failure of expected pharmacological action" per se within the duration of initial APR-246 treatment/exposure of 6 cycles will not be reported as an AE or SAE. However, the signs and symptoms and/or clinical sequelae resulting from lack of efficacy will be reported if they fulfill the definition of an AE or SAE.
- Stop dates for AEs can be either the last date that the AE occurred, or the next date which reflects a change in grade/status.

Events that **do not** meet the definition of an AE include:

- Medical or surgical procedure (e.g., endoscopy, appendectomy); the condition that leads to the procedure is either an AE or (if existing pre-study) a baseline medical history item.
- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital. Example: Hospital admission for sole purpose of red blood cell transfusion).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.
- Signs/symptoms of Gr. 1 or Gr. 2 expected side effects of Azacitidine, as noted in visit notes by treating MD and/or designated staff. This includes (for example, but not limited to) Gr. 1-2: Nausea, constipation, injection site reactions, decreased neutrophil count, decreased platelet count. Must be located in source document that items are specifically attributable to Azacitidine.

- Signs/symptoms of Gr. 1 or Gr. 2 expected side effects of red blood cell and/or platelet transfusion needs. This includes (for example, but not limited to) Gr. 1-2: dizziness, headache, hypotension, hypertension, dyspnea, muscle weakness, ataxia, fall, petechiae, bruising. Must be located in source document that items are specifically attributable to the need for red blood cell transfusion and/or platelet transfusion.
- Items which are transient (example: seasonal allergies; joint pain during thunderstorms) will be listed as baseline medical history items and are not considered new AEs if only occurring during certain times of year or with certain events if ongoing chronically, and do not increase in grade and are not attributable to study medication.

b. Serious Adverse Event (SAE) Definition

A serious adverse event is one that at any dose (including overdose):

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event
- Suspected positive pregnancy

NOTE: The term "life-threatening" the definition refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

- Requires hospitalization or prolongation of existing hospitalization. NOTE: In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out-patient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
- Results in disability/incapacity

NOTE: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.

- All treatment related grade 4 non-hematologic laboratory abnormalities assessed using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v 4.03.

Medical or scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

c. Relationship to Investigational Product

It is a regulatory requirement for investigators to assess relationship to investigational product based on information available. The assessment should be reviewed on receipt of any new information and amended if necessary. "A reasonable possibility" is meant to convey that there are facts/evidence or arguments to suggest a causal relationship. Facts/evidence or arguments that may support "a reasonable possibility" include, e.g., a temporal relationship, a pharmacologically-predicted event, or positive dechallenge or rechallenge. Confounding factors, such as concomitant medication, a concurrent illness, or relevant medical history, should also be considered.

d. Laboratory and Other Safety Assessment Abnormalities Reported as AEs and SAEs

Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECGs, vital signs measurements), including those that worsen from baseline should be recorded as per the NCI-CTCAE 4.03 criteria. However, these laboratory results are to be recorded as AEs or SAEs if deemed clinically significant in the medical and scientific judgment of the investigator or treating physician. Any clinically significant safety

assessments that are associated with the underlying disease are **not** to be reported as AEs or SAEs, except for findings judged by the investigator or treating physician to be more severe than expected for the subject's condition or death. Data will be collected for typical disease-related events such as anemia, leukopenia or worsening of thrombocytopenia. All infections experienced during the study are to be recorded as AEs or SAEs.

e. Disease-Related Events and/or Disease-Related Outcomes Not Qualifying as SAEs

During the study period, the following conditions will not qualify as an AE or SAE provided they are not considered attributable to study medication:

- Events that occur prior to the 1st dose of APR-246.
- Cases of disease progression.

f. Pregnancy

Any pregnancy that occurs during study participation must be reported. To ensure subject safety, each pregnancy must be reported to the FDA with CC notification to Aprea within 2 weeks of learning of its occurrence. The pregnancy must be followed up to determine outcome (including premature termination) and status of mother and child. Pregnancy complications and elective terminations for medical reasons must be reported as an AE or SAE. Spontaneous abortions must be reported as an SAE. Any SAE occurring in association with a pregnancy, brought to the investigator's attention after the subject has completed the study and considered by the investigator as possibly related to the investigational product, must be promptly reported to the pharmacovigiliance groups at the H. Lee Moffitt Cancer Center and Aprea. In addition, the investigator must attempt to collect pregnancy information on any female partners of male study subjects who become pregnant while the subject is enrolled in the study. Pregnancy information must be reported to the H. Lee Moffitt Cancer Center as described above.

g. Prompt Reporting of Serious Adverse Events and Other Events to Aprea

The investigator must inform Aprea of any SAE within 24 hours of being aware of the event. This must be documented in Oncore, printed, signed, dated and emailed to Aprea via Theradex at: SafetyDeskEurope@theradex.co.uk . This form must be completed and supplied to Aprea within 24 hours/1 business day. The initial report must be as complete as possible, including an assessment of the causal relationship between the event and the investigational product, if available.

Information not available at the time of the initial report must be documented in Oncore as a follow up SAE (also printed, signed, dated and emailed to Aprea via Theradex at: SafetyDeskEurope@theradex.co.uk . If the SAE is deemed reportable to the FDA, then a MedWatch will also need to be completed and sent to Moffitt Cancer Center for review (see IND section 17.5 for further details) and FDA submission.

A final report to document resolution of the SAE is required. Any serious adverse events which occur during the clinical study or within 30 days of receiving the last dose of study medication, whether or not related to the study drug, must be reported by the investigator. In addition, any SAEs which occur as a result of protocol specific diagnostic procedures or interventions must also be reported. SAEs brought to the attention of the investigator at any time after cessation of APR-246 and considered by the investigator to be related or possibly related to APR-246 must be reported to Aprea with subsequent to the FDA if and when they occur. Additionally, in order to fulfill international reporting obligations, SAEs that are related to study participation (e.g., procedures, invasive tests, and/or change from existing therapy) or are related to a concurrent medication will be collected and recorded from the time the subject consents to participate in the study until he/she is discharged.

	Initial Reports		**Follow-up Information on a Previous Report**	
Type of Event	**Time Frame**	**Documents**	**Time Frame**	**Documents**
All SAEs	24 hours*	"SAE" data collection tool	24 hours*	Updated "SAE" data collection tool
Pregnancy	2 Weeks*	Pregnancy Notification Form	2 Weeks*	Pregnancy Follow-up Form

*From the time point when the SAE or pregnancy became known to reporter.

h. **Regulatory Reporting Requirements for SAEs**

Prompt notification of SAEs by the investigator to Aprea is essential so that legal obligations and ethical responsibilities towards the safety of subjects are met. The sponsor-investigator has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. The sponsor-investigator will comply with specific regulatory requirements relating to safety reporting to the regulatory

authority/Institutional Review Board (IRB), the FDA, notification to Aprea, and sub-investigators. Investigator safety reports are prepared for suspected unexpected serious adverse reactions according to local regulatory requirements and those policies set forth by the FDA and are forwarded to investigators and Aprea as necessary. An investigator who receives an investigator safety report describing an SAE(s) or other specific safety information (e.g., summary or listing of SAEs) from the H. Lee Moffitt Cancer Center will file it with the CIB and will notify the IEC /IRB, if appropriate according to local requirements.

12. PHARMACEUTICAL INFORMATION

a. Study Treatments and Preparation

[redacted]

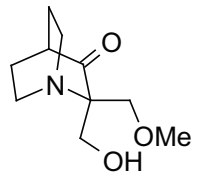

Figure 3. Structure of APR-246

b. Study drug compliance and accountability

1. Study drug accountability

The investigator or designee must maintain an accurate record of the shipment and

dispensing of study treatment in a drug accountability ledger. At study close-out, and, as appropriate during the course of the study, the investigational pharmacy will destroy all used and unused study treatment, packaging, and drug labels per the MCC drug destruction policy.

2. Disposal and destruction

Study drug destruction at the investigational site will only be permitted if authorized by Aprea after the site's drug destruction policy has been reviewed and deemed acceptable and if permitted by local regulations.

13. Study Calendar

All screening evaluations will be performed within 4 weeks prior to the start of APR-246 and azacitidine treatment. Subjects must have a bone marrow biopsy and aspirate (including cytogenetics) performed sometime within 4 weeks prior to the start of treatment. All transfusion and pre-transfusion hemoglobin or platelet count must be recorded for the 8 weeks prior to initiation of study treatment. Strict adherence to the visit schedule is required. In the event that a visit or test cannot be scheduled on the exact visit day, a window of ± 7 working days is allowable. Tests (including bone marrow biopsies and aspirates) done within the screening period prior to signing informed consent are allowed for use in this study.

1. **Baseline Assessment**: within 4 weeks of starting treatment

Medical history including:

- Disease characteristics such as WHO/FAB subtype, R-IPSS score, prior treatments.
- ECOG performance status
- Concurrent medication review
- Routine physical examination
- EKG
- Bone marrow exam, including cytomorphology, cytogenetic assessment, and flow cytometry analysis
- Documentation of a *TP53* gene mutation by NGS based on central or local evaluation
- Laboratory assessments:
  - ► CBC with differential (including platelet count)
  - ► Clinical chemistries including BUN, creatinine, sodium, potassium, alkaline phosphatase, ALT, AST, total bilirubin and albumin

- ► Urine or serum pregnancy test for females of childbearing potential will be performed at Screening or on first day of study medication, prior to first dose of study medication

- Record any blood and blood supportive care products for the 8 weeks prior to start of study medication.

2. **Treatment Period** (Day -14 - Cycle 6): APR-246 and azacitidine will be administered over a 28 day treatment cycle. Patients will have medical history, physical exam, performance status every 4 weeks. Subjects will have a CBC with differential (including platelet count) and a complete metabolic profile (CMP) performed. A BM aspirate and biopsy with cytogenetic analysis will be performed after cycle 3 and 6 (C3D22 and C6D22). An NGS myeloid panel will be performed on PB prior to cycle 1 and after cycle 3 and 6 (C3D22 and C6D22). If *TP53* mutation is only present on BM analysis at screening, serial NGS myeloid panel can be performed on bone marrow. These analyses will be used to assess pathologic response, cytogenetic response, molecular response and disease progression.
3. **End of Treatment**: Subjects will complete a response assessment within 4 weeks after their last dose of APR-246 and azacitidine. Subjects discontinuing study early should complete their end of treatment visit within 4 weeks after their last dose of investigational product. Physical exam, vital signs, adverse event reporting, CBC, CMP and BM aspirate and biopsy with cytogenetic analysis will be performed. If end of treatment tests are declined or not feasible, not considered deviation.

Continuation Phase: After completing 6 cycles of therapy, subjects responding or who have stable disease may continue APR-246 and azacitidine. Bone marrow biopsy and aspirate will be repeated after every 3 cycles. An NGS myeloid panel should be performed on PB every 3 cycles. A CBC and CMP will be obtained every 4 weeks.

4. **End of treatment assessment**: includes best response achieved, date of first response, date of loss of response, reason for discontinuation.

Off treatment follow-up: include vital status, date of death/last contact, transformation to AML and the date of transformation to AML if applicable.

Study Calendar Evaluation*	**Baseline/ Screening ***	**Lead-in phase*** **						**Cycle 1**							**Cycle 2 and Subsequent Cycles**					**Every 3 Cycles**	**End of Treatment ******
		Day -14	Day -13	Day -12	Day-11	Day -10	Day -7	Day 1	Day 2	Day 3	Day 4[a]	Day 8	Day 15	Day 22	Prior to/ Day 1[b]	Days 1-4[a]	Day 8	Day 15	Day 22		
Informed consent	X																				
Baseline medical history [c]	X																				
Physical exam [d]	X	X						X							X						X
Height	X																				
Weight	X	X						X							X						X
Vital Signs[d]	X	X	X	X	X			X	X	X	X				X	X					X
ECOG PS	X	X						X							X						X
APR-246		X	X	X	X			X	X	X	X					X					
Azacitidine[a]											X					X					
Bone marrow Biopsy and Aspiration**	X					X **														X	X
Flow Cytometry and Cytogenetics	X																			X	X
NGS Myeloid Panel[e]	X																			X	X
Correlative Studies[f]	X					X **														X	
Response Assessment																				X	X
Hematology [g]	X	X					X	X				X	X	X	X		X	X	X		X
Blood chemistry [h]	X	X					X	X				X	X	X	X		X	X	X		X
Pregnancy test [i]	X																				
EKG[j]	X				X			X			X				X						
Transfusion Log	X	<----------From 8 weeks prior to randomization throughout end of treatment date -------->																			X
Adverse events		<----------Starting C1D-14 (post 1st dose) through 30 days post end of treatment--------->																			X

APR-246 will be administered as monotherapy in the lead-in phase IV over 4 days (days -14 to -10) during the Phase 1b portion only***.
For combination therapy, APR-246 is administered IV on days 1-4 of each 28 day cycle.

a: Azacitidine is administered SC or IV over 7 days starting day 4 (7 consecutive days, 4-10; or 2+5 (i.e., days 4-5 and 8-12))

b: Day 1 evaluations for subsequent cycles are to be done within 7 days prior to next cycle drug administration.

c: Full medical history will be obtained by the APP/MD at baseline for safety and eligibility purposes, though only baseline medical history items will be recorded on CRFs – this will include any issues/items from 4 weeks prior to screening date.

d: Physical exam and vital signs (including blood pressure, heart rate, respiration rate and temperature) will be completed for safety purposes though not recorded onto CRFs and items noted will be recorded as AEs where appropriate. Vitals will be collected prior to APR-246, 2 hours into infusion and at end of infusion (+/- 30 mins at all time points).

e: NGS myeloid panel will evaluate recurrent gene mutations and *TP53* VAF (secondary endpoints #4-6) at study entry and every 3 cycles from peripheral blood (PB) mononuclear cells (MNC). If *TP53* mutation is only present on BM analysis at screening, serial NGS myeloid panel can be performed on bone marrow MNC. Samples will be sent to Genoptix as the central laboratory for mutational analysis.

f: Correlative studies (secondary endpoints #7-8) will be performed on BM MNC during screening, day #-10 of lead in phase and every 3 cycles. If

specimen not collected (e.g. dry tap), not considered deviation.

g: Hematology including hemoglobin, WBC with differential, and platelet count.

h: Blood chemistry including sodium, potassium, BUN, glucose, ALT/AST, alkaline phosphatase, total protein, total bilirubin, albumin, creatinine, and calcium.

i: Pregnancy test; for women of childbearing potential, a negative pregnancy test (urine or serum) must be documented between Screening and first day of treatment on study. Must be documented prior to 1st dose of medication on study

j: EKG prior to initial dose of APR-246 and after infusion on day -11 of the lead in phase. EKG will be performed on day 1 and at end of infusion on day 4 of cycle 1 and then on day 1 evaluations of subsequent cycles.

Concurrent medications will not be captured on CRFs but will be reviewed with subjects in clinic at start of cycle visits to determine if they are reveal any new AEs or SAEs. For patients with documented CR or PR at disease assessment following cycle 3, hematology and blood chemistry assessments will be required for D1 of each subsequent cycle and more frequently per the discretion of the treating physician.

* All screening/baseline evaluations will be performed within 4 weeks prior to the start of APR-246 and azacitidine treatment. All assessments are to be performed on day 1 of each week. In the event that a visit or test cannot be scheduled on the exact visit day, a window of ±7 working days is allowable.

**Bone marrow aspirate will be only be required for day #-10 in the phase 1b component of the trial. In the phase 2 component, bone marrow assessment completed within 1 month of signing consent will be accepted to fulfill screening requirement as long as it has been completed following the last MDS treatment (excluding ESAs) and there are unstained core biopsy or clot sections available for p53 immunohistochemistry.

***Lead-in phase will only be part of the Phase 1b portion of the study.

****Continuation Phase: After completing cycle 6 response assessments, subjects who have stable disease or better may continue treatment with APR-246 and azacitidine on 28 day cycles. Bone marrow biopsy and aspirate will be repeated after every 3 cycles. A CBC and CMP will be obtained at Day 1 of each cycle or more often per study investigator.

End of Cycle 6 Evaluation and End of Treatment: Subjects discontinuing treatment early should complete their end of treatment visit within approximately 28 days of their last dose of investigational product. Physical exam, vital signs, adverse event reporting, CBC, and blood chemistry and BM aspirate and biopsy with cytogenetic and NGS analysis should be performed if feasible.

Off Treatment assessment: includes best response, date of first response, date of loss of response, reason for discontinuation.

Off study CRF: vital status, date of death/last contact, transformation to AML and the date of transformation to AML if applicable.

14. MEASUREMENT OF EFFECT

Definitions: Response and progression will be assessed according to modified International Working Group (IWG) 2006 criteria[34] (Appendix B).

15. STATISTICAL CONSIDERATIONS

1. Study Design

This study will be a multi-institution, open-label, phase Ib/II clinical trial conducted in 2 parts: a Phase 1b part followed by a Simon's two-stage Phase 2 design. The study will assess the safety and efficacy of APR-246 in combination with azacitidine for the treatment of *TP53* mutant myeloid neoplasms. All patients will have pre-screening NGS on peripheral blood (PB) or bone marrow (BM) samples to determine *TP53* mutational status and therefore eligibility to participate in this study. A PB sample will be obtained prior to treatment for NGS by the central laboratory to evaluate baseline VAF and for serial analysis. The initial Phase 1b part will evaluate APR-246 administered with azacitidine. Cohorts of at least 3 evaluable patients will be enrolled using a modified 3 + 3 design. The MTD is defined as the dose level below which DLT is manifested in ≥33% of the patients or at dose level 1 if DLT is manifested in <33% of the patients.

Following completion of the Dose Finding Phase, we will conduct a dose expansion, whereby patients will be treated with APR-246 administered at the MTD with azacitidine on a 28 day cycle utilizing the same dosing schedule as in Phase 1b. All patients who complete at least one treatment cycle of APR-246 and azacitidine and who have at least one post-treatment clinical response assessment will be considered eligible for the efficacy-evaluable population and will be summarized for all efficacy variables. Patients who fail to complete one treatment cycle will also be considered efficacy-evaluable if they show clear evidence of clinically significant disease progression. Patients who are not efficacy-evaluable will be replaced. A Simon's two-stage minimax design will be applied, as follows:

Stage 1: Enroll a total of 24 evaluable patients at the MTD (including patients who were enrolled during the Phase 1 part of the study). If less than 6 of 24 patients achieve CR, then the study will be terminated early with the conclusion that the regimen does not warrant further investigation. If 6 or more patients achieve CR, then enrollment will be permitted to continue to Stage 2.

Stage 2: Enroll 21 more evaluable patients for a total of 45. If less than 14 of 45 patients achieve CR, then there is insufficient evidence to support continued study of this treatment. If at least 14 of 45 patients achieve CR, then there is sufficient evidence to support further study of APR-246 in combination with azacitidine in Phase 3. Up to 39 additional evaluable patients will be treated at the level of the MTD in the

Phase 2 portion of the trial.

This rule has the following operating characteristics: 90% power, with alpha=0.05 under the null hypothesis that the proportion of patients achieving CR is ≤ 20% versus the alternative hypothesis that it is ≥ 40%. This design has a probability of early termination equal to 0.66 if the true CR rate is 20%.

2. Statistical Analysis Methods

Demographic and clinical variables for the study patients will be summarized using descriptive statistics (mean, standard deviation, median, inter-quartile range, range, frequency counts and percentages). Safety and efficacy data will be analyzed overall as well as separately for each dose cohort when appropriate.

3. Safety Analysis

This analysis will include all subjects who have received any protocol treatment, regardless of patient eligibility. The number (%) of subjects with adverse events, serious adverse events, and adverse events leading to treatment discontinuation will be reported. Adverse events summary will be reported by type and severity. Laboratory parameters will also be summarized using descriptive statistics.

4. Efficacy Analysis: ITT

This analysis will include all subjects who have received any protocol treatment, regardless of patient eligibility or duration of treatment. Those who have no response assessment data due to reasons such as drop out of the study, withdrawal consent, or lost to follow-up will be treated as non-responders for various response evaluations. The proportion of subjects achieving the primary endpoint will be summarized. A 95% exact binomial confidence interval of the proportion will also be provided for all participants treated at the MTD. In addition, a second analysis of evaluable subjects will be performed. Evaluable subjects are defined as those who complete at least 3 cycles of therapy and complete the first on treatment bone marrow biopsy and aspirate to evaluate study drug response.

Analyses of the following secondary endpoints will be performed as well:

i. Duration of response defined as the time between achieving response and progression of disease.
ii. OS at 8 months
iii. Proportion of subjects achieving hematological improvement (HI), partial response (PR), complete response (CR), and/or marrow CR (mCR) by the IWG 2006 criteria (see appendix B)
iv. Proportion of subjects who obtain a response as defined by IWG 2006 criteria with a baseline *TP53* VAF ≥ 20% versus < 20% or high versus low nuclear p53 protein expression

v. Correlation of *TP53* VAF with nuclear p53 protein expression as determined by IHC
vi. Proportion of subjects who achieve ≥ 50% reduction in TP53 VAF.
vii. Progression free survival (PFS) and OS in clonal responders (≥ 50% reduction in *TP53* VAF) versus non-responders
viii. Profile expression of genes related to p53-mediated signal transduction before and after APR-246 treatment
ix. Profile ROS production before and after APR-246 treatment
x. AML transformation according to World Health Organization (WHO) criteria
xi. OS
xii. LFS
xiii. Determine recurrent gene mutations in *ASXL1, BCOR, BRAF, CALR, CBL, CEBPA, CSF3R, DNMT3A, ETV6, EZH2, GATA2, GNAS, IDH1, IDH2, JAK2, KIT, KRAS, MPL, NF1, NPM1, NRAS, PDGFRA, PDGFRB, PHF6, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SMC1A, SMC3, SRSF2, STAG2, STAT3, STAT5b, TET2, TP53, U2AF1, WT1, and ZRSR2* at study entry and serially throughout treatment to assess changes in somatic mutation landscape.

Preliminary data on the above endpoints will prove to be very useful for further investigation of this protocol's treatment. Such data will be summarized appropriately in an exploratory fashion. Both point estimates and 95% confidence intervals will be reported, if feasible. Time-to-event endpoints such as OS and LFS will be summarized using the Kaplan-Meier product-limit method.

16. Laboratory Correlates

Unless otherwise specified, all laboratory correlates will be performed in the laboratory of Dr. Alan List or at Genoptix medical laboratory.

1. Mutation analyses

Genoptix medical laboratory (2110 Rutherford Rd, Carlsbad, CA 92008) will be the central laboratory for this study regarding molecular profiling. All patients will undergo baseline NGS for confirmation of *TP53* mutation, baseline *TP53 VAF*, and identification of additional somatic mutations. These analyses will not impact enrollment into the clinical trial. Importantly, there will be serial evaluation following cycle 3 and cycle 6 where NGS will be repeated to evaluate for clonal reduction of *TP53* VAF as well as changes in the underlying mutational architecture of these patients. These analyses will be utilized for secondary endpoints #4-7. **For correlative genomic studies that will be performed at Genoptix medical laboratory, 2-3 mL of peripheral blood will be obtained in a standard Vacutainer tube with EDTA (purple top) as anticoagulant. If *TP53* mutation is only present on BM analysis at screening, serial NGS myeloid panel**

can be performed on bone marrow MNC. A FedEx shipping label will be included in the shipper box with a research specific requisition. Recurrent gene mutations of *ASXL1, BCOR, BRAF, CALR, CBL, CEBPA, CSF3R, DNMT3A, ETV6, EZH2, GATA2, GNAS, IDH1, IDH2, JAK2, KIT, KRAS, MPL, NF1, NPM1, NRAS, PDGFRA, PDGFRB, PHF6, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SMC1A, SMC3, SRSF2, STAG2, STAT3, STAT5b, TET2, TP53, U2AF1, WT1,* and *ZRSR2* will be evaluated at each NGS analysis. DNA and RNA will be isolated from BM-MNC by the Ficoll method following cycle 3 and cycle 6 and stored in the laboratory of Dr. Alan List. In patients who become negative for *TP53* mutation by NGS (VAF sensitivity for this assay is 1% in patients with previously identified variants), we will perform PCR-based barcoding or digital droplet PCR to identify rare variants in an effort to determine the depth of remission induced by treatment with APR-246 and azacitidine.

2. Immunohistochemistry for p53 expression

BM core biopsies will be centrally evaluated at Moffitt Cancer center by Dr. Ling Zhang to evaluate for p53 protein expression as a biomarker of response, correlation with *TP53* VAF, and serially over the course of treatment (secondary endpoints # 4-5). Three sections from each core biopsy will be obtained at baseline and following cycle 3 and 6 of therapy. To evaluate for p53 protein expression, p53 IHC will be performed using a standard protocol (p53 antibody, clone Bp53-11, Ventana Medical Systems, Tucson, AZ, USA). Nuclear p53 expression will be assessed quantitatively by percent p53 positivity and semi-quantitatively with an IHC score of stain intensity (0, 1+, 2+, and 3+) multiplied by percent positive hematopoietic cells.

Shipping

Bone marrow core biopsies will be shipped to the below address.

H. Lee Moffitt Cancer Center
Dr. Ling Zhang
12902 Magnolia Drive
Tampa, FL 33612
Phone: (813) 745-2852

3. *In vivo* evaluation of APR-246

As the primary mechanism of action of APR-246 is restoration of wild-type function of p53, we will utilize the lead in phase of APR-246 monotherapy to assess the activation of downstream targets of p53. An additional 20ml of bone marrow aspirate will be obtained in purple EDTA tubes at the pre-study bone marrow (prior to Cycle #1) and on day -10 following four days of APR-246 as well as at disease assessments (following cycle 3 and cycle 6; see study calendar). Utilizing the bone marrow aspirates, the mononuclear

cells will be collected with isolation of DNA and RNA as previously described and stored in the laboratory of Dr. Alan List. Evaluation of p53-mediated signal transduction will be analyzed using Qiagen human p53 Signaling Pathway RT² Profiler PCR Array (secondary endpoint #8). As production of ROS leading to DNA damage is an additional mechanism of action of APR-246, we will evaluate for ROS production before and after APR-246 monotherapy via flow cytometry (CellROX® Deep Red Reagant, Life Technologies; secondary endpoint #9).

i. Shipping

Heparinized bone marrow aspirates should be shipped overnight on cool packs, wrapping the vials to insure the marrow does not freeze. Specimens must be shipped via next day delivery on the day the specimen is obtained. DO NOT USE DRY ICE OR WET ICE. A Fedex account number will be provided for overnight shipping. Call Kathy McGraw or the List laboratory at (813) 745-8271 on the day of expected shipment to confirm delivery date. Shipping can only be done Monday through Thursday for arrival Tuesday through Friday. Saturday arrivals cannot be processed. Be sure all samples are labeled appropriately with patient identifier information and designation of bone marrow.

Shipping Addresses:
H. Lee Moffitt Cancer Center
Kathy McGraw or David Sallman, Dr. Alan List Laboratory
SRB3/23234List/x8271
12902 Magnolia Drive
Tampa, FL 33612
Phone: 813-745-8271

ii. Banking

The residuals and/or derivatives of bone marrow and blood samples collected for the correlative studies will be retained in the List laboratory for possible use in approved future studies. If future use is denied or withdrawn by the patient, the samples will be removed from consideration for use in any future study.

17. REGULATORY AND REPORTING REQUIREMENTS

This research will be done in compliance with the applicable State and Federal laws and regulations and in compliance with ICH guidelines. The study description will be posted on the www.clinicaltrials.gov website in compliance with current regulations. The data and safety plan will be executed in accordance with ICH

guidelines and in compliance with policy and procedures at the H. Lee Moffitt Cancer Center & Research Institute. The following must be observed to comply with Food and Drug Administration (FDA) regulations for the conduct and monitoring of clinical investigations; they also represent sound research practice:

1. Informed Consent

The investigator is responsible for patient care and for obtaining consent by the patient. Written informed consent must be obtained prior to entry of any patient. The original consent form signed and dated by the subject and by the person consenting the subject prior to the subject's entry into the study must be maintained in the Investigator's study files.

2. Use of Specimens For Research

The patient is free at any time in the future to decide not to provide specimens or to withdraw his/her specimens from further scientific research. Such a decision will have no impact on his/her treatment or other aspects of participation in this study.

3. Institutional Review

No subject is to be enrolled on this protocol until the Center's Institution Review Board has approved it. The protocol for this study has been designed in accordance with the general ethical principles outlined in the Declaration of Helsinki. The Investigator will be responsible for preparing documents for submission to the relevant IRB and obtaining written approval for this study. The approval will be obtained prior to the initiation of the study. The approval for both the protocol and informed consent must specify the date of approval, protocol number and version, or amendment number. Any amendments to the protocol after receipt of IRB approval must be submitted by the Investigator to the IRB for approval. The Investigator is also responsible for notifying the IRB of any serious deviations from the protocol, or anything else that may involve added risk to subjects.

4. Drug Accountability

For each drug supplied for a study, an accountability ledger containing current and accurate inventory records covering receipt, dispensing, and the return of study drug supplies must be maintained. Drug supplies must be kept in a secure, limited access storage area under the recommended storage conditions. These Accountability Forms must be readily available for inspection and are open to FDA inspection at any time.

5. IND Safety Reporting Requirements

This protocol is associated with an Investigational New Drug Application (IND) supported by Aprea. IND Safety Reports are required for any adverse experience associated (or possibly associated) with the use of investigational product(s) that is both serious and unexpected. To meet the IND Safety reporting requirements set forth in 21 CFR§312.32, an FDA Form 3500A (MedWatch form) will be completed and provided to the Moffitt Office of Institutional Regulatory Affairs for Investigational Drugs and Devices. All other adverse events will be reported to the FDA as part of the annual reporting requirement of the IND.

The initial report will include details of the current illness and serious adverse events, and an assessment of the causal relationship between the event and the investigational product(s). Information not available at the time of the initial report (e.g., an end date for the adverse event or laboratory values received after the report) will be documented on the follow-up report. A final report to document resolution of the serious adverse event is required if it is not documented in the initial report. The Moffitt Office of Institutional Regulatory Affairs will provide the report to the FDA as an official submission to the IND. This will occur independently of any other use of the MedWatch form or required reporting of serious adverse events. Submission of the MedWatch form to any other agency, including the FDA, does not fulfill this IND Safety Reporting requirement. Follow-up to the safety report must also be submitted to the Office of Institutional Regulatory Affairs in a timely manner. Upon completion of the report, a copy of all initial and follow-up MedWatch forms will be scanned and emailed to the Office of Institutional Regulatory Affairs and a telephone call will be made to confirm receipt. For unexpected fatal or life threatening experiences associated with the use of the investigational product(s), the Office of Institutional Regulatory Affairs will be contacted upon notice of the event. Notification to the FDA will be made as soon as possible, but no later than 7 calendar days after initial receipt of the information.

6. Hospital/Clinic Records

Hospital records for patients on this study are the responsibility of the investigator. They will be available for review by the sponsors of the trial, health care personnel involved in this study, the IRB, DHHS, and the FDA.

7. Investigator Study Files

The Principal Investigator is responsible for maintaining study files for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or if no

application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified.

The following documents should be kept in the study files:

- A completed, signed FDA Form 1572 (Statement of Investigator) and copies of all current curricula vitae of all sub investigators listed on the Statement of the Investigator.
- The original protocol and all amendments
- Final IRB approval, annual renewals and all IRB correspondence
- Blank Case Report Forms
- Copy of all IRB approved Informed Consent forms with applicable version date
- Updated laboratory certification and laboratory values (covering entire time of study)
- Copy of all patient's signed informed consent forms
- The final completed case report form for all patients

8. STUDY MONITORING

The Protocol Review & Monitoring Committee will monitor this study. Regulatory documents and case report forms will be reviewed routinely by the MCC Clinical Research Monitoring Core for accuracy, completeness and source verification of data entry, validation of appropriate informed consent process, adherence to study procedures, and reporting of SAEs and protocol deviations according to MCC Monitoring Policies. As part of the responsibilities assumed by participating in the study, the Investigator agrees to maintain and have available for monitoring adequate case records (accurate source documents and CRFs) for the subjects treated under this protocol. CRFs will only be required for patients who have received at least one dose of study medication. In addition, the Investigator agrees to maintain all administrative documents, e.g., IRB/IEC correspondence, investigational product and supplies shipment manifests, monitoring logs, or Moffitt Cancer Center/designee correspondence. The PI will be primarily responsible for monitoring of adverse events, protocol violations, and other immediate protocol issues. The study coordinator will collect information of subjects enrolled at Moffitt and other institutions through the use of electronic or paper AE forms, CRF forms, End of Study forms, and Informed Consent forms.

Internal Monitoring

Data will be captured in Oncore, Moffitt's Clinical Trials Database. The Case Report Forms will be reviewed by Moffitt's Internal Monitors, periodically, throughout the conduct of the trial. The monitoring will include source data verification, utilizing research subjects' medical records.

On-site Audits

The Investigator should promptly notify Moffitt Cancer Center or its authorized representative of any audits scheduled by any regulatory authorities and promptly forward copies of any audit reports received to Moffitt Cancer Center or its authorized representative.

Data & Safety Monitoring Plan

Identification of oversight responsibility:

The PI has primary responsibility.

The MCC Protocol Monitoring Committee (PMC):

The PMC meets monthly and reviews accrual, patterns and frequencies of all adverse events, protocol violations and when applicable, internal audit results.

Description of internal (PI) safety review and monitoring process:

Responsible for identifying and reviewing adverse events biweekly:

Principal Investigator

Study team

To be reviewed:

Adverse events by grade (Gr. 3 or above using CTCAE v4.03) and attribution (expected or unexpected)

Relationship to study drug/intervention

Application of dose finding escalation/de-escalation rules

Application of study designed stopping/decision rules

Whether the study accrual pattern warrants continuation/action

Protocol violations

AEs will be reported along with all other data in the Oncore database. The PI or PI designate will report all adverse events to the Clinical Research Office (CRO). The CRO will report all SAEs to Aprea, and all reportable SAEs to the IRB. AE information will be entered into the CRO database. AE information will be managed by the CRO and will be made available to the PMC or appropriate monitoring body by designated members of the PMC or the study statisticians.

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APPENDIX A

Performance Status Criteria

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

APPENDIX B

RESPONSE CRITERIA FOR SUBJECTS WITH MDS AND CMML ACCORDING IWG 2006 CRITERIA[34]

ALTERING DISEASE NATURAL HISTORY	
Complete remission (CR)	Bone marrow: ≤ 5% myeloblasts with normal maturation of all cell lines Persistent dysplasia will be noted Peripheral blood: Hemoglobin ≥ 11 g/dL Platelets ≥ 100 x 10^9/L Neutrophils ≥ 1.0 x 10^9/L Blasts 0%
Partial remission (PR)	All CR criteria if abnormal before treatment, except: Bone marrow blasts decreased by ≥ 50% over pretreatment but still > 5% Cellularity and morphology not relevant
Marrow CR	Bone marrow: ≤ 5% myeloblasts and decrease by ≥ 50% over pretreatment Peripheral blood: if HI responses, they will be noted in addition to marrow CR
Stable disease (SD)	Failure to achieve at least PR, but no evidence of progression for > 8 weeks
Failure	Death during treatment Disease progression characterized by worsening of cytopenias, increase in % of bone marrow blasts, or progression to a more advanced MDS FAB subtype than pretreatment
Disease Progression (PD)	For subjects with: Less than 5% blasts: ≥ 50% increase in blasts to > 5% blasts 5%-10% blasts: ≥ 50% increase in blasts to > 10% blasts 10%-20% blasts: ≥ 50% increase in blasts to > 20% blasts 20%-30% blasts: ≥ 50% increase in blasts to > 30% blasts Any of the following: At least 50% decrement from maximum remission/response levels in granulocytes or platelets Reduction in hemoglobin (Hgb) concentration by ≥ 2 g/dL - Transfusion dependence
CYTOGENETIC RESPONSE	
Complete	Disappearance of the chromosomal abnormality without appearance of new ones
Partial	At least 50% reduction of the chromosomal abnormality
HEMATOLOGICAL IMPROVEMENT (HI)	
Erythroid response (HI-E) (Pretreatment < 11 g/dL)	Hgb increase by ≥ 1.5 g/dL Relevant reduction of units of RBC transfusions by an absolute number of at least 4 RBC transfusions/8 weeks compared with the pretreatment transfusion number in the previous 8 weeks. Only RBC transfusions given for a Hgb of ≤ 9.0 g/dL pretreatment will count in the RBC transfusion evaluation
Platelet response (HI-P) (Pretreatment < 100 x 10^9/L)	Absolute increase of ≥ 30 x 10^9/L for subjects starting with > 20 x 10^9/L Increase from < 20 x 10^9/L to > 20 x 10^9/L and by at least 100%
Neutrophil response (HI-N) (Pretreatment < 1.0 x 10^9/L)	At least 100% increase and an absolute increase of > 0.5 x 10^9/L

PROGRESSION/RELAPSE CRITERIA FOR SUBJECTS WITH MDS/CMML

ALTERING DISEASE NATURAL HISTORY	
Disease Progression (PD)	For subjects with: Less than 5% blasts: ≥ 50% increase in blasts to > 5% blasts 5%-10% blasts: ≥ 50% increase in blasts to > 10% blasts 10%-20% blasts: ≥ 50% increase in blasts to > 20% blasts 20%-30% blasts: ≥ 50% increase in blasts to > 30% blasts Any of the following: At least 50% decrement from maximum remission/response levels in granulocytes or platelets Reduction in hemoglobin (Hgb) concentration by ≥ 2 g/dL Transfusion dependence
Disease transformation	Transformation to AML (30% or more blasts)
Relapse after CR or PR	At least one of the following: Return to pretreatment bone marrow blast % Decrement of ≥ 50% from maximum remission/response levels in granulocytes or platelets - Reduction in Hgb concentration by ≥ 1.5 g/dL or transfusion dependence
HEMATOLOGICAL IMPROVEMENT	
Progression/relapse after HI	At least one of the following: At least 50% decrement from maximum response levels in granulocytes or platelets Reduction in Hgb by ≥ 1.5 g/dL - Transfusion dependence

APPENDIX C: 2016 WHO CLASSIFICATION FOR CMML AND MDS[35]

WHO CMML

WHO Subtype	Peripheral Blood	Bone Marrow
#Chronic Myelomonocytic Leukemia *CMML-0 **CMML-1 ***CMML-2	*<2% blasts **≥2% and <5% blasts ***≥5% and <20% blasts persistent monocytosis > 1 x 10^9/L and >10% of differential +/- cytopenias Leukocytosis frequent	* <5% blasts **≥5% and <10% blasts ***<20% blasts >10% dysplasia in affected lineage ***Auer Rods The absence of the Philadelphia chromosome of bcr-abl fusion gene.

#Not meeting WHO criteria for *BCR-ABL1* CML, PMF, PV, or ET. No evidence of *PDGFRA*, *PDGFRB*, or *FGFR1* rearrangement or *PCM1-JAK2* (should be specifically excluded in cases with eosinophilia). If myelodysplasia is absent or minimal, CMML diagnosis can be made with acquired clonal cytogenetic/molecular genetic abnormality or monocytosis x 3 months when all other causes of monocytosis have been excluded. Blasts and blast equivalents include myeloblasts, monoblasts, and promonocytes.

WHO MDS

WHO Category	Peripheral blood	Bone marrow
MDS with single lineage dysplasia (MDS-SLD)	Cytopenia (1-2 lines) <1% blasts	Dysplasia (1 line) < 5% blasts RS <15%/<5%†
MDS with ring sideroblasts (MDS-RS)*	Cytopenia (1-3 lines) < 1% blasts	Dysplasia (1-3 lines) < 5% blasts RS >15%/>5%†
MDS with multi-lineage dysplasia (MDS-MLD)	Cytopenia (1-3 lines) < 1%blasts	Dysplasia (2-3 lines) < 5% blasts RS <15%/<5%†
MDS with excess blasts type I & II (MDS-EB-1 & MDS-EB-2**)	Cytopenia (0-3 lines) Type I: 2-4% blasts Type II: 5-19% blasts	Dysplasia (1-3 lines) Type I 5-9% blasts Type II 10-19% blasts
MDS with isolated del(5q)***	Cytopenia (1-2 lines) < 1% blasts	Dysplasia (1-3 lines) < 5% blasts
MDS unclassified (MDS-U)****	Cytopenia (0-3 lines) 1% or < 1% blasts	Dysplasia (1-3 lines) < 5% blasts

Cytopenias defined as: hemoglobin < 10 g/dL; platelet count < 100 x 10^9/L; and absolute neutrophil count < 1.8 x 10^9/L. To be classified as dysplasia, >10% of any cell lineage must be dysplastic. PB monocytes must be < 1 X 10^9/L. †If *SF3B1* mutation is present. *Subclassified as MDS-RS with single or multi-lineage dysplasia (MDS-RS-SLD or MDS-RS-MLD). Pancytopenia with RS is classified as MDS-RS-MLD. **Presence of Auer rods is classified as MDS-EB-2. *** Del(5q) alone or with 1 additional abnormality except -7 or del(7q). ****1% peripheral blasts needs to be confirmed on 2 separate occasions to make a diagnosis of MDS-U. MDS-U diagnosis is also made with SLD and pancytopenia or MDS defining cytogenetic abnormality in the absence of cytopenias.