Phase 1 Study to Evaluate Safety and Efficacy of APR-548 in Combination with Azacitidine for the Treatment of *TP53*-Mutant Myelodysplastic Syndromes

Protocol No.:	A20-11202
IND No.:	150967
Sponsor:	Aprea Therapeutics, Inc. [redacted]
Responsible Medical Officer:	[redacted]
Medical Monitor:	[redacted]
Protocol Version:	6.0
Replaces:	5.0
Date:	10 September 2021

CONFIDENTIAL

INVESTIGATOR'S STATEMENT

1. I have carefully read this protocol entitled "Phase 1 Study to Evaluate Safety and Efficacy of APR-548 in Combination with Azacitidine for the Treatment of *TP53*-Mutant Myelodysplastic Syndromes" and agree that it contains all the necessary information required to conduct the study. I agree to conduct this study as outlined in the protocol.
2. I understand that this study will not be initiated without approval of the appropriate Institutional Review Board (IRB)/Independent Ethics Committee (IEC), and that all administrative requirements of the governing body of the Institution will be complied with fully.
3. Informed written consent will be obtained from all participants in accordance with institutional guidelines, United States (US) Food and Drug Administration (FDA) requirements as specified in Title 21 Code of Federal Regulations (CFR), Part 50, the European Union Directive 2001/20/European Commission (EC) and its associated Detailed Guidances, European Union Good Clinical Practice (GCP) Directive 2005/28/EC, the International Council for Harmonization (ICH) Guideline for GCP, Section 4.8, and the terms of the Declaration of Helsinki (2013).
4. I will enroll participants who meet the protocol criteria for entry.
5. I understand that my signature on each completed electronic Case Report Form (eCRF) indicates that I have carefully reviewed the complete set of eCRFs and accept full responsibility for the contents thereof.
6. I understand that the information presented in this study protocol is confidential, and I hereby assure that no information based on the conduct of the study will be released without prior consent from the Sponsor unless this requirement is superseded by the FDA, a Competent Authority of the European Union or another Regulatory Authority.

Protocol Version 6.0: 10 September 2021

Name: _____ Telephone: _____

_____ _____

Address: _____

_____

Signature: _____ Date: _____

Sponsor/Representative:

Signature: [redacted]

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CLINICAL STUDY SYNOPSIS

Title	Phase 1 Study to Evaluate Safety and Efficacy of APR-548 in Combination with Azacitidine for the Treatment of *TP53*-Mutant Myelodysplastic Syndromes
Sponsor	Aprea Therapeutics, Inc.
Monitor/ Contract Research Organization (CRO)	[redacted]
Number of Study Centers	Up to 10
Clinical Phase	1
Sample Size	Up to 46 subjects
Investigational Agent	APR-548
Study Design	This is an open-label first-in-human (FIH) phase 1 clinical trial that utilizes a standard 3 + 3 design. The study will assess safety, pharmacokinetics (PK), and clinical activity of orally (p.o.) administered APR-548 alone and in combination with azacitidine for the treatment of *TP53*-mutant myelodysplastic syndromes (MDS). *Safety Run-In Periods (28-day cycles)* Safety Run-In Monotherapy: 3+3 dose escalation of APR-548 as monotherapy on Days 1-4. Safety Run-In Combination Therapy: 3+3 dose escalation of APR-548 in combination with azacitidine. APR-548 on Days 1-4 in combination with azacitidine on Days 1-7. APR-548 will initially be given p.o. once daily (QD) during Days 1 – 4 of a 28-day safety run-in period. The first 3 subjects enrolled on each dose level will be evaluated for a 28-day dose-limiting toxicity (DLT) safety follow-up period prior to enrolling additional subjects on that dose level or prior to dose escalation. If no DLT is observed, subjects will proceed to the safety run-in combination therapy portion with APR-548 in combination with azacitidine administered subcutaneously (s.c.) or intravenously (i.v.) at the standard dose of 75 mg/m^2 on Days 1-7 of the 28-day cycle. The first 3 subjects on each dose level of the combination with APR-548 and azacitidine will be evaluated for a 28-day DLT safety follow-up period prior to dose escalation with the combination treatment. During the safety run-in of the monotherapy and during the safety run-in of the combination treatment, each subject within the dose level will be evaluated for safety and tolerability of APR-548. APR-548 dose escalation will be guided by an assessment of safety, PK and pharmacodynamic (PD) data, and may proceed until DLTs are observed, or the pre-defined maximum exposure of APR-548 in plasma

	(average maximum concentration [C_{max}] and area under the curve [AUC] 8 µM and 16 µM·h, respectively) is reached, or the minimum biologically effective dose (mBED) is achieved (the dose at which no further incremental effect on markers of apoptosis and/or proliferation is observed, in the monotherapy safety run-in only). The Data Review Team (DRT), consisting of the Medical Monitor, Site Principal Investigators, retina specialist and other clinical research personnel that the Sponsor deems appropriate, will review the available PK, PD and safety data for each 3-6 subject cohort in both the safety run-in for the monotherapy and the combination portions, and increases in dose will be guided by the observed safety, PK and PD data, until the recommended phase 2 dose (RP2D) is determined. The RP2D of APR-548 will be defined as the lowest of: 1. The mBED in the monotherapy safety run-in, 2. The dose that achieves the pre-defined level of maximum exposure in the monotherapy or combination therapy safety run-ins, or 3. The dose at which there are <2 DLTs in 3-6 subjects in a cohort, in the monotherapy or combination therapy safety run-ins. Up to 10 subjects may be enrolled at the RP2D per decision of the DRT to gain additional confidence in the RP2D. Subjects in Cohort 1 will receive a starting APR-548 dose of 50 mg/day (Dose level 1). If there is a DLT in ≤1/3 subjects in Cohort 1, Cohort 1 will be expanded to enroll 3 more subjects. If ≥2 subjects out of the total 3–6 subjects have a DLT, dose escalation will not occur. If ≤1 DLT occurs in the 6 subjects treated at that dose level, the subsequent subject group (Cohort 2) will be enrolled upon DRT review of data as described above, at the APR-548 dose of 150 mg/day (Dose level 2). If ≥2 of 6 subjects at a dose level have a DLT, or if the pre-defined maximum exposure of APR-548 in plasma is reached or the minimum biological effective dose is achieved (in monotherapy safety run-in only), then dose escalation ceases. This study will evaluate up to 6 dose levels. Any subsequent dose escalation will not exceed 100% and will follow the same cohort escalation plan as outlined above and in Figure 1.

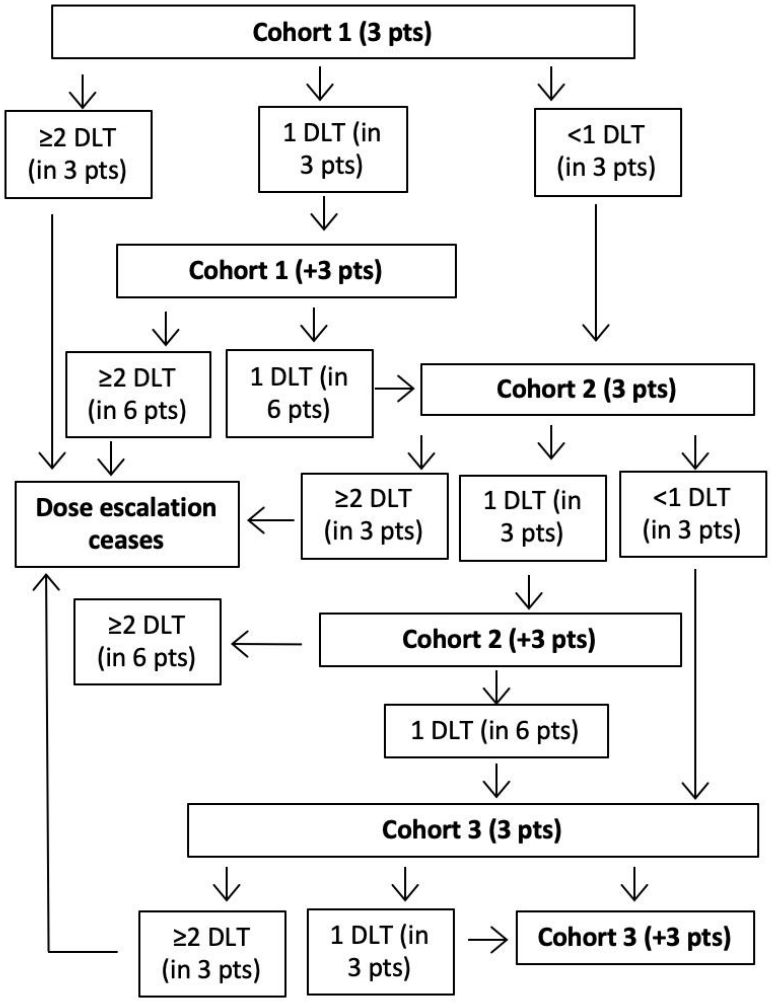

Figure 1. Dose escalation schema for the safety run-in and combination portion

Cohort 1 – Dose Level 1 (APR-548 50 mg/day); Cohort 2 – Dose Level 2 (APR-548 150 mg/day); Cohort 3 – Dose Level 3 (APR-548 300 mg/day).
Additional cohorts may be added utilizing the same dose escalation scheme

Dose-Limiting Toxicity

All treatment-emergent adverse events (TEAEs) are relevant to the determination of DLTs unless they are clearly and solely due to the disease under study. During the 28-day safety run-in period for APR-548 monotherapy and during the 28-day safety run-in period for APR-548 in combination with azacitidine, a DLT is defined as any of the following TEAEs defined by the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE), as follows:

- Non-hematological NCI-CTCAE ≥Grade 3 adverse event (AE), including ocular AEs that are different from retinopathy or optic nerve disorder classifications.
- Prolonged myelosuppression with the persistence of ≥Grade 4 neutropenia or

	thrombocytopenia in the absence of disease lasting 14 days from the initial administration of APR-548 (only during the safety run-in period for APR-548 monotherapy). • Grade 3 nausea/vomiting/diarrhea/fatigue/central nervous system (CNS) AE. Grade 3 nausea/vomiting/diarrhea may be excluded if parenteral nutrition, tube feeding, or prolonged hospitalization is not required. • New or worsening retinopathy or optic nerve disorder AE of any grade. • Grade 3 metabolic/electrolyte abnormalities that do not resolve to ≤Grade 1 or baseline within 72 hours. • Grade ≥4 neutropenia lasting past day 28 in the absence of disease. • Any AE, regardless of the NCI-CTCAE Grade, resulting in discontinuation, dose reduction or treatment with less than 75% of planned doses of APR-548, unless clearly and solely related to the disease under study. Investigator should contact the Medical Monitor to discuss any findings suggestive of ocular-associated AEs. Any ≥Grade 2 ocular-associated AEs are considered AEs of special interest and should be reported as serious adverse events (SAEs). Subjects with signs and/or symptoms of vision changes should be seen immediately by ophthalmologist/retinal specialist. APR-548 may be permanently discontinued, following a discussion with the Medical Monitor, if: • Any retinopathy or optic nerve disorder develops, regardless of perceived attribution to study drug, and • A clinical retina specialist or ophthalmologist determines a retinal or optic nerve finding is substantially changed from the subject's baseline test. Additionally, TEAEs that meet the above criteria, but occur after the DLT evaluation period will not be defined as DLTs, but will be reported as AEs/SAEs and will be reviewed across all cohorts during the study to help determine the AE profile for the respective treatment regimens under study. A subject that discontinues APR-548 therapy during the DLT assessment period without DLT is considered evaluable for the purpose of safety only if at least 75% of the scheduled dose of APR-548 was administered in the monotherapy safety run-in period. The DRT will also review any other emergent toxicities that are not explicitly defined by the DLT criteria to determine if any warrant a DLT designation. Following discussions with the DRT, the schedule of APR-548 may be modified, including multiple daily and weekly doses, based on available PK, PD, and safety data. Subjects may continue treatment as long as safety remains acceptable, progression has not occurred, and the subject has not withdrawn consent. Response and progressive disease will be assessed based on the Guidelines for Implementation of International Working Group (IWG) response criteria (Appendix V) after every two treatment cycles for the first year of study treatment, then every three cycles thereafter. Subjects may remain on study treatment after relapse or progression if they are continuing to derive clinical benefit in the opinion of the investigator.

	Investigators may choose to transition subjects towards a stem cell transplantation (SCT) as appropriate after a response by the study treatment has been achieved. Subjects who undergo SCT will be removed from study treatment and will be followed per the study calendar.
Study Objectives	**Primary objective:** To investigate the PK, safety and tolerability of APR-548 as monotherapy and in combination with azacitidine and determine the RP2D of APR-548 in subjects with *TP53*-mutant MDS. **Secondary objectives:** 1. To assess preliminary clinical activity of APR-548 administered in combination with azacitidine: a. Rate of complete remission (CR) b. Duration of CR (DOCR) c. Overall response rate (ORR) d. Duration of ORR e. Rate of and time to development of acute myeloid leukemia (AML), as defined World Health Organization (WHO) criteria f. Overall survival (OS) g. Relapse-free survival (RFS) h. Rate of transition to hematopoietic stem cell transplant (HSCT) i. Rate of red blood cell (RBC) and/or platelet transfusion independence (TI) for 8 weeks 2. To characterize PK of APR-548 after single and multiple dosing when given p.o. 3. To evaluate the PK drug-drug interaction between APR-548 and azacitidine. **Exploratory objectives:** 1. To assess PD markers of APR-548 in blood and bone marrow samples. 2. To assess molecular markers of response to APR-548 in baseline and serial blood samples and bone marrow samples.
Study Endpoints	**Primary endpoints:** 1. Occurrence of DLTs, classified and graded according to the NCI-CTCAE version 5.0 2. Frequency of TEAEs and SAEs related to APR-548 alone and in combination with azacitidine 3. RP2D of APR-548 **Secondary endpoints:** 1. Rate of CR 2. DOCR, defined as the time from documentation of CR to disease relapse 3. Duration of response (DOR), defined as the time from documentation of tumor response to disease relapse/progression or death as a result of any cause 4. ORR is the proportion of subjects achieving hematological improvement (HI), partial remission (PR), CR, marrow CR by modified IWG 2006 criteria 5. Rate of and time to AML transformation, according to WHO criteria

	6. OS 7. RFS 8. Proportion of subjects who transition to HSCT 9. Subjects who achieve RBC and/or platelet TI during the 8-week period after enrollment 10. PK parameters: C_{max}, time to C_{max} (T_{max}), AUC, oral volume of distribution (V_d/F), oral clearance (CL/F) and elimination half-life ($T_{1/2}$) of APR-548 in the absence and presence of azacitidine 11. PK parameters: C_{max}, T_{max}, AUC, $T_{1/2}$ of APR-548 and the metabolites M8 (reduced MQ-glutathione adduct), M10 (reduced MQ-cysteine adducts), MQ water adducts (MQ-H2O) and MQ glutathione adducts (MG-GSH) **Exploratory endpoints:** 1. PD markers of APR-548 may be explored using flow cytometry and molecular techniques including, but not limited to, markers of apoptosis and proliferation 2. Exploratory analyses of molecular markers of response may include: *TP53* variant allele frequency (VAF) by next-generation sequencing (NGS), mutations and VAF of other genes by NGS, and gene and protein expression
Eligibility criteria	**Inclusion criteria:** 1. Provision of signed and dated, written informed consent prior to any study specific procedures. 2. Documented diagnosis of *TP53*-mutant MDS, according to WHO criteria that is previously untreated and meets IPSS-R classification of intermediate, high, or very high risk disease, or *TP53*-mutant MDS that is relapsed and/or refractory to prior treatments. Eligible *TP53* mutations are defined in Appendix VII. 3. Adequate organ function as defined by the following laboratory values: a. Creatinine clearance ≥60 mL/min (by Cockcroft-Gault method, Appendix I), b. Total serum bilirubin ≤1.5 × upper limit of normal (ULN) unless due to Gilbert's syndrome or MDS organ involvement, c. Alanine aminotransferase and aspartate aminotransferase ≤2.5 × ULN, unless due to MDS organ involvement. 4. Age ≥18 years at the time of signing the informed consent form. 5. Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1 or 2 (Appendix II). 6. Projected life expectancy of ≥12 weeks. 7. Clear ocular media and adequate pupil dilation to permit fundus examination and retinal imaging. 8. Female subjects must be surgically sterile, postmenopausal (for at least 1 year), or have negative results for a pregnancy test at screening, on a serum or urine sample obtained within 7 days prior to initiation of study treatment. 9. Heterosexual female subjects of childbearing potential must agree to use adequate contraception or practice sexual abstinence as the preferred and usual lifestyle of the subject during the study and for up to 90 days after study treatment discontinuation. Adequate contraception is defined by the concomitant use of 2 effective methods of contraception, which can be

comprised of two barrier methods, or a barrier method plus a hormonal method or an intrauterine device that meets <1% failure rate for protection from pregnancy in the product label (Appendix III). Females of non-childbearing potential are those who are postmenopausal greater than 1 year or who have had a bilateral tubal ligation or hysterectomy or bilateral oophorectomy. Female subjects must refrain from egg cell donation while on study treatment and for at least 90 days after the last dose of APR-548 or azacitidine, whichever is longer.

10. Male subjects with female partners of childbearing potential must have had a prior vasectomy or agree to use an adequate method of contraception (i.e., double barrier method: condom plus spermicidal agent) starting with the first dose of study therapy through 90 days after the last dose of APR-548 or azacitidine, whichever is longer. Male subjects should agree to refrain from sperm donation during the study and for at least 90 days after the last dose of APR-548 or azacitidine, whichever is longer. Should a female partner of a male subject become pregnant or suspect she is pregnant while participating in the study, he should inform his treating physician and the female partner should contact her physician immediately.

Exclusion criteria:

1. Any of the following cardiac abnormalities:
    a. Myocardial infarction within six months prior to enrollment;
    b. New York Heart Association Class III or IV heart failure (Appendix IV) or known left ventricular ejection fraction <40%;
    c. A history of familial long QT syndrome;
    d. Symptomatic atrial or ventricular arrhythmias not controlled by medications;
    e. Corrected QT interval Fridericia's formula (QTcF) ≥470 msec, unless due to underlying bundle branch block and/or pacemaker and with the approval of the Medical Monitor.
2. Concomitant malignancies or previous malignancies with less than a 1-year disease-free interval at the time of signing informed consent. Subjects with adequately treated basal or squamous cell carcinoma of the skin, or adequately treated carcinoma *in situ* (e.g., cervix) may enroll irrespective of the time of diagnosis. Subjects with controlled, advanced prostate cancer are permitted.
3. Known hypersensitivity to mannitol.
4. Known hypersensitivity to fluorescein sodium.
5. Use of cytotoxic chemotherapeutic agents, or experimental agents for the treatment of *TP53*-mutant MDS within 14 days or 5 half-lives of the product (whichever is shorter) of the first day of study drug treatment.
6. Prior exposure to eprenetapopt (APR-246).
7. Ongoing or prior history of ocular or hepatic graft-versus-host disease following allogeneic SCT. Subjects receiving systemic immunosuppressive therapy for treatment of (not prophylaxis) of graft-versus-host disease must be off systemic immunosuppressive therapy for a minimum of 6 weeks.

	8. A female subject who is pregnant or breast-feeding. 9. Known history of human immunodeficiency virus, active hepatitis B or active hepatitis C infection. 10. Malabsorption syndrome or other condition likely to affect gastrointestinal absorption of APR-548. 11. Known history or current evidence of ocular disease in either eye that, in the opinion of the investigator, may confound retinal assessments (e.g., glaucoma, advanced age-related macular degeneration, diabetic retinopathy, uveitis, or the presence of any condition that precludes adequate visualization of the fundus such as dense cataracts or corneal scarring). 12. Family history of Leber hereditary optic neuropathy, autosomal dominant optic atrophy, late-onset retinal degeneration, familial dysautonomia or other hereditary mitochondrial disease, unless the causative mutation(s) in the family have been determined and the participant has tested negative for the mutation(s). 13. Known prior or current retinal or optic nerve disease (e.g., retinitis pigmentosa, diabetic retinopathy, optic neuritis) that could impact interpretation of new onset retinal or optic nerve disease. 14. Corrected visual acuity worse than 20/40. 15. Other severe acute or chronic medical or psychiatric condition or laboratory abnormality that, in the opinion of the investigator, may increase the risk associated with study participation or study treatment administration or may interfere with the interpretation of study results and/or would make the subject inappropriate for enrollment into this study. 16. Active uncontrolled infection.
Treatment Plan	Treatment will be administered on an outpatient basis but may be administered in the inpatient setting for subjects who are hospitalized and meet criteria for treatment. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the subject's disease under study. In the 28-day safety run-in period, APR-548 will be given p.o. QD on Days 1 through 4. Following discussions with the DRT, the schedule of APR-548 may be modified, including multiple daily and weekly doses. Following the 28-day safety run-in period, subjects proceed to the combination portion. In the combination portion, APR-548 will be given p.o. QD on Days 1 through 4 of each 28-day cycle. Azacitidine will be given at the standard dose of 75 mg/m^2 over 7 consecutive days (Days 1-7) as a s.c. injection or i.v. infusion. Following discussions with the DRT, the schedule of APR-548 may be modified, including multiple daily and weekly doses. Subjects in the 28-day safety run-in periods will be followed for at least 28 days before the safety of each cohort can be fully assessed and decisions made for dose

escalation in the next cohort. The mBED of APR-548 will be defined as the dose at which no further incremental effect on markers of apoptosis and/or proliferation is observed. Dose escalation of the monotherapy safety run-in may proceed until the earliest occurrence of 1) the mBED is reached, 2) ≥2 DLTs in 3-6 subjects in a cohort, or 3) average APR-548 exposure of exceeds 1/10 of the no-observed-adverse-effect level (NOAEL) or 1/5 the NOAEL for an individual subject (NOAEL relates to the dose level at which no AEs including eye toxicity were observed in the dog). For subjects that proceed from the monotherapy safety run-in to the combination safety run-in, if the dose escalation of the 28-day safety run-in proceeds more rapidly than the combination portion such that the dose of APR-548 undergoing evaluation in the 28-day safety run-in exceeds that being assessed in the combination portion, the subject will receive the dose of APR-548 currently being assessed for safety in the combination portion. If a subject in the 28-day safety monotherapy run-in period has a dose reduction of APR-548 due to an AE, the subject will proceed with the reduced dose of APR-548 in the combination safety run-in of APR-548 with standard dose azacitidine. Dose escalation of the combination safety-run in may proceed until the earliest occurrence of 1) ≥2 DLTs in 3-6 subjects in a combination cohort, or 2) average APR-548 exposure in a combination cohort exceeds 1/10 of the NOAEL or 1/5 the NOAEL for an individual subject. In addition, the dose of APR-548 in the combination portion will not exceed the mBED established in the monotherapy safety run-in.

Cohort	**Dose Level** [a]	**APR-548 (mg)** [b]	**Expected Human PK**		**Safety Factor to APR-548 exposure at NOAEL in Dogs**	
			C_{max} (μM)	AUC (h·μM)	C_{max} (74.3 μM)	AUC (160 h·μM)
1	1 (Starting dose)	50 mg/day	1.2	2.7	62	59
2	2	150 mg/day	3.7	8	20.1	20
3	3	300 mg/day	7.4	16	10	10
4	4	450 mg/day [c]	11.1	24	6.7	6.7
Max dose		Average limit	8	16	9.2	10
Max dose		Individual limit	15	32	5	5

[a] Additional cohorts may be added guided by the DRT, with maximum exposure not exceeding pre-specified threshold
[b] Dose levels are preliminary. Exact dose levels will be determined on safety and exposure data
[c] Expected Human PK will be updated based on ongoing PK analysis and dose level of 450 mg/day will only be administered if expected human PK is below pre-specified thresholds after this update.

Subjects in the combination portion may receive a higher dose of APR-548 than originally assigned after completion of at least two cycles of combination therapy at the assigned dose level if safety data for a higher dose level of APR-548 in combination with azacitidine have been reviewed by the DRT and concluded as safe.

Subjects in the 28-day safety run-in periods (monotherapy or combination portions) who miss ≥25% of APR-548 doses for a nonmedical reason (e.g., subject's preference) or noncompliance will be replaced.

	The RP2D of APR-548 will be defined as the lowest of: 1) the mBED in the monotherapy safety run-in, 2) the dose that achieves the pre-defined level of maximum exposure in the monotherapy or combination therapy safety run-ins, or 3) the dose at which there are <2 DLTs in 3-6 subjects in a cohort, in the monotherapy or combination therapy safety run-ins.
Duration of Follow-Up	Subjects will be followed as per the Schedule of Assessments. After a subject is removed or withdrawn from study treatment, the subject will be followed until death or withdrawal of consent for study participation, whichever occurs first. Off-treatment data on OS will be updated every 6 months or until death or withdrawal of consent for study participation, whichever occurs first. If a subject is removed from the study treatment due to unacceptable AE(s), the event(s) will be followed until resolution or stabilization of the AE(s). Subjects who respond and discontinue study treatment for reasons other than disease relapse should continue to have response assessments, and survival should be collected every 2 months until disease relapse, withdrawal of consent for study participation, or death, whichever occurs first. After disease relapse, data for survival should be collected every 6 months until death or withdrawal of consent for study participation. *Criteria for Removal from Study Treatment* Study treatment can continue for subjects deriving clinical benefit in the opinion of the investigator, unless one or more withdrawal criteria are met, or at the subject's discretion, or if the study is terminated. 1. Study Treatment Discontinuation Study treatment must be discontinued if: • There is evidence of disease progression according to IWG 2006 criteria. (Subjects who have PD but who are continuing to derive clinical benefit in the opinion of the investigator may continue to receive study treatment). • A female subject becomes pregnant. • A subject is 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. • The subject starts new treatment for their disease under study. • The subject receives HSCT. 2. Subject Withdrawal from Study Treatment If the subject is permanently withdrawn from study treatment, but does not withdraw consent from the study, the investigator should make every effort to have the subject complete all withdrawal assessments at the time of withdrawal and complete all scheduled follow-up visits. 3. Study Completion Subject must be taken off the study if: • The subject dies during the study.

	• The subject is lost to follow-up. • The subject withdraws consent for study participation. 4. Subject Withdrawal from Study A subject may voluntarily withdraw from study treatment or withdraw consent from study participation at any time. The investigator may also, at his or her discretion, discontinue a subject from study treatment at any time. The investigator and/or designated staff will record the date and the reason for subject withdrawal from the study.
Statistics	This is a FIH, phase 1, open-label, safety, and preliminary efficacy study of APR-548 in combination with azacitidine in subjects with *TP53*-mutant MDS. The RP2D of APR-548 will be defined as the lowest of: 1. The mBED in the monotherapy safety run-in, 2. The dose that achieves the pre-defined level of maximum exposure in the monotherapy or combination therapy safety run-ins, or 3. The dose at which there are <2 DLTs in 3-6 subjects in a cohort, in the monotherapy or combination therapy safety run-ins. The study will implement the following stopping criteria: • Any death within 30 days from the first dose of APR-548. • A medically equivalent SAE experienced by >1 subject within the first 30 days of study treatment (monotherapy and 1st cycle of combination treatment). • Any >Grade 3 AE experienced by >2 subjects within the first 30 days of study treatment. • Any ≥Grade 3 ocular associated AE that does not return to ≤Grade 1 or baseline within 7 days. If a stopping criterion is met, enrollment will be temporarily suspended until the DRT has performed a prompt cumulative review of safety data and the circumstances of the event in question, to determine whether dosing and/or the protocol should be modified. *Determination of Sample Size* This trial assumes a sample size of up to 46 subjects, 6 subjects in each of 6 dose escalation cohorts plus 10 additional subjects at the RP2D. *Analysis Populations* Safety population: Subjects will be evaluable for safety if they receive at least one dose of APR-548. The safety population will be the primary analysis population used for all analyses such as subject disposition, subject demographics, exposure, safety parameters and efficacy parameters. The safety population will be the primary analysis population for efficacy. Efficacy-evaluable (EE) population: All subjects who complete at least one treatment

	cycle of APR-548 and who have at least one post-treatment clinical response assessment. The EE population will be the secondary analysis population for efficacy. PK population: Subjects will be evaluable for PK if at least one sample for PK evaluation has been obtained. *Safety Analyses* Safety data including AEs, vital signs, laboratory data, electrocardiogram (ECG), ophthalmologic assessment findings, and other physical exam findings will be tabulated for the safety population. AEs will be tabulated by System Organ Class (SOC), preferred term, severity, and relationship to treatments. The tabulation of laboratory parameters will include the normal ranges for each parameter. Each value will be classified as falling above, below, or within the normal range. Laboratory parameters will also be tabulated by maximum NCI-CTCAE v5.0 severity grade. *Efficacy Analyses* CR rate will be summarized for the safety and EE subjects as the proportion (%) of subjects with CR. In addition to presenting the CR rate, its associated exact 95% confidence intervals (CI) for each treatment arm will also be presented. DOR is defined as the time from the date when criteria for response are met to the date of relapse/PD or death due to any cause, whichever occurs first. Subjects alive with no relapse/PD will have their DOR censored at the date of the last clinical assessment. The DOCR will be summarized in each treatment arm by providing the median DOR together with associated 95% CI, using Kaplan-Meier methodology. Overall response will be summarized in number (%) of subjects in each category of responses (CR, PR, marrow complete response [mCR] with HI, mCR without HI, HI). ORR will be analyzed by using the similar method as primary endpoint CR. DOR, as defined above, will also be evaluated in regard to ORR. Time to AML is calculated from first day of study treatment to first onset of AML. Kaplan-Meier methodology will be utilized. Rate of AML transformation will be analyzed by using the similar method as primary endpoint CR. Survival data are collected at treatment and follow-up periods. Subjects will be followed until death or withdrawal of consent from the study, whichever occurs first. OS is defined as the number of days from the first day of treatment to the date of death due to any cause. Kaplan-Meier methodology will be utilized. RFS is defined as the time from the date of randomization to disease relapse or death, whichever occurs first. If neither event occurs, RFS will be censored at the date of the last clinical assessment. Kaplan-Meier methodology will be utilized. Time to AML, OS and RFS will be analyzed using the similar methods as DOR. Transition rate to HSCT will be analyzed using the similar methods as CR.

	Pharmacokinetic Analysis Intense PK sampling for APR-548 and the metabolites M8 and M10 will be performed in the safety run-in period on Days 1, 2, 4 and 5 of monotherapy, and on Cycle 1 of combination therapy on Days 1, 2, 4, 5. Sparse PK sampling for APR-548 and the metabolites M8 and M10 will be performed in every subsequent cycle on Day 1. Intense PK sampling for azacitidine will be performed in Cycle 1 on Days 1, 2, 4 and 5. PK sampling for MQ-H2O, MQ-Cys and MQ-GSH will be implemented for individual subjects at selected centers. Samples will be collected in the safety run-in period on Days 4 and 5 of monotherapy and on Cycle 1 of combination therapy on Days 4 and 5. Non-compartmental methods will be used to derive PK parameters for APR-548, the metabolites and azacitidine (C_{max}, T_{max}, $T_{1/2,}$ AUC, CL/F and Vd/F, as appropriate). The PK of the study drugs and metabolites will be summarized using descriptive statistics (mean, standard deviation, $CV_{\%}$ mean, geometric mean, CV% geometric mean). The concentration data for APR-548 will be evaluated using population pharmacokinetics (popPK) analysis in combination with data from other studies.

LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	Adverse event
AML	Acute myeloid leukemia
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area under the curve
BMMC	Bone marrow mononuclear cell
BUN	Blood urea nitrogen
CFR	Code of Federal Regulations
CHF	Congestive heart failure
CI	Confidence interval
CL	Oral clearance
C_{max}	Maximum concentration
CMML	Chronic myelomonocytic leukemia
CNS	Central nervous system
CR	Complete remission
CRO	Contract Research Organization
Cys	Cysteine
DLT	Dose-limiting toxicity
DOCR	Duration of complete remission
DOR	Duration of response
DRM	Data review meeting
DRT	Data Review Team
EC	European Commission
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Form
EDC	Electronic data capture
EE	Efficacy-evaluable
FDA	Food and Drug Administration
FIH	First-in-human
GCP	Good Clinical Practice
GSH	Glutathione
HI	Hematological improvement
HMA	Hypomethylating agent
HSCT	Hematopoietic stem cell transplantation
IB	Investigator's Brochure
ICH	International Council for Harmonization
IEC	Independent Ethics Committee
IPSS	International Prognostic Scoring System
IPSS-R	Revised International Prognostic Scoring System

IRB	Institutional Review Board
i.v.	Intravenous
IWG	International Working Group
LFT	Liver function test
mBED	Minimum biologically effective dose
mCR	Marrow complete response
MDS	Myelodysplastic syndrome
MQ	2-methylene-quinuclidin-3-one
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NGS	Next-generation sequencing
NOAEL	No-observed-adverse-effect level
OS	Overall survival
ORR	Overall response rate
PBMC	Peripheral blood mononuclear cell
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
p.o.	Orally *(per os)*
popPK	Population pharmacokinetics
PR	Partial remission
PRBC	Packed red blood cells
QD	Once daily (*quaque die*)
QTcF	Corrected QT interval by Fridericia formula
RBC	Red blood cell
RFS	Relapse-free survival
RP2D	Recommended phase 2 dose
SAE	Serious adverse event
s.c.	Subcutaneous
SCT	stem cell transplantation
SD-OCT	Spectral domain optical coherence tomography
SOC	System Organ Class
TEAE	Treatment-emergent adverse event
T_{max}	Time to maximum concentration
TrxR1	Thioredoxin reductase 1
$T_{1/2}$	Half-life
TI	Transfusion independence
ULN	Upper limit of normal
US	United States
USPI	United States Prescribing Information
VAF	Variant allele frequency
V_d/F	Volume of distribution
WHO	World Health Organization

1.0 GENERAL INFORMATION

1.1 Protocol Number and Title of the Study

Protocol No. A20-11202

Protocol Title: Phase 1 Study to Evaluate the Safety and Efficacy of APR-548 in Combination with Azacitidine for the Treatment of *TP53*-Mutant Myelodysplastic Syndromes

1.2 Sponsor

Aprea Therapeutics, Inc.
[redacted]

1.3 Medical Monitor

[redacted]

1.4 Signature Authorization

The protocol will be signed by Aprea Therapeutics, Inc.

2.0 BACKGROUND INFORMATION

2.1 Disease Background

Myelodysplastic syndromes (MDS) are a heterogeneous group of neoplasms arising from a clonal hematopoietic precursor[1,2]. MDS is characterized clinically by cytopenia 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 (IPSS-R), 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

Patients with higher risk MDS fall into IPSS categories of intermediate-2-risk and high-risk groups, corresponding largely to IPSS-R groups very high, high, and, sometimes, intermediate.

To date, the only disease modifying agents in MDS include lenalidomide in patients with isolated deletion of 5q (del(5q)) and the hypomethylating agents (HMA) azacitidine and decitabine.

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, $p<0.0001$)[6].

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[7]. Current NGS myeloid panels incorporate 20-50 genes and can identify mutations in the vast majority of patients[8,9]. In MDS, mutations of *ASXL1*, *ETV6*, *EZH2*, *RUNX1* and *TP53* were found to be independently associated with decreased survival[10]. Of these, recent investigations have suggested that the mutational status of *TP53* is the most important negative prognostic factor in MDS patients. *TP53* mutations are detected in up to 20% of MDS cases, are associated with higher risk MDS, complex cytogenetics and poor overall survival (OS) compared to wild-type *TP53* MDS.

TP53 mutations are strongly associated with poor outcomes in azacitidine treated patients[11]. In addition, Bejar and colleagues recently confirmed decreased OS in *TP53*-mutant MDS patients treated with HMA without effect on response rates[12]. 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[13]. 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.

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)[10,14–17]. Together, these data highlight the dismal outcomes of *TP53*-mutant MDS patients and the dire need for the development of novel therapeutic strategies, particularly in this patient population.

2.3 APR-548

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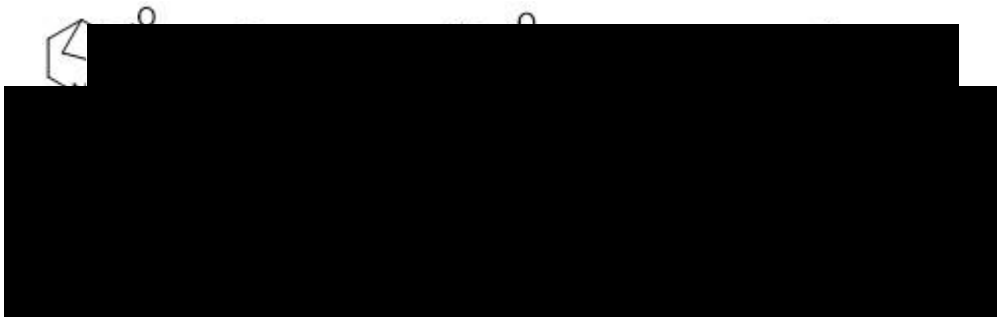
2.3.1 Pharmacology and Mode of Action

[redacted]

[redacted]



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2.3.2 Pharmacokinetics

[redacted]

[redacted]

[redacted]

[redacted]

2.3.3 Safety Pharmacology and Toxicology

[redacted]

[redacted]

[redacted]

2.4 Azacitidine

Azacitidine is a nucleoside metabolic inhibitor that was approved for the treatment of patients with MDS of all subtypes in 2004 by the US FDA and for higher-risk MDS, chronic myelomonocytic leukemia (CMML) and AML with 20% to 30% blasts and multi-lineage dysplasia by the European Medicines Agency in 2009.

Azacitidine is a pyrimidine nucleoside analogue of cytidine that exerts its antineoplastic activity by hypomethylation of the DNA, which has a cytotoxic effect on abnormal hematopoietic cells[30]. At low doses, azacitidine is able to induce reactivation of cell cycle-regulating genes that were initially silenced due to hypermethylation, which may induce cell differentiation, reduce proliferation and/or increase apoptosis of the daughter cells[31].

In two early trials by Silverman et al. (CALGB 8421 and CALGB 8921)[32], median duration of response (DOR) was 14.7 and 17.3 months for patients treated with i.v. versus subcutaneous (s.c.) azacitidine, respectively. In the randomized, multicenter, phase 3, CALGB 9221 trial, patients with MDS who received azacitidine 75 mg/m^2/day s.c. for 7 days every 28 days had a significantly higher overall response rate (ORR) than those receiving supportive care alone (60% versus 5%). Hematological response data from these three trials were retrospectively reanalyzed using International Working Group (IWG) 2000 criteria. Among 99 patients who received azacitidine and 41 patients who received supportive care (without subsequently crossing over to azacitidine), the ORR (CR plus partial remission [PR] plus hematological improvement [HI]) was 47% versus 17%, with CR rates of 10% versus 0%, PR rates of 1% versus 0% and HI rates of 36% versus 17%[33].

In the randomized, open-label, multicenter, phase 3 trial AZA-001, in adults with higher-risk (i.e., IPSS intermediate-2-risk or high-risk classification) MDS/AML, median OS was

significantly better with s.c. azacitidine 75 mg/m^2 per day for 7 days every 28 days than with conventional care (21.1 versus 11.5 months; p=0.0045) and with azacitidine versus low-dose cytarabine (24.5 versus 15.3 months; p=0.0006). The median time to AML transformation was significantly longer in patients receiving azacitidine than in those receiving conventional care (17.8 versus 11.5)[6,34].

Azacitidine is rapidly absorbed after s.c. or i.v. administration with a V_d of 76 ± 26 L and a $T_{1/2}$ of 41 ± 8 minutes. C_{max} is reached after about 30 minutes. Azacitidine and its metabolites are known to be substantially excreted by the kidney, and the risk of adverse reactions to this drug may be greater in patients with impaired renal function. Urinary excretion is the primary route of elimination; the cumulated urinary excretion is 85% of radioactive dose with less than 1% recovered in feces over 3 days.

Azacitidine undergoes spontaneous hydrolysis in aqueous solution and is deaminated by cytidine deamidases. Preliminary data indicate that drug-drug interaction with APR-548 as perpetrator are not likely, but the drug-drug interaction will be investigated as part of the current trial.

2.5 Rationale for the Starting Dose and Maximum Exposure of APR-548

[redacted]

2.6 Rationale for Combination of APR-548 with Azacitidine

[redacted].

2.7 Rationale for Dose of APR-548 in Combination with Azacitidine

[redacted]

2.8 Potential Risks and Benefits

2.8.1 Potential Risks

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]ions[redacted]

[redacted]

[redacted] could be considered to originate from the CNS should be treated as clinically indicated.

[redacted]

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2.8.2 Potential Benefits

Patients with *TP53*-mutant MDS have a poor prognosis and unmet medical need. APR-548 has demonstrated *in vitro* synergy with azacitidine, a standard of care agent for *TP53*-mutant MDS. The purpose of this clinical trial is to define the safety and tolerability of APR-548 in combination with azacitidine in patients with *TP53*-mutant MDS and to determine preliminary efficacy. Given that *TP53*-mutant MDS patients have a limited life expectancy and lack of effective treatments, the benefit/risk assessment supports the use of APR-548 in this trial.

2.9 Characteristics of a Well-Conducted Trial

The following characteristics of an adequate and well-conducted trial will be implemented:

1. The investigators will be well qualified by scientific training and experience.
2. Detailed electronic eCRFs will be completed for every subject.
3. Requirements for institutional ethics review as set forth by the appropriate IRB/IEC, Title 21 CFR Part 56, the European Union Directive 2001/20/EC and its associated Detailed Guidances, European Union GCP Directive 2005/28/EC, the ICH Guideline for GCP, Sections 3 and 4, and the terms of the Declaration of Helsinki (2013), will be followed.
4. Requirements for informed consent in accordance with institutional guidelines, FDA requirements as specified in Title 21 CFR, Part 50, the European Union Directive 2001/20/EC and its associated Detailed Guidances, European Union GCP Directive 2005/28/EC, the ICH Guideline for GCP, Section 4.8, and the terms of the Declaration of Helsinki (2013), will be followed.
5. Safety data will be recorded and evaluated.
6. Routine monitoring visits will be conducted by the Sponsor's representative to ensure data accuracy.
7. Drug accountability will be strictly maintained.
8. This trial will be conducted according to GCP, the protocol and applicable regulatory requirements.

2.10 Subject Population

This study will enroll adult male and female subjects of age ≥18 years with documented diagnosis of *TP53*-mutant MDS, according to the World Health Organization (WHO) classification (<20% blasts). Subjects with relapsed/refractory *TP53*-mutant MDS will be allowed in the study.

3.0 TRIAL OBJECTIVES AND PURPOSE

3.1 Primary Objective

To investigate the PK, safety and tolerability of APR-548 as monotherapy and in combination with azacitidine and determine the recommended phase 2 dose (RP2D) of APR-548 in subjects with *TP53*-mutant MDS.

3.2 Secondary Objectives

1. To assess preliminary clinical activity of APR-548 administered in combination with azacitidine.
    a. Rate of CR
    b. Duration of CR (DOCR)
    c. ORR
    d. Duration of ORR
    e. Rate of and time to development of AML transformation, as defined by WHO criteria
    f. OS
    g. Relapse-free survival (RFS)
    h. Rate of transition to hematopoietic stem cell transplant (HSCT)
    i. Rate of red blood cell (RBC) and/or platelet transfusion independence (TI) for 8 weeks
2. To characterize PK of APR-548 after single and multiple dosing when given orally (p.o.)
3. To evaluate the PK drug-drug interaction between APR-548 and azacitidine.

Exploratory objectives:

1. To assess PD markers of APR-548 in blood and bone marrow samples.
2. To assess molecular markers of response to APR-548 in baseline and serial blood samples and bone marrow samples.

4.0 TRIAL DESIGN

4.1 Overview of Trial Design

This is an open-label FIH phase 1 clinical trial that will assess safety, PK, and clinical activity of p.o. administered APR-548 in combination with azacitidine for the treatment of *TP53*-mutant MDS.

Treatment will be administered on an outpatient basis but may be administered in the inpatient setting for subjects who are hospitalized and meet criteria for study treatment. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the subject's disease under study.

The study is guided by a 3+3 dose escalation of APR-548 in the safety run-in monotherapy and the safety run-in combination therapy portions.

APR-548 will initially be given p.o. QD during Days 1 – 4 of a 28-day safety run-in period. The first 3 subjects enrolled on each dose level will be evaluated for a 28-day DLT safety follow-up period prior to enrolling additional subjects on that dose level or prior to dose escalation. If no DLT is observed, subjects will proceed to the safety run-in combination therapy portion with APR-548 in combination with azacitidine administered s.c. or i.v. at the standard dose of 75 mg/m^2 on Days 1-7 of the 28-day cycle. The first 3 subjects on each dose level of the combination with APR-548 and azacitidine will be evaluated for a 28-day DLT safety follow-up period prior to enrolling additional subjects on that dose level or prior to dose escalation with the combination treatment.

During the safety run-in of the monotherapy and during the safety run-in of the combination treatment, each subject within the dose level will be evaluated for safety and tolerability of APR-548. APR-548 dose escalation will be guided by an assessment of safety, PK and PD data, and may proceed until DLTs are observed or the pre-defined maximum exposure of APR-548 in plasma (average C_{max} and AUC 8 µM and 16 µM·h, respectively) is reached, or the minimum biologically effective dose (mBED) is achieved (the dose at which no further incremental effect on markers of apoptosis and/or proliferation is observed, in the monotherapy safety run-in only). The DRT, consisting of the Medical Monitor, Site Principal Investigators, retina specialist and other clinical research personnel that the Sponsor deems appropriate, will review available PK, PD and safety data for each 3-6 subject cohort in both the safety run-in for the monotherapy and the combination portions, and increases in dose will be guided by the observed safety, PK and PD data, until the RP2D is determined. The RP2D of APR-548 will be defined as the lowest of:

1. mBED in the monotherapy safety run-in,
2. The dose that achieves the pre-defined level of maximum exposure in the monotherapy or combination therapy safety run-ins, or
3. The dose at which there are <2 DLTs in 3-6 subjects in a cohort, in the monotherapy

or combination therapy safety run-ins.

Up to 10 subjects may be enrolled at the RP2D per decision of the DRT to gain additional confidence in the RP2D.

Subjects in Cohort 1 will receive a starting dose of 50 mg/day (Dose level 1). If there is a DLT in ≤1/3 subjects in Cohort 1, Cohort 1 will be expanded to enroll 3 more subjects. If ≥2 subjects out of the total 3–6 subjects have a DLT, dose escalation will not occur. If ≤1 DLT occurs in the 6 subjects treated at that dose level, the subsequent subject group (Cohort 2) will be enrolled upon DRT review of data as described above, at the APR-548 dose of 150 mg/day (Dose level 2). If ≥2 of 6 subjects at a dose level have a DLT, or if the pre-defined maximum exposure of APR-548 in plasma is reached or the mBED is achieved (in monotherapy safety run-in only), then dose escalation ceases. This study will evaluate up to 6 dose levels. Any subsequent dose escalation will not exceed 100% and will follow the same principle as described above. The table below shows the planned dose levels based on the expected APR-548 human PK and under the assumption that the doses are well tolerated. Once the RP2D is established, if only 3 subjects were treated at the RP2D, an additional 3 subjects will be included in the cohort and if ≤1 DLT occurs in the 6 subjects treated at that dose level, the RP2D will be defined.

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The DRT will also review any other emergent toxicities that are not explicitly defined by the DLT criteria to determine if any warrant a DLT designation.

If the dose escalation of the 28-day safety run-in proceeds more rapidly than the combination portion such that the dose of APR-548 undergoing evaluation in the 28-day safety run-in exceeds that being assessed in the combination portion, the subject will receive the dose of APR-548 currently being assessed for safety in the combination portion. If a subject in the 28-day safety run-in period has a dose reduction of APR-548 due to AE, the subject will proceed with Cycle 1 of the combination portion with the reduced dose of APR-548 in combination with standard dose azacitidine.

Following discussions with the DRT, the schedule of APR-548 may be modified, including multiple daily and weekly doses, based on available PK, PD, and safety data.

Subjects in the 28-day safety run-in period and in Cycle 1 of the combination portion who miss ≥25% of APR-548 doses for a nonmedical reason (e.g., subject's preference) or noncompliance will be replaced.

Subjects in the combination portion may receive a higher dose of APR-548 than originally assigned after completion of at least two cycles of combination therapy at the assigned dose level if safety data for a higher dose level of APR-548 in combination with azacitidine have been reviewed by the DRT and concluded as safe.

4.1.1 Treatment Duration

Subjects may continue treatment with APR-548 and azacitidine until disease progression, development of other unacceptable toxicity, confirmed pregnancy, HSCT, death, withdrawal of consent, or study termination, whichever occurs first. Subjects who experience disease progression per the applicable response criteria who are, in the opinion of the investigator, benefiting from treatment, may be allowed to continue on study drug with approval of the Medical Monitor.

Following discontinuation of study drug, subjects are to attend a follow-up visit 28 days after the last dose of study drug for final assessments. When study drug is withheld from a subject in order to resolve a toxicity and the subject does not subsequently restart treatment, end of treatment is defined as the date when the study drug was first held. Subjects should proceed with end of treatment assessments, safety, and survival follow-up. If the decision to not restart study treatment comes outside of the 28-day safety follow-up window, the subject should have an End of Treatment visit and proceed with survival follow-up.

Subjects who achieve an adequate response to treatment with APR-548 and meet other criteria required to undergo HSCT may proceed to HSCT after discontinuation of APR-548. If a subject proceeds to HSCT, evaluation of the extent of disease, response to treatment, and any new antineoplastic therapies will be collected at least monthly as long as the subject remains in remission and assessed as part of the study until relapse, death, withdrawal of

consent, loss to follow-up, or end of study, whichever comes first. If a subject discontinues APR-548 to undergo HSCT, but is then deemed ineligible for HSCT, the subject may restart APR-548 with Medical Monitor approval.

All subjects that enter survival follow-up will be contacted every two months for assessment of survival status and new antineoplastic therapies. Please see Section 6.9 for details on long-term follow-up.

4.2 End of Study

The end of the study is defined as the date of the last visit of the last subject undergoing the trial.

4.3 Drug Products

4.3.1 APR-548

[redacted]

4.3.2 Azacitidine

Chemical Name: 4-amino-1-β-D-ribofuranosyl-s-triazin-2(1H)-one.

Formulation, preparation, storage and stability: Please see commercial package insert approved by regulatory agencies[35].

Route of Administration: s.c. injection or i.v. infusion administered s.c. or i.v. for 7 consecutive days (Days 1-7). S.c. route is preferred but will allow for i.v. at the investigator's discretion.

However, the same route should be maintained over the 7-day treatment period (whichever route of administration is used on Day 1, the same route should be followed on all other days). On the days of concomitant APR-548 administration, azacitidine will be administered 1 hour (±15 min) after APR-548 intake. Missed doses, such as for a weekend or holiday, for any reason but toxicity may, at the investigator's decision, be replaced by adding an additional dosing day for azacitidine so that the subject receives the total 7 days of treatment per cycle. If the azacitidine dose interruption is >3 days, discuss with the Medical Monitor.

4.4 Duration of Therapy

Subjects may remain on study treatment to the end of the trial while deriving clinical benefit, unless unacceptable toxicity, death, or subject withdrawal. Subjects may remain on study treatment after disease progression if they are continuing to derive clinical benefit in the opinion of the investigator.

4.5 Trial Discontinuation

The Sponsor may terminate this study prematurely. Written notification of the termination is required. Conditions that may warrant termination include, but are not limited to:

- The discovery of an unexpected, significant, or unacceptable risk to the subjects enrolled in the study.
- Failure of the investigator to enter subjects at an acceptable rate.
- Insufficient adherence to protocol requirements (non-compliance).
- Lack of evaluable and/or complete data.
- Decision to modify the developmental plan of the drug.
- A decision on the part of the Sponsor to suspend or discontinue development of the drug.

The Sponsor reserves the right to discontinue the study for clinical or administrative reasons at any time. If the site does not recruit at a reasonable rate, the study may be discontinued at that site. Should the study be terminated and/or the site closed for whatever reason, all documentation and study drug pertaining to the study must be returned to the Sponsor or its representative, and the Investigators, IRB/IEC and Regulatory Authorities will be promptly informed of the termination and the reason for the decision. The Investigator should promptly inform the subjects and assure appropriate therapy and follow-up. Subjects should then be withdrawn from the study.

4.6 Drug Accountability/Disposition of APR-548 Supplies

The investigator or designee will maintain accurate records of receipt and the condition of the study drug supplied for this study, including dates of receipt. In addition, accurate records will be kept of when and how much study drug is dispensed and administered to

each subject in the study. Any reasons for departure from the protocol dispensing regimen must also be recorded.

At the completion of the study, there will be a final reconciliation of all study drugs.

4.7 Inclusion Criteria

1. Provision of signed and dated, written informed consent prior to any study specific procedures.
2. Documented diagnosis of *TP53*-mutant MDS, according to WHO criteria that is previously untreated and meets IPSS-R classification of intermediate, high, or very high risk disease, or *TP53*-mutant MDS that is relapsed and/or refractory to prior treatments. Eligible *TP53* mutations are defined in Appendix VII.
3. Adequate organ function as defined by the following laboratory values:
   a. Creatinine clearance ≥60 mL/min (by Cockcroft-Gault method, Appendix I),
   b. Total serum bilirubin ≤1.5 × upper limit of normal (ULN), unless due to Gilbert's syndrome or MDS organ involvement,
   c. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤2.5 × ULN, unless due to MDS organ involvement.
4. Age ≥18 years at the time of signing the informed consent form.
5. Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1 or 2 (Appendix II).
6. Projected life expectancy of ≥12 weeks.
7. Clear ocular media and adequate pupil dilation to permit fundus examination and retinal imaging.
8. Female subjects must be surgically sterile, postmenopausal (for at least 1 year), or have negative results for a pregnancy test at screening, on a serum or urine sample obtained within 7 days prior to initiation of study treatment.
9. Heterosexual female subjects of childbearing potential must agree to use adequate contraception or practice sexual abstinence as the preferred and usual lifestyle of the subject during the study and for up to 90 days after study treatment discontinuation. Adequate contraception is defined by the concomitant use of 2 effective methods of contraception, which can be comprised of two barrier methods, or a barrier method plus a hormonal method or an intrauterine device that meets <1% failure rate for protection from pregnancy in the product label (Appendix III). Females of non-childbearing potential are those who are postmenopausal greater than 1 year or who have had a bilateral tubal ligation or hysterectomy or bilateral oophorectomy. Female subjects must refrain from egg cell donation while on study treatment and for at least 90 days after the last dose of APR-548 or azacitidine, whichever is longer.
10. Male subjects with female partners of childbearing potential must have had a prior vasectomy or agree to use an adequate method of contraception (i.e., double barrier method: condom plus spermicidal agent) starting with the first dose of study therapy through 90 days after the last dose of APR-548 or azacitidine, whichever is longer. Male

subjects should agree to refrain from sperm donation during the study and for at least 90 days after the last dose of APR-548 or azacitidine, whichever is longer. Should a female partner of a male subject become pregnant or suspect she is pregnant while participating in the study, he should inform his treating physician and the female partner should contact her physician immediately.

4.8 Exclusion Criteria

1. Any of the following cardiac abnormalities:
   a. Myocardial infarction within six months prior to enrollment;
   b. New York Heart Association Class III or IV heart failure (Appendix IV) or known left ventricular ejection fraction <40%;
   c. A history of familial long QT syndrome;
   d. Symptomatic atrial or ventricular arrhythmias not controlled by medication(s);
   e. QTcF ≥470 msec, unless due to underlying bundle branch block and/or pacemaker and with the approval of the Medical Monitor.
2. Concomitant malignancies or previous malignancies with less than a 1-year disease-free interval at the time of signing informed consent. Subjects with adequately treated basal or squamous cell carcinoma of the skin, or adequately treated carcinoma *in situ* (e.g., cervix) may enroll irrespective of the time of diagnosis. Subjects with controlled, advanced prostate cancer are permitted.
3. Known hypersensitivity to mannitol.
4. Known hypersensitivity to fluorescein sodium.
5. Use of cytotoxic chemotherapeutic agents, or experimental agents for the treatment of *TP53*-mutant MDS within 14 days or 5 half-lives of the product (whichever is shorter) of the first day of study drug treatment.
6. Prior exposure to eprenetapopt (APR-246).
7. Ongoing or prior history of ocular or hepatic graft-versus-host disease following allogeneic SCT. Subjects receiving systemic immunosuppressive therapy for treatment of (not prophylaxis) of graft-versus-host disease must be off systemic immunosuppressive therapy for a minimum of 6 weeks.
8. A female subject who is pregnant or breast-feeding.
9. Known history of human immunodeficiency virus, active hepatitis B or active hepatitis C infection.
10. Malabsorption syndrome or other condition likely to affect gastrointestinal absorption of APR-548.
11. Known history or current evidence of ocular disease in either eye that, in the opinion of the investigator, may confound retinal assessments (e.g., glaucoma, advanced age-related macular degeneration, diabetic retinopathy, uveitis, or the presence of any condition that precludes adequate visualization of the fundus such as dense cataracts or

corneal scarring).

12. Family history of Leber hereditary optic neuropathy, autosomal dominant optic atrophy, late-onset retinal degeneration, familial dysautonomia or other hereditary mitochondrial disease, unless the causative mutation(s) in the family have been determined and the participant has tested negative for the mutation(s).
13. Known prior or current retinal or optic nerve disease (e.g., retinitis pigmentosa, diabetic retinopathy, optic neuritis) that could impact interpretation of new onset retinal or optic nerve disease.
14. Corrected visual acuity worse than 20/40.
15. Other severe acute or chronic medical or psychiatric condition or laboratory abnormality that, in the opinion of the investigator, may increase the risk associated with study participation or study treatment administration or may interfere with the interpretation of study results and/or would make the subject inappropriate for enrollment into this study.
16. Active uncontrolled infection.

4.9 Inclusion of Women, Minorities and Children

Both men and women ≥18 years of age and members of all races and ethnic groups are eligible for this study. Children are not eligible for this study because the safety and tolerability of the proposed dosing schedule has not been determined in adults.

4.10 Withdrawal Criteria

Protocol therapy can continue for subjects receiving clinical benefit, unless one or more study treatment discontinuation criteria are met, or at the subject's discretion, or if the study is terminated. Subjects are free to discontinue treatment or withdraw from the study at any time and for any reason, without penalty to their continuing medical care. Any discontinuation of treatment or withdrawal from the study must be fully documented in the eCRF and should be followed up by the investigator. The investigator may withdraw a subject at any time if this is considered to be in the subject's best interest.

4.10.1 Study Treatment Discontinuation

Study treatment must be discontinued if:

- Evidence of disease progression according to IWG 2006 criteria. Subjects who have PD but who are continuing to derive clinical benefit in the opinion of the investigator may continue to receive study treatment.
- A female subject becomes pregnant.
- A subject is 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.
- The subject starts new treatment for their disease under study.

- The subject receives HSCT.

4.10.2 Subject Withdrawal from Study Treatment

If the subject is permanently withdrawn from study treatment, but does not withdraw consent from the study, the investigator should make every effort to have the subject complete all withdrawal assessments at the time of withdrawal and complete all scheduled follow-up visits.

4.10.3 Study Completion

Subject must be taken off the study if:

- The subject dies during the study.
- The subject is lost to follow-up.
- The subject withdraws consent for study participation.

4.10.4 Subject Withdrawal from Study

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

4.10.5 Withdrawn Subjects

When a subject is removed from the study treatment, the investigator will clearly document the reason in the medical record and complete the appropriate eCRF page describing the reason for discontinuation. In addition, every effort should be made to complete the appropriate assessments listed in Section 6.8.

Subjects who miss ≥25% of APR-548 doses for a nonmedical reason (e.g., subject's preference) or noncompliance will be replaced.

4.11 Noncompliance

All instances of protocol deviations will be recorded according to guidelines per Sponsor's representative.

5.0 TREATMENT OF SUBJECTS

5.1 Drug Preparation and Administration

Study treatment may be administered on an outpatient basis. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the subject's disease.

5.1.1 APR-548

The APR-548 starting dose is 50 mg/day administered p.o during Days 1–4 for the 28-day safety run-in and in the 28-day cycles in the combination portion.

APR-548 is distributed to sites as a powder in bottle for oral solution. APR-548 should be reconstituted with cold sterile water as follows:

- 150 mg bottle once reconstituted with 30 mL of cold sterile water will be 5 mg/mL.
- 150 mg bottle once reconstituted with 10 mL of cold sterile water will be 15 mg/mL.
- 300 mg bottle once reconstituted with 10 mL of cold sterile water will be 30 mg/mL.

The prepared solution should be taken with an additional 200 mL of water at least 2 hours after and 1 hour before any food intake.

Detailed instructions on vial concentration, preparation and dispensing can be found in the Pharmacy Binder.

5.1.2 Azacitidine

Azacitidine is given at the standard dose of 75 mg/m^2 s.c. or i.v. over 7 consecutive days, Days 1 – 7. Cycles should be repeated every 28 days. S.c. route is preferred but i.v. is allowed at the investigator's discretion. The same route should be maintained over the 7-day treatment period (whichever route of administration is used on Day 1; the same route should be followed on all other days). On days of concomitant APR-548 intake, azacitidine should be administered 1 hour (±15 min) after APR-548. Detailed instructions on preparation and administration can be found in the current United States Prescribing Information (USPI)[35]. Missed doses, such as for a weekend or holiday, for any reason but toxicity may, at the investigator's decision, be replaced by adding an additional dosing day for azacitidine so that the subject receives the total 7 days of treatment per cycle. If the azacitidine dose interruption is >3 days, discuss with the Medical Monitor.

5.1.3 Dose-Limiting Toxicity

All treatment-emergent adverse events (TEAEs) are relevant to the determination of DLTs unless they are clearly and solely due to the disease under study. During the 28-day safety run-in period for APR-548 monotherapy and during the 28-day safety run-in period for APR-548 in combination with azacitidine, a DLT is defined as any of the following TEAEs defined by the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 5.0, as follows:

- Non-hematological NCI-CTCAE ≥Grade 3 AE, including ocular AEs that are different from retinopathy or optic nerve disorder classifications.
- Prolonged myelosuppression with the persistence of ≥Grade 4 neutropenia or thrombocytopenia in the absence of disease lasting 14 days from the initial

administration of APR-548 (only during the safety run-in period for APR-548 monotherapy).
- Grade 3 nausea/vomiting/diarrhea/fatigue/CNS AE. Grade 3 nausea/vomiting/diarrhea may be excluded if parenteral nutrition, tube feeding, or prolonged hospitalization is not required.
- New or worsening retinopathy or optic nerve disorder AE of any grade.
- Grade 3 metabolic/electrolyte abnormalities that do not resolve to ≤Grade 1 or baseline within 72 hours.
- Grade ≥4 neutropenia lasting past day 28 in the absence of disease.
- Any AE, regardless of the NCI-CTCAE Grade, resulting in discontinuation, dose reduction or treatment with less than 75% of planned doses of APR-548, unless clearly and solely related to disease under study.

Investigator should contact the Medical Monitor to discuss any findings suggestive of ocular-associated AEs. Any ≥Grade 2 ocular-associated AEs are considered AEs of special interest and should be reported as SAEs. Subjects with signs and/or symptoms of vision changes should be seen immediately by ophthalmologist/retinal specialist. APR-548 may be permanently discontinued, following a discussion with the Medical Monitor, if:
- Any retinopathy or optic nerve disorder develops, regardless of perceived attribution to study drug, and
- A clinical retina specialist or ophthalmologist determines a retinal or optic nerve finding is substantially changed from the subject's baseline test.

Additionally, TEAEs that meet the above criteria, but occur after the DLT evaluation periods will not be defined as DLTs, but will be reported as AEs/SAEs and will be reviewed across all cohorts during the study to help determine the AE profile for the respective treatment regimens under study. A subject that discontinues APR-548 therapy during the DLT assessment period without DLT is considered evaluable for the purpose of safety only if at least 75% of the scheduled dose of APR-548 was administered in the monotherapy safety run-in period.

The DRT also will review any other emergent toxicities that are not explicitly defined by the DLT criteria to determine if any warrant a DLT designation.

5.1.4 Dose Escalation Strategy

The RP2D of APR-548 will be defined as the lowest dose of APR-548 that results in:
1. The mBED in the monotherapy safety run-in,
2. The dose that achieves the pre-defined level of maximum exposure in the monotherapy or combination therapy safety run-ins, or
3. The dose at which there are <2 DLTs in 3-6 subjects in a cohort, in the monotherapy or combination therapy safety run-ins.

Up to 10 subjects may be enrolled at the RP2D per decision of the DRT to gain additional confidence in the RP2D.

DRT consisting of the Medical Monitor, Site Principal Investigators, independent retina specialist and other clinical research personnel that the Sponsor may deem appropriate, will hold data review meetings (DRMs) on an interim basis at a frequency dependent on study accrual. At these meetings, the DRT will review AEs and DLTs and make recommendations regarding the dose for the next dosing cohort and decide when the RP2D is reached.

5.2 Dose Interruptions/Withholding

Study treatment, including APR-548 and azacitidine, may be withheld from a subject in the event of intercurrent illness, TEAE, administrative reasons, or other reasons. If the subject's condition subsequently improves, or the situation that resulted in withholding study drug resolves, the investigator may resume dosing as soon as possible, unless the reason for dose interruption falls into one of the categories noted below.

Dosing with APR-548 may be permanently discontinued in any subject who develops any retinopathy or optic nerve disorder (of ≥Grade 2), regardless of perceived attribution to study drug, and if a clinical retina specialist or ophthalmologist determines a retinal or optic nerve finding is substantially and clinically significantly changed from the subject's baseline test, and that continued participation in the trial would increase the risk for such a finding to worsen (see Table 7 for details).

Dose management guidelines in Table 2 or Table 3 should be followed for each occurrence of a ≥Grade 3 hematologic or non-hematologic toxicity where relationship to APR-548 and/or azacitidine cannot be excluded (e.g., due to underlying disease).

Dose management guidelines in Table 4, Table 5 and Table 6, respectively, should be followed for each occurrence of infections, nausea/vomiting/diarrhea, and neutropenia with and without fever.

Dose management guidelines in Table 8, Table 9 and Table 10, respectively, should be followed for each occurrence of CNS-associated TEAEs, renal TEAEs (serum creatinine elevations or acute kidney injury), or hepatic TEAEs (elevations in bilirubin, ALT and/or AST).

Subjects experiencing ≥2 occurrences of the same TEAE where attribution to APR-548 and/or azacitidine cannot be excluded (e.g., due to underlying disease), the investigator should consult with the study Medical Monitor before study treatments are resumed or discontinued.

Treatment may be discontinued if a TEAE has not improved (≤Grade 1 or baseline level) or resolved after ≤4 weeks. Consult with the study Medical Monitor to discuss the subject's condition before permanently discontinuing study treatment.

Table 2. Dose management guidelines for new onset hematologic toxicities

Toxicity	Occurrence	APR-548	Azacitidine
Grade 3 or 4 hematologic toxicity *	1st occurrence • Hold all study treatments until blood count(s) return to baseline or Grade ≤1. Treat promptly.	• Consider reducing APR-548 by one dose level from starting dose for all future cycles of treatment if toxicity does not resolve to Grade ≤1 within 7 days from onset date despite maximal medical intervention(s). • If unable to reduce APR-548 dose, discuss with study Medical Monitor before resuming or discontinuing treatment.	• Follow dose modification guidelines per USPI[36].
	2nd or later occurrence • Hold all study treatments until blood count(s) return to baseline or Grade ≤1. Treat promptly.	• Consult with study Medical Monitor before resuming or discontinuing study treatments.	

* Grade 3 and Grade 4 neutropenia with fever or Grade 4 neutropenia without fever should follow the guidelines provided in Table 6.

Table 3. Dose management guidelines for non-hematologic toxicities

Toxicity	Occurrence	APR-548	Azacitidine
Grade 3 or 4 non-hematologic toxicity *	1st occurrence • Hold all study treatments until toxicity returns to baseline or Grade ≤ 1. Treat promptly.	• Consider reducing APR-548 by one dose level from starting dose for all future cycles of treatment if toxicity does not resolve to Grade ≤1 within 7 days from onset date despite maximal medical intervention(s). • If unable to reduce APR-548 dose, discuss with study Medical Monitor before resuming treatment.	• Follow dose modification guidelines per USPI[36].
	2nd or later occurrence • Hold all study treatments until toxicity returns to	• Consult with study Medical Monitor before resuming study treatments.	

	baseline or Grade ≤ 1. Treat promptly.	

*For infections and infestation, nausea/vomiting/diarrhea, ≥ Grade 3 neutropenia, CNS-associated TEAEs, Renal TEAEs, or hepatic TEAEs, refer to Table 4, Table 5, Table 6, Table 8, Table 9 and Table 10, respectively for dose management guidelines.

Table 4. APR-548 and azacitidine dose management guidelines for infection and/or infestation adverse events

Toxicity Grade	Occurrence	APR-548	Azacitidine
Grade 3 or 4	1st occurrence	• Interrupt dosing and monitor subject; treat promptly. • Resume at current dose level if toxicity returns to baseline or Grade ≤1 in less than 7 days from onset date. • Reduce APR-548 by one dose level from starting dose for all future cycles of treatment if toxicity does not resolve to Grade ≤1 within 7 days of onset date. • If unable to reduce APR-548 dose, discuss with study Medical Monitor before resuming or discontinuing treatment. • Initiate or reassess infection prophylaxis measures. Refer to Section 5.3.3 for supportive management guidelines specific to infections or infestations.	Follow dose modification guidelines per USPI[36].
	2nd occurrence	• Hold all study treatments and monitor subject; treat promptly. • Assess current infection prophylaxis measures being administered and adjust treatment plan as clinically indicated. Refer to Section 5.3.3 for supportive management guidelines specific to infections or infestations. • While APR-548 and azacitidine are interrupted, consult with study Medical Monitor before resuming or discontinuing study treatments.	

Table 5. APR-548 dose management guidelines for nausea, vomiting, and/or diarrhea adverse events

Worst toxicity grade	Dose modification guidelines for APR-548 *
Grade 1	• Maintain dose level of APR-548 and monitor subject; provide supportive care measures as clinically indicated.
Grade 2	• Interrupt APR-548 dosing and monitor subject; treat promptly. • Resume at current dose level if toxicity resolves to Grade ≤1 or baseline, with or without use of supportive care measures, within 72 hours of onset. • If toxicity does not resolve to Grade ≤1 within 72 hours despite maximal medical intervention(s), then reduce APR-548 by 1 dose level once the toxicity resolves to

	Grade ≤1 or baseline. • If unable to reduce APR-548 dose, discuss with study Medical Monitor before resuming or discontinuing treatment. • For subsequent doses of APR-548, pre-medication with prophylactic supportive therapy should be given if clinically indicated.	
Grade 3	1st occurrence	• Interrupt APR-548 dosing and monitor subject; treat promptly. • Resume at current dose level if toxicity resolves to Grade ≤1 or baseline, with supportive care measures, within 72 hours of onset. • If toxicity does not resolve to Grade ≤1 within 72 hours despite maximal medical intervention(s), then reduce APR-548 by 1 dose level once the toxicity resolves to Grade ≤1 or baseline. • If unable to reduce APR-548 dose, discuss with study Medical Monitor before resuming or discontinuing treatment. • For subsequent doses of APR-548, pre-medication with prophylactic supportive therapy should be given.
	2nd occurrence	• Interrupt APR-548 dosing and monitor subject; treat promptly. • Reduce APR-548 by 1 dose level if the toxicity resolves to Grade ≤1 or baseline within 72 hours of onset. • If toxicity does not resolve to Grade ≤1 despite maximal medical intervention(s) within 72 hours of onset, then consult with study Medical Monitor before resuming or discontinuing APR-548. • If unable to reduce APR-548 dose, discuss with study Medical Monitor before resuming or discontinuing treatment. • Prior to subsequent doses of APR-548, reassess prophylactic supportive therapy regimen and adjust as clinically indicated.
	3rd occurrence	• Interrupt APR-548 dosing and monitor subject; treat promptly. • Consult with study Medical Monitor before resuming or discontinuing APR-548.
Grade 4	• Interrupt APR-548 dosing and monitor subject; treat promptly. • Consult with study Medical Monitor before resuming or discontinuing APR-548.	

* Refer to Section 5.3.5 for details on supportive management guidelines specific to nausea, vomiting, and/or diarrhea.

Table 6. Dose management guidelines for APR-548 and azacitidine for worsening neutropenia from baseline with or without fever

Toxicity	Occurrence	APR-548	Azacitidine
Grade 3 or 4 neutropenic fever or Worsening neutropenia from baseline to Grade 4 without fever	1st occurrence	• Interrupt dosing and monitor subject; treat promptly. • Resume at current dose level if neutropenia and fever (if present) resolve to baseline within 7 days after onset. • If toxicity does not resolve to baseline despite maximal medical intervention(s) within 7 days of onset, then reduce • APR-548 by 1 dose level once the toxicity resolves to baseline. • If unable to reduce APR-548 dose, discuss with study Medical Monitor before resuming or	Follow dose modification guidelines per USPI[36].

		discontinuing treatment. • Initiate or reassess prophylaxis measures. Refer to Sections 5.3.3 and 5.3.4 for supportive management guidelines specific to infection prophylaxis and neutropenia, respectively.	
	2nd occurrence	• Hold all study treatments and monitor subject; treat promptly. • Assess current infection prophylaxis measures being administered and adjust treatment plan as clinically indicated. Refer to Sections 5.3.3 and 5.3.4 for supportive management guidelines specific to infection prophylaxis and neutropenia, respectively. • While APR-548 and azacitidine are interrupted, consult with study Medical Monitor before resuming or discontinuing study treatments.	

Table 7. Management of ocular-associated adverse events

Parameter	Worst toxicity	Occurrence	Dose Modifications for APR-548
Retinopathy and optic nerve disorders	Grade 1	1st occurrence	Interrupt treatment with APR-548. Monitor and treat patient promptly as clinically indicated. Consult with study medical monitor before resuming dosing of APR-548 at same dose level once event has stabilized or resolved to baseline.
		2nd occurrence	Interrupt treatment with APR-548. Monitor and treat patient promptly as clinically indicated. Consult with study medical monitor to determine if patient should resume at same dose level or if APR-548 should be reduced by 1 dose level.
		3rd occurrence	Interrupt treatment with APR-548. Monitor and treat patient promptly as clinically indicated. Consult with study medical monitor to determine if APR-548 treatment should be reduced by one dose level or permanently discontinued.
	Grade 2	1st occurrence	Interrupt treatment with APR-548. Monitor and treat patient promptly as clinically indicated. Notify medical monitor and report as SAE (AESI). Once resolved to baseline parameters, then reduce dose of APR-548 by one level. If unable to reduce dose of APR-548, discuss with study medical monitor before resuming or discontinuing treatment with APR-548.
		2nd occurrence	Permanently discontinue treatment with APR-548. Monitor and treat patient promptly as clinically indicated. Notify medical monitor and report as SAE (AESI).
	≥Grade 3	N/A	Permanently discontinue subject from APR-548.
Other	Grade 1		Interrupt treatment with APR-548 for new finding or worsening from baseline. Monitor and treat patient promptly as clinically indicated. May resume at current dose level once stabilized or resolved to baseline. Consult with study medical monitor before resuming or reducing dose of APR-548 for a second or greater occurrence of same event.
	Grade 2	1st occurrence	Interrupt treatment with APR-548 for new finding or worsening from baseline. Monitor and treat patient promptly as clinically indicated. Notify Medical Monitor and report as SAE (AESI).

			Once resolved to baseline parameters, then: First occurrence, reduce dose of APR-548 by one level. If unable to reduce dose of APR-548, discuss with study medical monitor before resuming or discontinuing treatment with APR-548.
		2nd occurrence	Permanently discontinue treatment with APR-548. Monitor and treat patient promptly as clinically indicated. Notify Medical Monitor and report as SAE (AESI).
	≥Grade 3	N/A	Permanently discontinue subject from APR-548.

Table 8. Management of CNS adverse events (e.g., dizziness, tremor, confusion, and ataxia)

Worst toxicity	Dose Modifications for APR-548
Grade 1	Maintain dose level
Grade 2	If resolved to ≤Grade 1 with medical therapy, continue same dose level If not resolved despite treatment interruption and maximal medical therapy, interrupt treatment with APR-548 and contact the study Medical Monitor.
Grade ≥3	Interrupt treatment with APR-548 and give medical therapy. If resolved (to ≤Grade 1) with medical therapy, reduce APR-548 by one dose level when treatment is restarted. If not resolved despite treatment interruption and maximal medical therapy, consult study Medical Monitor to determine if a reduction of APR-548 by one dose level could be undertaken once the CNS AE resolves to ≤Grade 1 or if APR-548 should be permanently discontinued.
Grade 4	Permanently discontinue subject from APR-548.

Table 9. Management of serum creatinine elevations and acute kidney injury

Parameter	Worst toxicity[†]	Occurrence	Dose Modifications for APR-548
Serum Creatinine Elevations	Grade 1 (<1.5 × baseline)	N/A	Maintain dose level of APR-548
	Grade 2 (>1.5 to 3.0 × baseline)	1st occurrence	Omit dose until resolved to ≤Grade 1 or baseline, then maintain dose level. Monitor subject.
		2nd occurrence	Reduce by 1 dose level. If unable to reduce by 1 dose level, consult with study Medical Monitor before resuming or discontinuing study treatment.
	Grade 3 (>3.0 – 6.0 × baseline)	1st occurrence	Interrupt dosing with APR-548 and monitor subject. Treat promptly. Reduce by 1 dose level. If event resolved to ≤Grade 1 or baseline less than 7 days from onset. If unable to reduce by 1 dose level, consult with study Medical Monitor before resuming or discontinuing study treatment. If not resolved or resolved in >7 days, evaluate if other contributing factors are present. If none, or after consideration of best interest of the subject, discontinue subject from APR-548. If other factors present, consider continuing after ↓ 1 dose level.
		2nd occurrence	Permanently discontinue treatment with APR-548.
	Grade 4 (>6.0 × baseline)	N/A	Permanently discontinue subject from APR-548.
Acute kidney injury (including acute tubular necrosis)	≤Grade 2	1st occurrence	Omit dose until resolved to ≤Grade 1 with medical therapy. Consult with study Medical Monitor to discuss if a dose reduction should be undertaken.
		2nd occurrence	Permanently discontinue treatment with APR-548.
	Grade 3	1st occurrence	Omit dose until resolved to ≤Grade 1 with medical therapy. Consult with study Medical Monitor to determine if dose reduction or permanent discontinuation of APR-548 should be undertaken.
		2nd occurrence	Permanently discontinue treatment with APR-548.
	Grade 4	N/A	Permanently discontinue subject from APR-548

[†] Common Terminology Criteria for Adverse Events (CTCAE) version 5.
[*] If dose reduction by 1 dose level is not available, consultation with study Medical Monitor should occur prior to resuming treatment with APR-548.

Table 10. Management of liver enzymes elevation

Parameter	Worst toxicity	Occurrence	Dose Modifications for APR-548
Bilirubin‡	Grade 1 (>ULN - 1.5 × ULN)	N/A	Maintain dose level with liver function tests (LFTs)≠ monitored as per protocol
	Grade 2 (>1.5 - 3.0 × ULN) with ALT or AST ≤3.0 × ULN	1st occurrence	Omit dose until resolved to ≤Grade 1 or baseline, then: If resolved in ≤7 days, maintain dose level. If resolved in >7 days, then ↓ 1 dose level. If unable to reduce by 1 dose level, then consult with study Medical Monitor before resuming or discontinuing study treatment.
		2nd occurrence	Omit dose until resolved to ≤Grade 1 or baseline, then ↓ 1 dose level. If unable to reduce by 1 dose level, then consult with study Medical Monitor before resuming or discontinuing study treatment.
	Grade 3 (>3.0 - 10.0 × ULN) with ALT or AST ≤3.0 × ULN	1st occurrence	Omit dose until resolved to ≤Grade 1 or baseline, then: If resolved in ≤7 days, ↓ 1 dose level. If no subsequent event, consider re-escalation to original dose level after consultation with study Medical Monitor. Omit dose until resolved to ≤Grade 1, then: ↓ 1 dose level. If not resolved or resolved in ≤7 days, evaluate if other contributing factors are present. If none, or after consideration of best interest of the subject, discontinue subject from APR-548.
		2nd occurrence	Evaluate if other contributing factors are present. If none, or after consideration of best interest of the subject, discontinue subject from APR-548. If other factors present, consider continuing after ↓ 1 dose level. If unable to reduce by 1 dose level, then consult with study medical monitor before resuming or discontinuing study treatment.
	Grade 4 (>10.0 × ULN)	N/A	Permanently discontinue subject from APR-548
AST or ALT	Grade 1 (>ULN - 3.0 × ULN if baseline was normal; 1.5 - 3.0 × baseline if baseline was abnormal)	N/A	Maintain dose level with LFTs≠ monitored per protocol
	Grade 2 (>3.0 - 5.0 × ULN if baseline was normal; >3.0 - 5.0 × baseline if baseline was abnormal) in the absence of total bilirubin	1st occurrence	Omit dose until resolved to ≤Grade 1 or baseline, then, if resolved in ≤7 days, maintain dose level. If resolved in >7 days, then ↓ 1 dose level. If unable to reduce by 1 dose level, then consult with study Medical Monitor before resuming or discontinuing study treatment
		2nd occurrence	Omit dose until resolved to ≤Grade 1 or baseline, then ↓ 1 dose level. If unable to reduce by 1 dose level, then consult with study Medical Monitor before resuming or discontinuing study treatment.

Parameter	Worst toxicity	Occurrence	Dose Modifications for APR-548
	elevation to > 2.0 × ULN		
	Grade 3 (>5.0 - 20.0 × ULN if baseline was normal; >5.0 – 20.0 x baseline if baseline was abnormal) in the absence of total bilirubin elevation to > 2.0 × ULN	1st occurrence	Omit dose until resolved to ≤Grade 1 or baseline. For 1st occurrence, if resolved in ≤7 days, then maintain dose level. If not resolved or resolved in <7 days, evaluate if other contributing factors are present. If none, or after consideration of best interest of the subject, discontinue subject from APR-548. If other factors present, consider continuing after ↓ 1 dose level. If unable to reduce by 1 dose level, then consult with study Medical Monitor before resuming or discontinuing study treatment.
		2nd occurrence	Evaluate if other contributing factors are present. If none, or after consideration of best interest of the subject, discontinue subject from APR-548. If other factors present, consider continuing after ↓ 1 dose level. If unable to reduce by 1 dose level, then consult with study Medical Monitor before resuming or discontinuing study treatment.
	Grade 4 (>20.0 × ULN if baseline was normal; >20.0 × baseline if baseline was abnormal)	N/A	Permanently discontinue subject from APR-548

◊ Please note that the Investigator is responsible for determining whether a subject meets potential Hy's Law criteria at any point during the study. Hy's law identifies subjects at risk for severe drug-induced liver injury and is defined as AST or ALT ≥3× ULN together with Total Bilirubin Level ≥2×ULN, where no other reason, other than the suspected drug, can be found to explain the combination of increases, e.g., elevated alkaline phosphatase indicating cholestasis, viral hepatitis, or another drug. APR-548 should be permanently discontinued in any subject who meets potential Hy's Law criteria.
† Common Terminology Criteria for Adverse Events (CTCAE) version 5.
‡ For subjects with Gilbert's syndrome, these dose modifications apply to changes in direct bilirubin only.
≠ LFTs include albumin, ALT, AST, total bilirubin (fractionated if total bilirubin >2.0 × ULN), alkaline phosphatase.

5.3 Supportive Management

5.3.1 Growth Factors

Use of hematopoietic growth factors is allowed according to institutional practice.

5.3.2 Blood Products

The use of blood products, including 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 10×10^9/L for platelet transfusion and a hemoglobin threshold of 8.0 g/dL for PRBC transfusion or as clinically indicated at the discretion of treating physician.

[redacted]

The following recommendations were taken from National Comprehensive Cancer Network Guidelines for Prevention and Treatment of Cancer-Related Infections (Version 1.2021).

Table 11. Recommendations for prevention and treatment of cancer-related infections

Organism	Recommendations
Bacterial	Consider fluoroquinolone or other suitable anti-bacterial prophylaxis during neutropenia
Fungal	Consider prophylaxis during neutropenia and for anticipated mucositis. Assess risk for Pneumocystis jirovecii pneumonia and select agent(s) as clinically indicated.
Viral	Start treatment with anti-viral medication in the setting of neutropenia and continually assess the risk for viral infection during treatment with anti-cancer therapy.

Please refer to Section 5.4 for a description of drugs that are not allowed to be used while a subject is on study treatment. Refer to Section 5.2 for dose modification guidelines for APR-548 and azacitidine therapy in the setting of severe or life-threatening infection.

5.3.4 Management of Neutropenia with or without Fever

Monitor complete blood counts at the time of study enrollment and throughout the course of study treatment. Supportive measures such as antimicrobials for prophylaxis or in the setting of prolonged myelosuppression, and/or at the first signs of infection are recommended to reduce the risk of a serious or severe infection that may lead to a fatal outcome in the setting of neutropenia with or without fever (see Section 5.3.3). Please refer to Section 5.4 for a description of drugs that are not allowed to be used while a subject is on study treatment.

Myeloid growth factors may be used in the setting of severe or prolonged neutropenia (see Section 5.3.1). Refer to Section 5.2 for dose modification guidelines for APR-548 and azacitidine in the setting of severe or life-threatening neutropenia with or without the presence of fever.

5.3.5 Management of Nausea, Vomiting and Diarrhea

Prior to starting study treatment, subjects should be evaluated to determine risk for nausea, vomiting, and/or diarrhea based on medical history and concomitant medications. Anti-nausea, anti-emetic, and/or anti-diarrheal medications should be initiated in subjects with intermediate or high risk for nausea, vomiting, or diarrhea. Subjects who experience nausea, vomiting, and/or diarrhea in association with APR-548 administration should be prescribed appropriate rescue treatment and prophylaxis (e.g., anti-nausea, anti-emetics, or anti-diarrheal medication). Monitor electrolyte levels and renal function during the course of study treatment and institute appropriate supportive care measures, such as electrolyte and/or fluid repletion, in the setting of vomiting and/or diarrhea.

Please refer to Section 5.4 for a description of drugs that are not allowed to be used while a subject is on study treatment. Refer to Section 5.2 for dose modification guidelines for APR-548 in the setting of nausea, vomiting, and/or diarrhea.

5.3.6 Management of CNS-Related Adverse Events

In prior APR-246 clinical studies, prochlorperazine 10 mg orally up to three times a day anti-emetics (e.g., ondansetron), or medication(s) to treat motion sickness (e.g., meclizine) have been used for prophylaxis and/or to treat CNS-related AEs that have occurred during treatment with APR-246. Prochlorperazine, anti-emetic, or motion sickness medication(s) should be administered as clinically indicated to prevent or treat CNS-related AEs while subjects are receiving treatment with APR-548. When prophylaxis is clinically indicated, it is recommended to start prochlorperazine, anti-emetic, or motion sickness medication(s) at least one day prior to the scheduled dose of APR-548 and continue giving through day 4 of each treatment cycle. Please follow dose administration guidelines in the current USPI for prochlorperazine, anti-emetic, or motion sickness medication(s).

Refer to Table 8 for dose modification guidelines for APR-548 in the setting of CNS-related AEs.

5.4 Concomitant Treatment

Subjects must not receive any other concurrent anti-cancer therapy, including investigational agents while on study treatment.

As the metabolic pathways for APR-548 are not yet known, concomitant administration of strong inhibitors or inducers of cytochrome P450 isoenzymes or transporter enzymes should be avoided during the study. If unavoidable, the concomitant administration should be ceased for 2 days prior to APR-548 administration in each cycle until one day thereafter.

The use of proton pump inhibitors is prohibited for 1 week prior to APR-548 treatment until the day after the last APR-548 administration within a cycle. Other acid-reducing agents (H_2-receptor antagonist and direct acting neutralizing agents) can be given up to 2 days prior to APR-548 administration. Then they should be stopped until the day after APR-548 administration within each cycle. If needed, direct acting neutralizing agents can be given on the APR-548 treatments days starting 4 hours after administration until bedtime. No acid reducing agents should be taken in the morning prior to APR-548 intake.

As the mechanism of action for APR-548 is associated with enhanced oxidative stress in tumor cells, use of the following medications that may antagonize therapeutic effects of APR-548 is discouraged, but not prohibited during study treatment administration:

- Antioxidants (including but not limited to coenzyme Q10)
- Supplementation with vitamin C, vitamin E, zinc and/or selenium outside of a daily multi-vitamin
- Iron chelators (e.g., deferoxamine or deferasirox)
- Melatonin

Investigator should contact the Medical Monitor to discuss the subject's concomitant treatment, if the subject is taking one or more of the medications listed above or plans to start taking any of these medications while on study treatment.

Subjects may continue their baseline medications as long as they are not prohibited. Palliative and supportive care (e.g., anti-emetics, bisphosphonates) for disease-related symptoms may be utilized as clinically indicated. AEs will be treated as clinically indicated. All concomitant medications should be recorded in the eCRF.

5.5 Monitoring Subject Compliance

This study will be monitored by Aprea Therapeutics, Inc. or its Contract Research Organization (CRO) according to ICH E6 guidelines of GCP. The study site monitor will regularly visit the study sites to ensure that the study is conducted according to the protocol and GCP principles. All instances of protocol deviations will be entered and reviewed by the investigator, Sponsor and appropriate [redacted]

6.0 STUDY EVALUATIONS

6.1 Schedule of Study Assessments

Study assessments are summarized in Table 12 and described in Sections 6.2 through 6.9.

Table 12. Schedule of assessments

Assessments	Screening[a]	Safety Run-In (APR-548 Monotherapy)[b]									APR-548 + Azacitidine Combination												End of treatment[x]	Long-term follow-up[y]
											Cycle 1 (Safety Run-In)					Cycle 2				Cycles 3+				
		D1	D2	D3	D4	D 5	D 8	D 15	D 21	D 28	D1	D2 - 7	D8	D15	D 22	D1[c]	D2 - 7	D8	D15	D1[d]	D2-7	D15[e]		
Informed consent	×																							
Mutant *TP53* confirmation[f]	×																							
Medical history[g]	×																							
Physical examination[h]	×	×						×	×		×			×	×	×				×			×	
Vital signs[h]	×	×	×	×	×			×	×		×	×		×	×	×	×			×	×		×	
Height[i]	×																							
Weight	×	×						×	×		×			×	×	×				×			×	
ECOG performance status	×	×						×	×		×			×	×	×				×			×	
APR-548[j]		×	×	×	×						×[j]	×				×[j]	×			×	×			
Azacitidine[k]											×	×				×	×			×	×			
Disease assessment[l]	×							×												×			×	
Hematology[m]	×	×					×		×		×		×	×		×		×	×	×		×	×	
Serum chemistry[n]	×	×					×		×		×		×	×		×		×	×	×		×	×	
Creatinine clearance[o]	×	×					×		×		×		×	×		×		×	×	×		×	×	
Coagulation profile[p]	×										×					×								
Pregnancy test[q]	×										×					×				×			×	
ECG[r]	×	×	×		×	×					×	×				×				×			×	
APR-548 PK sample[s]		×	×		×	×					×	×				×				×				
Azacitidine PK sample[s]											×	×												
PBMC/BMMC sample collection[t]	×							×												×			×	
Blood sample for PD markers[u]		×	×		×	×					×	×												
Bone marrow biopsy (optional)					×																			
Ophthalmological evaluation[v]	×							×		×				×		×			×	×		×[v]	×	
Clinical safety assessment[w]		Starting at the time of study treatment initiation through 30 days after last dose																						
Concomitant medications	Reviewed throughout study																							
Transfusion log	From 8 weeks prior to enrollment to end of treatment/off treatment																							
Survival																								×

Footnotes to schedule of assessments

a. All screening/baseline assessments are performed within 28 days prior to the start of study treatment. In the event that a visit or test cannot be scheduled on the exact visit day, a window of ±3 days is allowable.

b. A window of ±1 day is allowable for visits during the safety run-in (APR-548 monotherapy) period.

c. A window of ±2 days is allowable for this visit.

d. After the first cycle, Day 1 assessments of subsequent cycles are to be done within 3 days prior to next cycle drug administration.

e. A window of ±5 days is allowable for this visit.

f. Can be confirmed using archived sample.

g. Full medical history is obtained at screening for safety and eligibility purposes; this will include any clinically significant findings from 28 days prior to screening date.

h. Physical exam and vital signs (including blood pressure, heart rate, respiration rate and temperature) are completed for safety purposes and clinically significant items are recorded as AEs where appropriate. Vital signs are collected on days of APR-548 administration.

i. Can be recorded historically from up to 1 year prior to informed consent.

j. APR-548 is administered p.o. on Days 1–4 of each 28-day cycle. Cycle 1 Day 1 of the combination treatment may proceed following ophthalmic evaluation performed by retina specialist to ensure there are no retinal or optic nerve associated AEs, and to evaluate for other potential ocular AEs.

k. Azacitidine is administered s.c. or i.v. over 7 consecutive days, on Days 1-7 of each 28-day cycle. SC route is preferred but may be given i.v. at the investigator's discretion. The same route should be maintained over the 7-day treatment period (whichever route of administration is used on Day 1; the same route should be followed on all other days).

l. Subjects will have the extent of their disease assessed based on IWG 2006 criteria (Appendix V). Bone marrow aspirates and core sampling should be performed according to standard of care and analyzed at the local site's laboratory in accordance with the International Council for Standardization in Hematology (ICSH) Guidelines[37]. Disease assessment is performed at screening, based on peripheral blood and/or bone marrow according to standard of care. Optional disease assessment may be performed during monotherapy safety run-in at approximately Day 15 at the discretion of the investigator. Starting on Cycle 3 Day 1 (±7 days) disease assessment will be performed every two cycles (every 8 weeks), through Month 12. Thereafter, evaluation of disease response, including bone marrow and peripheral blood, will be conducted every three cycles (every 12 weeks) during treatment, independent of dose delays and/or dose interruptions, and/or at any time when progression of disease is suspected. Samples for exploratory endpoints are collected at each disease assessment timepoint.

m. Hematology must include complete blood count with white blood cell differential. Results of hematology parameters have to be reviewed prior to initiation of each treatment cycle.

n. Serum chemistry must include sodium, potassium, chloride, magnesium, CO_2, blood urea nitrogen (BUN), creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase. Results of serum chemistry parameters have to be reviewed prior to initiation of each treatment cycle.

o. Calculated using Cockcroft-Gault method (Appendix I).

p. Coagulation profile will include prothrombin time or INR, and activated partial thromboplastin time.

q. Serum or urine βhCG must be performed within 7 days prior to study treatment initiation in female subjects of childbearing potential.

r. Standard 12-lead ECGs in triplicate at screening, on Days 1, 2, 4 and 5 of the safety run-in period, and on Days 1, 2, 4 and 5 of Cycle 1 and on Day 1 of each subsequent cycle with subject in a semi-recumbent position prior to APR-548 administration. Please consult Table 15 for ECG collection schedule.

s. See Section 7.3 for PK collection schedule.

t. Peripheral blood/bone marrow (or both; whichever sample is collected for disease assessment) mononuclear cells collection for exploratory biomarker testing. Disease assessment on approximately Day 15 during the monotherapy safety-run in is optional.

u. Blood sample for PD markers, collected pre-dose, 8 hours post dose, and 24 hours post dose on Day 1 and 4 of the safety run-in and Cycle 1. The 24 h post-dose timepoints correspond to pre-dose Day 2 and Day 5, respectively

v. Please consult Section 7.6 for the schedule of ophthalmological evaluation.

w. AE description, grade, start date, resolution date, outcome, and relationship to study treatment should be documented. For the time period from signing of informed consent up to receipt of the first dose of APR-548, non-serious events should be recorded as medical history on the appropriate eCRF page within the clinical database. SAE(s) that occur during the screening period are required to be reported and should include an assessment on whether the SAE was related to a protocol-defined procedure or activity.

x. Visit conducted at 28 days after study treatment discontinuation. A window of ±14 days is allowable for End of Treatment ophthalmological evaluation.

y. Long-term follow-up can be done remotely (e.g., via telephone, via local practitioner or via review of medical records). All subjects who undergo HSCT following discontinuation of APR-548 are to be followed after the last dose until relapse or end of study. Subjects who respond and discontinue study treatment for reasons other than disease relapse should continue to have response assessments, and survival should be collected every 2 months until disease relapse, withdrawal of consent for study participation, or death, whichever occurs first. After disease relapse, data for survival should be collected every 6 months until death or withdrawal of consent for study participation. If a subject proceeds to HSCT, evaluation of the extent of disease, response to treatment, and any new antineoplastic therapies will be collected at least monthly as long as the subject remains in remission and assessed as part of the study until relapse, death, withdrawal

of consent, lost to follow-up, or end of study, whichever comes first. If a subject is removed from the study due to unacceptable AEs, the event(s) will be followed until resolution or stabilization of the AE.

6.2 Pre-Study Assessments

Prior to performing any procedures or assessments, the nature of the study and the potential risks associated with the trial will be explained to all subject candidates and written informed consent will be obtained. Subjects who choose to participate will have to consent to collection and storage of blood for correlative studies. Evaluations obtained as part of routine medical care and performed during the screening period may be used in place of the study specific evaluations. Subjects will acknowledge and agree to the possible use of this information for the study by giving informed consent.

6.3 Screening

All screening evaluations are to be performed within approximately 28 days of study treatment initiation, unless otherwise noted.

- Signed written informed consent
- Mutant *TP53* confirmation
- Medical history
- Physical examination
- Height: historical record can be used from up to 1 year in the past
- Weight
- ECOG performance status (Appendix II)
- Vital signs, including blood pressure, heart rate, respiration rate and temperature
- Hematology, including complete blood count with white blood cell differential
- Serum chemistry, including sodium, potassium, chloride, magnesium, CO_2, BUN, creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase
- Creatinine clearance (Cockcroft-Gault method; Appendix I)
- Coagulation profile
- Serum or urine βhCG must be performed within 7 days prior to study treatment initiation for female subjects of childbearing potential
- ECG: standard 12-lead ECGs with subject in a semi-recumbent position in triplicate
- Blood and bone marrow for exploratory biomarker sample testing
- Concomitant medication review
- Baseline disease assessment (bone marrow aspirates and core sampling, within 28 days of study treatment initiation)
- Baseline ophthalmologic evaluation, per Table 16
- Transfusion log

6.4 Safety Run-In Period (APR-548 Monotherapy)

6.4.1 Day 1

- Physical examination

- Weight
- ECOG performance status (Appendix II)
- Vital signs, including blood pressure, heart rate, respiration rate and temperature
- Hematology, including complete blood count with white blood cell differential
- Serum chemistry, including sodium, potassium, chloride, magnesium, CO_2, BUN, creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase
- Creatinine clearance (Cockcroft-Gault method; Appendix I)
- ECG: standard 12-lead ECG with subject in a semi-recumbent position, per Table 15
- APR-548 administration
- Blood sampling for APR-548 PK, per Table 13
- Blood sampling for PD markers (pre-dose, and 8 hours post-dose)
- Clinical safety assessment
- Concomitant medications review
- Transfusion log

6.4.2 Days 2 – 4

- Vital signs, including blood pressure, heart rate, respiration rate and temperature
- APR-548 administration
- Blood sampling for APR-548 PK, per Table 13
- ECG: standard 12-lead ECG with subject in a semi-recumbent position, per Table 15 (Days 2 and 4)
- Optional bone marrow biopsy (Day 4) for correlative analyses
- Blood sampling for PD markers (Day 2: 24 hours post Day 1 dose, Day 4: pre-dose and 8 hours post dose)
- Clinical safety assessment
- Concomitant medications review
- Transfusion log

6.4.3 Day 5

- Blood sampling for APR-548 PK, per Table 13
- Blood sampling for PD markers (Day 5: 24 hours post Day 4 dose)
- ECG: standard 12-lead ECG with subject in a semi-recumbent position, per Table 15
- Clinical safety assessment
- Concomitant medications review
- Transfusion log

6.4.4 Day 8

- Hematology, including complete blood count with white blood cell differential

- Serum chemistry, including sodium, potassium, chloride, magnesium, CO_2, BUN, creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase
- Creatinine clearance (Cockcroft-Gault method; Appendix I)
- Clinical safety assessment
- Concomitant medications review
- Transfusion log

6.4.5 Day 15

- Physical examination
- Weight
- ECOG performance status (Appendix II)
- Vital signs, including blood pressure, heart rate, respiration rate and temperature
- Optional disease assessment per IWG 2006 criteria (Appendix V)
- Blood and bone marrow for exploratory biomarker sample testing
- Ophthalmologic evaluation, per Table 16
- Clinical safety assessment
- Concomitant medications review
- Transfusion log

6.4.6 Day 21

- Physical examination
- Weight
- ECOG performance status (Appendix II)
- Vital signs, including blood pressure, heart rate, respiration rate and temperature
- Hematology, including complete blood count with white blood cell differential
- Serum chemistry, including sodium, potassium, chloride, magnesium, CO_2, BUN, creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase
- Creatinine clearance (Cockcroft-Gault method; Appendix I)
- Clinical safety assessment
- Concomitant medications review
- Transfusion log

6.4.7 Day 28

- Ophthalmologic evaluation, per Table 16
- Clinical safety assessment
- Concomitant medications review
- Transfusion log

6.5 Cycle 1 (APR-548 + Azacitidine Safety Run-In Period)

6.5.1 Day 1

- Physical examination
- Weight
- ECOG performance status (Appendix II)
- Vital signs, including blood pressure, heart rate, respiration rate and temperature
- Hematology, including complete blood count with white blood cell differential
- Serum chemistry, including sodium, potassium, chloride, magnesium, CO_2, BUN, creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase
- Creatinine clearance (Cockcroft-Gault method; Appendix I)
- Coagulation profile
- ECG: standard 12-lead ECG with subject in a semi-recumbent position, per Table 15
- APR-548 administration
- Azacitidine administration. Azacitidine is administered 1 hour (±15 min) after APR-548
- Blood sampling for APR-548 and azacitidine PK, per Table 13
- Blood sampling for PD markers (pre-dose, and 8 hours post-dose)
- Serum or urine βhCG for female subjects of childbearing potential
- Clinical safety assessment
- Concomitant medications review
- Transfusion log

6.5.2 Days 2 – 4

- Vital signs, including blood pressure, heart rate, respiration rate and temperature
- APR-548 administration
- Azacitidine administration. Azacitidine is administered 1 hour (±15 min) after APR-548
- Blood sampling for APR-548 and azacitidine PK, per Table 13
- ECG: standard 12-lead ECG with subject in a semi-recumbent position, per Table 15 (Days 2 and 4)
- Blood sampling for PD markers (Day 2: 24 hours post-dose; Day 4: pre-dose, and 8 hours post-dose)
- Clinical safety assessment
- Concomitant medications review
- Transfusion log

6.5.3 Days 5 – 7

- Azacitidine administration
- Blood sampling for APR-548 and azacitidine PK, per Table 13 (Day 5)

- Blood sampling for PD markers (Day 5; 24 hours post Day 4 dose)
- ECG: standard 12-lead ECG with subject in a semi-recumbent position, per Table 15 (Day 5)
- Blood sampling for PD markers (Day 5; 24 hours post-dose)
- Clinical safety assessment
- Concomitant medications review
- Transfusion log

6.5.4 Day 8

- Hematology, including complete blood count with white blood cell differential
- Serum chemistry, including sodium, potassium, chloride, magnesium, CO_2, BUN, creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase
- Creatinine clearance (Cockcroft-Gault method; Appendix I)
- Clinical safety assessment
- Concomitant medications review
- Transfusion log

6.5.5 Day 15

- Physical examination
- Weight
- ECOG performance status (Appendix II)
- Vital signs, including blood pressure, heart rate, respiration rate and temperature
- Hematology, including complete blood count with white blood cell differential
- Serum chemistry, including sodium, potassium, chloride, magnesium, CO_2, BUN, creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase
- Creatinine clearance (Cockcroft-Gault method; Appendix I)
- Ophthalmologic evaluation, per Table 16
- Clinical safety assessment
- Concomitant medications review
- Transfusion log

6.5.6 Day 22

- Physical examination
- Weight
- ECOG performance status (Appendix II)
- Vital signs, including blood pressure, heart rate, respiration rate and temperature
- Clinical safety assessment
- Concomitant medications review

- Transfusion log

6.6 Cycle 2

6.6.1 Day 1

- Physical examination
- Weight
- ECOG performance status (Appendix II)
- Vital signs, including blood pressure, heart rate, respiration rate and temperature
- Hematology, including complete blood count with white blood cell differential
- Serum chemistry, including sodium, potassium, chloride, magnesium, CO_2, BUN, creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase
- Creatinine clearance (Cockcroft-Gault method; Appendix I)
- Coagulation profile
- APR-548 administration. Cycle 1 Day 1 treatment may proceed following ophthalmologic evaluation performed by retina specialist to ensure there are no clinically significant ocular-associated AE
- Azacitidine administration on Days 1-7. Azacitidine is administered 1 hour (±15 min) after APR-548
- Blood sampling for APR-548 and azacitidine PK, per Table 13
- ECG: standard 12-lead ECG with subject in a semi-recumbent position, per Table 15
- Serum or urine βhCG for female subjects of childbearing potential
- Ophthalmologic evaluation, per Table 16
- Clinical safety assessment
- Concomitant medications review
- Transfusion log

6.6.2 Days 2 – 7

- Vital signs, including blood pressure, heart rate, respiration rate and temperature
- APR-548 administration on Days 2-4
- Azacitidine administration on Days 2-7. On the days of concomitant administration, azacitidine is administered 1 hour (±15 min) after APR-548
- ECG: standard 12-lead ECG with subject in a semi-recumbent position, per Table 15 (Days 2 – 4)
- Clinical safety assessment
- Concomitant medications review
- Transfusion log

6.6.3 Day 8

- Hematology, including complete blood count with white blood cell differential
- Serum chemistry, including sodium, potassium, chloride, magnesium, CO_2, BUN, creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase
- Creatinine clearance (Cockcroft-Gault method; Appendix I)
- Clinical safety assessment
- Concomitant medications review
- Transfusion log

6.6.4 Day 15

- Hematology, including complete blood count with white blood cell differential
- Serum chemistry, including sodium, potassium, chloride, magnesium, CO_2, BUN, creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase
- Creatinine clearance (Cockcroft-Gault method; Appendix I)
- Ophthalmologic assessment: complete ophthalmic exam, spectral domain optical coherence tomography (SD-OCT), color fundus photography, fundus autofluorescence and fluorescein angiography
- Clinical safety assessment
- Concomitant medications review
- Transfusion log

6.7 Cycle 3 and Onwards

6.7.1 Day 1

- Physical examination
- Weight
- ECOG performance status (Appendix II)
- Vital signs, including blood pressure, heart rate, respiration rate and temperature
- Hematology, including complete blood count with white blood cell differential
- Serum chemistry, including sodium, potassium, chloride, magnesium, CO_2, BUN, creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase
- Creatinine clearance (Cockcroft-Gault method; Appendix I)
- Disease assessment per IWG 2006 criteria (Appendix V). Disease assessment is performed every two cycles (every 8 weeks) through Month 12, based on peripheral blood and/or bone marrow according to standard of care. Thereafter, disease assessment will be conducted every three cycles (every 12 weeks).

- ECG: standard 12-lead ECG with subject in a semi-recumbent position, per Table 15
- APR-548 administration
- Blood sampling for APR-548 and azacitidine PK, per Table 13
- Azacitidine administration over 7 consecutive days, on Days 1-7. S.c. route is preferred but may be given i.v. at the investigator's discretion. The same route should be maintained over the 7-day treatment period (whichever route of administration is used on Day 1, the same route should be followed on all other days).
- Blood and bone marrow for exploratory biomarker sample testing
- Serum or urine βhCG for female subjects of childbearing potential
- Ophthalmologic evaluation, per Table 16
- Clinical safety assessment
- Concomitant medications review
- Transfusion log

6.7.2 Days 2 – 7

- Vital signs, including blood pressure, heart rate, respiration rate and temperature
- APR-548 administration on Days 2-4
- Azacitidine administration on Days 2-7. On the days of concomitant administration, azacitidine is administered 1 hour after APR-548
- Clinical safety assessment
- Concomitant medications review
- Transfusion log

6.7.3 Day 15

- Hematology, including complete blood count with white blood cell differential
- Serum chemistry, including sodium, potassium, chloride, magnesium, CO_2, BUN, creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase
- Creatinine clearance (Cockcroft-Gault method; Appendix I)
- Ophthalmologic evaluation, per Table 16 (only in Cycle 3)
- Clinical safety assessment
- Concomitant medications review
- Transfusion log

6.8 End of Treatment Visit

This visit should take place within 28 days of the last dose of study treatment, if treatment is stopped early for any reasons.

- Physical examination
- Weight

- Vital signs
- ECOG performance status (Appendix II)
- Hematology, including complete blood count with white blood cell differential
- Serum chemistry, including sodium, potassium, chloride, magnesium, CO_2, BUN, creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase
- Creatinine clearance (Cockcroft-Gault method; Appendix I)
- Disease assessment per IWG 2006 criteria (Appendix V)
- Blood and bone marrow for exploratory biomarker sample testing
- Ophthalmologic evaluation per Table 16
- ECG: standard 12-lead ECG with subject in a semi-recumbent position, per Table 15
- Serum or urine βhCG
- Clinical safety assessment
- Concomitant medications review
- Transfusion log

6.9 Long-Term Follow-Up

Long-term follow-up can be done remotely (e.g., via telephone, via local practitioner or via review of medical records). All subjects who undergo HSCT following discontinuation of APR-548 are to be followed after the last dose until relapse or end of study. Subjects who respond and discontinue study treatment for reasons other than disease relapse should continue to have response assessments, and survival should be collected every 2 months until disease relapse, withdrawal of consent for study participation, or death, whichever occurs first. After disease relapse, data for survival should be collected every 6 months until death or withdrawal of consent for study participation. If a subject proceeds to HSCT, evaluation of the extent of disease, response to treatment, and any new antineoplastic therapies will be collected at least monthly as long as the subject remains in remission and assessed as part of the study until relapse, death, withdrawal of consent, lost to follow-up, or end of study, whichever comes first. If a subject is removed from the study due to unacceptable AEs, the event(s) will be followed until resolution or stabilization.

7.0 STUDY ASSESSMENTS

7.1 Safety Assessments

7.1.1 Safety Analysis

Safety data will be tabulated for all subjects and include vital signs, laboratory parameters, ECG and AEs.

7.1.2 Reporting of Adverse Events

7.1.2.1 Adverse Events

An AE is any untoward medical occurrence in a subject administered a pharmaceutical product that does not necessarily have a causal relationship with the treatment. An AE can be any unfavorable and unintended sign (including a laboratory finding), symptom or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

The AE reporting period starts at the time of study treatment initiation. At each evaluation, subjects should be interviewed in a non-directed manner to elicit potential adverse reactions from the subject. The occurrence of an AE will be based on changes in the subject's physical examination, laboratory results, and/or signs and symptoms.

For the time period from signing of informed consent up to receipt of the first dose of APR-548, non-serious events should be recorded as medical history on the appropriate eCRF page within the clinical database. SAEs that occur during the screening period are required to be reported and should include an assessment on whether the SAE was related to a protocol-defined procedure or activity.

All AEs (except laboratory abnormalities that are assessed as not clinically significant by the investigator, or examining ophthalmologist as applicable), regardless of causal relationship, are to be recorded in the eCRF and source documentation. The investigator must determine the intensity of any AEs according to the NCI-CTCAE version 5.0 and their causal relationship to each study medication administered that has been administered to the subject at the time of event onset. Those AEs not covered by these criteria will be graded as follows:

1. Mild: Discomfort noticed, but no disruption of normal daily activity. Prescription drug not ordinarily needed for relief of symptom but may be given because of personality of subject.
2. Moderate: Discomfort sufficient to reduce or affect normal daily activity. Subject is able to continue in study; treatment for symptom may be needed.
3. Severe: Incapacitating, severe discomfort with inability to work or to perform normal daily activity. Severity may cause cessation of treatment with test drug; treatment for symptom may be given and/or subject hospitalized.
4. Life-Threatening: Symptom(s) place the subject at immediate risk of death from the reaction as it occurred; it does not include a reaction that, had it occurred in a more serious from, might have caused death.
5. Fatal: Event caused the death of the subject.

AEs will be followed until resolution or stabilization while the subject remains on-study. Once the subject is removed from study, events thought to be related to the study medication will

be followed until resolution or stabilization, unless, in the investigator's opinion the event is unlikely to resolve due to the subject's underlying disease, or until the subject starts a new treatment regimen or the subject is lost to follow-up.

The end of the AE and SAE reporting period is 30 days after last dose of study treatment, unless the subject has withdrawn consent for study participation or started new therapy for underlying disease. Progression of primary disease should be reported on a response assessment eCRF in this FIH study. Events unequivocally related only to progression of primary disease do not need to be reported separately on the AE CRF page. Hospitalization to start new therapy for primary disease after confirmed progression/relapse does not meet reporting criteria for an AE or SAE.

7.1.2.2 AE Relationship Attribution Definitions

An AE is considered to be associated with the use of the study treatment if the attribution is determined as possible or definite.

The relationship of each AE to study treatment should be evaluated by the Investigator using the following criteria:

- Definitely related: A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to the medication administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug should be clinically plausible.
- Possibly related: A clinical event, including laboratory test abnormality, with a reasonable time sequence to the medication administration, but which could also be explained by concurrent disease or other drugs or chemicals. Information on the drug withdrawal may be lacking or unclear.
- Unlikely related: A clinical event, including laboratory test abnormality, with little or no temporal relationship to medication administration, and which other drugs, chemicals, or underlying disease provide plausible explanations.
- Not related: A clinical event, including laboratory test abnormality that has no temporal relationship to the medication or has more likely alternative etiology.

7.1.2.3 Definition of an Unexpected Adverse Event

An unexpected AE is defined as any adverse drug experience, the specificity or severity of which is not consistent with the reference safety information in the current Investigator's Brochure (IB) for a study product or products administered to a subject; or, if an IB is not available, the specificity or severity of which is not consistent with the risk information described in this protocol or in the regulatory agency study authorization application.

For study treatments used in this protocol that have been approved for use in the US by the FDA (i.e., azacitidine), the reference safety information that will be used for making expectedness decisions is the most current version of the product USPI that can be found on the FDA website.

Unexpected, as used in this definition, refers to an adverse drug experience that has not been previously observed (e.g., included in the IB or other product reference safety information such as the USPI) rather than from the perspective of such experience not being anticipated from the pharmacological properties of the pharmaceutical product.

7.1.2.4 Serious Adverse Event

An SAE is defined as any untoward medical occurrence that at any dose:

1. Results in death,
2. Is life-threatening (i.e., 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 was more severe),
3. Requires inpatient hospitalization or prolongation of existing hospitalization excluding that for pain management, disease staging/re-staging procedures, or catheter placement unless associated with other serious events,
4. Results in persistent or significant disability/incapacity, or
5. Is a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious adverse drug events when, based on appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

7.1.2.5 Pregnancy

Any pregnancy detected during the study, or that occurs within 30 days after stopping study medication, must be reported immediately to the investigator. Pregnancy, in and of itself, is not regarded as an AE, unless there is suspicion that study medication may have interfered with the effectiveness of a contraceptive medication. If the subject becomes pregnant while on-study, the study drug should be immediately discontinued. Pregnancy information about a female subject or a female partner of a male subject should be reported immediately from the time the investigator first becomes aware of a pregnancy or its outcome. This will be performed by the investigator per instructions from the Sponsor's monitoring CRO.

Any pregnancy complication, spontaneous abortion, elective termination of a pregnancy for medical reasons, outcome of stillbirth, congenital anomaly/birth defect, or SAE in the mother will be recorded as an SAE and will be reported as described in Section 7.1.2.6.

7.1.2.6 Reporting of Serious Adverse Events

AEs classified as serious require expeditious handling and reporting to Sponsor's monitoring CRO to comply with regulatory requirements.

For any SAE that occurs while a subject is on-study; within 30 days of the last study treatment administration, regardless of any opinion as to the relationship of the SAE to the study treatment; or if any SAE that the investigator feels is related to the study treatment occurs later than 30 days after the last study treatment administration, [redacted] Safety Desk must be notified immediately (within 24 hours of becoming aware of the event). The primary mechanism for reporting an SAE to the sponsor will be the electronic data collection tool. An SAE eCRF should be completed in the electronic data capture (EDC) system within 24 hours of site becoming aware of the event. EDC Alerts will be sent to Safety desk for all SAEs.

If the EDC system is inaccessible/unavailable, then the site will use the paper SAE form in order to report the SAE within 24 hours. Emailing the paper SAE forms is the preferred method of notification.

SAEs will be reported to: Email: [redacted]

7.1.2.7 Safety Monitoring Plan

The Medical Monitor is responsible for ongoing safety monitoring for the study per the detailed safety plan. This monitoring will include a review of all SAEs as they are reported by the study site. The Medical Monitor will also be in contact with site monitors and will be available to discuss any issues concerning safety with site staff. Safety data will be reviewed periodically by [redacted] and the Sponsor Medical Officer.

DRT consisting of the Medical Monitor, Site Principal Investigators, independent retina specialist and other clinical research personnel that the Sponsor may deem appropriate, will hold DRMs on an interim basis at a frequency dependent on study accrual. At these meetings, the DRT will review AEs, DLTs and other safety data as applicable and make recommendations regarding dose escalation and the RP2D.

The DRT will produce summaries or minutes of these meetings. These summaries will be available for inspection when requested by any of the regulatory bodies charged with the safety of human subjects and the integrity of data.

7.2 Efficacy Assessments

7.2.1 Complete Remission Rate

CR rate will be summarized for the safety and efficacy-evaluable (EE) subjects as the proportion (%) of subjects with CR per the IWG 2006 criteria (Appendix V)[38]. In addition to presenting the CR rate, its associated exact 95% CI will also be presented.

7.2.2 Duration of Response

DOR is defined as the time from the date when criteria for response are met to the date of PD or death due to any cause, whichever occurs first. Subjects alive with no PD will have their DOR censored at the date of the last clinical assessment. The DOCR will be summarized by providing the median DOR together with associated 95% CI, using Kaplan-Meier methodology.

7.2.3 Overall Response

Overall response will be summarized in number (%) of subjects in each category of responses (CR, PR, marrow complete response [mCR] with HI, mCR without HI, HI). ORR will be analyzed by using the similar method as primary endpoint CR. DOR, as defined above, will also be evaluated in regard to ORR. Time to AML is calculated from first day of study treatment to first onset of AML. Kaplan-Meier methodology will be utilized. Rate of AML transformation will be analyzed by using the similar method as primary endpoint CR.

7.2.4 Overall Survival

Survival data are collected at treatment and follow-up periods. Subjects will be followed until death or withdrawal of consent from the study, whichever occurs first.

OS is defined as the number of days from the first day of treatment to the date of death due to any cause. Kaplan-Meier methodology will be utilized.

7.2.5 Relapse-Free Survival

RFS is defined as the time from the date of randomization to disease relapse or death, whichever occurs first. If neither event occurs, RFS will be censored at the date of the last clinical assessment. Kaplan-Meier methodology will be utilized.

7.2.6 Rate of AML Transformation, Transition to HSCT, and Transfusion Independence

Rate of AML transformation, transition rate to HSCT, and rate of RBC TI and/or platelet TI will be analyzed using the similar methodology as CR rate.

7.3 Pharmacokinetics

Whole blood concentrations of APR-548 will be measured in the safety run-in period and in Cycle 1 of the combination portion, at the timepoints provided in Table 13. Plasma samples from Cycle 1 for analysis of azacitidine will be taken as indicated in Table 13.

Table 13. PK blood sampling timepoints for APR-548 and azacitidine in the safety run-in period and subsequent cycles

Period	Day	Time relative to APR-548 dose (h)									
		Pre-dose	0.25 [a]	0.5 [a]	0.75 [a]	1 [b]	1.5 [b]	2 [b]	4 [b]	6 [b]	8 [b]
Safety Run-In (APR-548 monotherapy)	1	×	×	×	×	×	×	×	×	×	×
	2	×									
	4	×	×	×	×	×	×	×	×	×	×
	5	×									
Cycle 1 (APR-548 + Azacitidine)	1	×	×	×	×	×[c,d]	×	×[d]	×[d]	×[d]	×[d]
	2	×[d]									
	4	×	×	×	×	×[c,d]	×	×[d]	×[d]	×[d]	×[d]
	5	×[d]									
Subsequent cycles	1	×				×		×			

a. A window of ±5 minutes is allowable for this collection.
b. A window of ±15 minutes is allowable for this collection.
c. Cycle 1 of combination therapy. Whole blood sample for APR-548 analysis and plasma sample for azacitidine analysis directly prior to azacitidine administration.
d. Cycle 1 of combination therapy. Plasma samples for azacitidine administration to be taken at the same time as whole blood samples for APR-548.

The PK sampling timepoints (0.25, 0.75, 1.5 hours post dose) can be reduced once the C_{max} and $T_{1/2}$ are established. The Sponsor will provide a written memo to sites, which should be submitted to IRBs and can be implemented per local site requirements.

PK blood sample for the measurements of whole blood concentrations of the unstable MQ adducts MQ-H2O, MQ-Cys and MQ-GSH will be implemented at selected centers which are equipped to conduct the critical sampling handling procedures described in the lab manual. All subjects enrolled in these centers will participate in this assessment and it is anticipated that data from at least 6 subjects will be available. The PK sampling schedule is provided in Table 14.

Table 14. PK blood sampling timepoints for MQ-H2O, MQ-Cys and MQ-GSH for subjects at selected centers

Period	Day	Time relative to APR-548 dose (h)									
		Pre-dose	0.25 [a]	0.5 [a]	0.75 [a]	1 [b]	1.5 [b]	2 [b]	4 [b]	6 [b]	8 [b]
Safety Run-In (APR-548 monotherapy)	4	×	×	×	×	×	×	×	×	×	×
	5	×									
Cycle 1 (APR-548 + Azacitidine)	4	×	×	×	×	×	×	×	×	×	×
	5	×									

a. A window of ±5 minutes is allowable for this collection.
b. A window of ±15 minutes is allowable for this collection.
The PK sampling timepoints (0.25, 0.75, 1.5 hours post dose) can be reduced once the C_{max} and $T_{1/2}$ are established. The Sponsor will provide a written memo to sites, which should be submitted to IRBs and can be implemented per local site requirements.

7.4 Pharmacodynamics

Blood samples for PD biomarkers are collected pre-dose, 8 hours post dose, and 24 hours post dose on Day 1 and Day 4 in safety run-in and Cycle 1 (the 24 h post-dose timepoints correspond to pre-dose Day 2 and Day 5, respectively). Flow cytometry analysis will be completed by central laboratory.

Peripheral blood/bone marrow (whichever sample is collected for disease response assessment) mononuclear cells collection for exploratory biomarker sample testing, including DNA mutation panel, RNA expression profile, *TP53* VAF by NGS, will be done at each response assessment. Descriptive statistics will be written. Not all listed analyses may be performed.

7.5 Electrocardiographic Assessment

Table 15 describes the routine ECG requirements.

Table 15. ECG assessment requirements

Time Point	ECG, number	Timing
Screening	Triplicate	Within 28 days of study treatment
Safety Run-In, Day 1	Triplicate	Pre-dose; 0.5, 1, 2, 3 and 4 hours after APR-548 administration (±10 min)
Safety Run-In, Day 2	Triplicate	Pre-dose
Safety Run-In, Day 4	Triplicate	Pre-dose; 0.5, 1, 2, 3 and 4 hours after APR-548 administration (±10 min)
Safety Run-In, Day 5	Triplicate	Pre-dose
Cycle 1, Day 1	Triplicate	Pre-dose; 0.5, 1, 2, 3 and 4 hours after APR-548 administration (±10 min)
Cycle 1, Day 2	Triplicate	Pre-dose
Cycle 1, Day 4	Triplicate	Pre-dose; 0.5, 1, 2, 3 and 4 hours after APR-548 administration (±10 min)
Cycle 1, Day 5	Triplicate	Pre-dose
Cycles 2+, Day 1	Triplicate	Pre-dose, 1- and 2-hours post dose (±10 min)
End of Treatment	Triplicate	-

Schedule of ECG assessments may be modified upon Sponsor notification, based on available data.

The following ECG parameters are to be captured in eCRF: heart rate, PR interval, RR interval, QT interval and QT interval corrected for heart rate using Fridericia's formula: $QTc=QT/RR^{1/3}$.

If a subject starts treatment with a medication known to prolong QT interval at any time during the study treatment period, an additional pre- and post-dose (4 hours after APR-548 administration, ±10 min) ECG should be performed on the next APR-548 treatment day unless such pre- and post-dose ECGs are already required on that next APR-548 treatment day per the schedule of study assessments.

7.6 Ophthalmological Evaluation

Table 16 describes the schedule of ophthalmological evaluation.

Table 16. Ophthalmological evaluation

Assessments [a]	Screening [b, c]	Safety Run-In [d]		Cycle 1 (1st combination cycle)	Cycle 2 & 3		Subsequent Cycles	End of treatment [i]
		D15 [e]	D28 [e]	D15 [e]	D1 [f]	D15 [f]	D1 [f]	
Complete ophthalmic exam [b]	×	×	×	×	×	×	×	×
Visual field test [g]	×	×		×	×	×	×	×
SD-OCT [h]	×	×	×	×	×	×	×	×
Color fundus photography	×	×	×	×	×	×	×	×
Fundus autofluorescence	×	×		×	×	×	×	×
Fluorescein angiography	×			×	×	×		

a. All evaluations are to be performed prior to administration of a next scheduled dose.
b. Complete ophthalmic exam includes best corrected distance visual acuity utilizing Early Treatment Diabetic Retinopathy Study (ETDRS) scale, intraocular pressure measurement using Tono-Pen or Goldmann (the same modality should be used throughout the study), slit lamp exam (anterior and posterior segments), dilated fundus (using indirect ophthalmoscopy). Additional testing may be performed at discretion of the retina specialist. Applicable ophthalmologic assessment may be repeated at an unscheduled visit during the study if clinically indicated. An ophthalmic CRF is completed by the examining retina specialist at each visit.
c. Performed within 14 days of initial APR-548 administration.
d. Applies to the 28-day safety run-in period for APR-548 monotherapy and the 28-day safety run-in period for APR-548 in combination with azacitidine.
e. A window of ±4 days is allowable for this visit. This visit has to be completed prior to Cycle 1 Day 1 (1st combination cycle).
f. A window of ±5 days is allowable for this visit.
g. An automated, threshold perimetry with Humphrey visual field analyzer using 30-2 testing protocol.
h. Spectral domain optical coherence tomography of the macula and optic nerve, including ganglion cell complex analysis with study-specified and standard data acquisition algorithms utilized.
i. Visit conducted at 28 days (±14 days) after study treatment discontinuation.

Please consult the A20-11202 study ophthalmic reference manual and ocular assessment worksheets for details. Ocular assessment worksheets are to be completed by retina specialist and submitted to the site for each visit.

8.0 STATISTICS

Demographic data and disease-related characteristics will be summarized using descriptive statistics (count and percent, mean, median, standard deviation, minimum, maximum). Continuous variables will be presented by *n*, mean, median, standard deviation and range (minimum and maximum), and categorical variables will be presented by count and percentage of subjects as appropriate. Data will be presented by each dose level in safety lead-in dose-finding portion and in the expansion portion. All subject data, efficacy and safety data will be summarized.

8.1 Sample Size

This trial assumes a sample size of up to 46.

8.2 Analysis Populations

Safety population: Subjects will be evaluable for safety if they receive at least one dose of APR-548. The safety population will be the primary analysis population used for all analyses such as subject disposition, subject demographics, exposure, safety parameters and efficacy parameters. The safety population will be the primary analysis population for efficacy.

EE population: All subjects who complete at least one treatment cycle of APR-548 and who have at least one post-treatment clinical response assessment. The EE population will be the secondary analysis population for efficacy.

PK population: Subjects will be evaluable for PK if at least one sample for PK evaluation has been obtained.

8.3 Endpoints

8.3.1 Primary

1. Occurrence of DLTs, classified and graded according to the NCI-CTCAE version 5.0
2. Frequency of TEAEs and SAEs related to APR-548 alone and in combination with azacitidine
3. RP2D of APR-548

8.3.2 Secondary

1. Rate of CR
2. DOCR, defined as the time from documentation of CR to disease relapse
3. DOR, defined as the time from documentation of tumor response to disease relapse/progression or death as a result of any cause
4. ORR is the proportion of subjects achieving HI, PR, CR, marrow CR by modified IWG 2006 criteria
5. Rate of and time to AML transformation, according to WHO criteria
6. OS
7. RFS
8. Proportion of subjects who transition to HSCT
9. Subjects who achieve RBC and/or platelet TI during the 8-week period after enrollment
10. PK parameters: C_{max}, T_{max}, AUC, oral V_d/F, oral clearance (CL/F) and elimination $T_{1/2}$ of APR-548 in the absence and presence of azacitidine.

11. PK parameters: C_{max}, T_{max}, AUC, $T_{1/2}$ of APR-548 and the metabolites M8 (reduced MQ-glutathione adduct), M10 (reduced MQ-cysteine adducts), MQ water adducts (MQ-H2O) and MQ glutathione adducts (MG-GSH).

8.3.3 Exploratory

1. PD markers of APR-548 may be explored using flow cytometry and molecular techniques including, but not limited to, markers of apoptosis and proliferation
2. Exploratory analyses of molecular markers of response may include: *TP53* VAF by NGS, mutations and VAF of other genes by NGS, and gene and protein expression

8.4 Safety Stopping Criteria

The study will implement the following stopping criteria:

- Any death within 30 days from the first dose of APR-548.
- A medically equivalent serious AE experienced by >1 subject within the first 30 days of study treatment (monotherapy and 1st cycle of combination treatment).
- Any > Grade 3 AE experienced by >2 subjects within the first 30 days of study treatment.
- Any ≥ Grade 3 ocular associated AE that does not return to ≤ Grade 1 or baseline within 7 days.

If a stopping criterion is met, enrollment will be temporarily suspended until the DRT has performed a prompt cumulative review of safety data and the circumstances of the event in question, to determine whether dosing and/or the protocol should be modified.

8.5 Safety

Safety data including AEs, vital signs, laboratory data, ECG, ophthalmologic assessment findings, and other physical exam findings will be summarized for the safety population. AEs will be tabulated by system organ class (SOC), preferred term, severity, and relationship to treatments. The tabulation of laboratory parameters will include the normal ranges for each parameter. AE terms will be coded using the Medical Dictionary for Drug Regulatory Activities, version 22.1 or higher. AEs will be summarized by SOC, preferred term, severity, and relationship to treatment. SAEs, deaths, and AEs leading to APR-548 dose modifications including interruptions, reductions, and early discontinuation of study treatment will be summarized. Laboratory parameters will be summarized by maximum NCI-CTCAE version 5.0 severity grade and also by change from pre-treatment to scheduled time points using descriptive statistics. Laboratory parameter listings will include the normal ranges for each parameter. Each value will be classified as falling above, below, or within the normal range.

AEs are to be collected from the time of first dose of APR-548 and SAEs are to be collected from the time of signing informed consent, throughout study enrollment, and up to 30 days after last dose of APR-548 or azacitidine, whichever is later. Data summaries will include only TEAEs, defined as events occurring at the first administration of APR-548 up to and including 30 days after last dose of study treatment.

8.6 Efficacy

CR rate will be summarized for the safety and EE subjects as the proportion (%) of subjects with CR. In addition to presenting the CR rate, its associated exact 95% CI for each treatment arm will also be presented.

DOR is defined as the time from the date when criteria for response are met to the date of PD or death due to any cause, whichever occurs first. Subjects alive with no PD will have their DOR censored at the date of the last clinical assessment. The DOCR will be summarized in each treatment arm by providing the median DOR together with associated 95% CI, using Kaplan-Meier methodology.

Overall response will be summarized in number (%) of subjects in each category of responses (CR, PR, mCR with HI, mCR without HI, HI). ORR will be analyzed by using the similar method as primary endpoint CR. DOR, as defined above, will also be evaluated in regard to ORR. Time to AML is calculated from first day of study treatment to first onset of AML. Kaplan-Meier methodology will be utilized. Rate of AML transformation will be analyzed by using the similar method as primary endpoint CR.

Survival data are collected at treatment and follow-up periods. Subjects will be followed until death or withdrawal of consent from the study, whichever occurs first.

OS is defined as the number of days from the first day of treatment to the date of death due to any cause. Kaplan-Meier methodology will be utilized.

RFS is defined as the time from the date of randomization to disease relapse or death, whichever occurs first. If neither event occurs, RFS will be censored at the date of the last clinical assessment. Kaplan-Meier methodology will be utilized.

Time to AML, OS and RFS will be analyzed using the similar methods as DOR.

Transition rate to HSCT will be analyzed using the similar methods as CR.

8.7 Pharmacokinetic Analysis

Intense PK sampling for APR-548 and the metabolites M8 and M10 will be performed in the safety run-in period on Days 1, 2, 4 and 5 of monotherapy, and on Cycle 1 of combination therapy on Days 1, 2, 4, 5. Sparse PK sampling for APR-548 and the metabolites M8 and M10

will be performed in every subsequent cycle on Day 1. Intense PK sampling for azacitidine will be performed in Cycle 1 on Days 1, 2, 4 and 5.

PK sampling for MQ-H2O, MQ-Cys and MQ-GSH will be implemented for individual subjects at selected centers. Samples will be collected in the safety run-in period on Days 4 and 5 of monotherapy and on Cycle 1 of combination therapy on Days 4 and 5.

Non-compartmental methods will be used to derive PK parameters for APR-548, the metabolites and azacitidine (C_{max}, T_{max}, $T_{1/2}$, AUC, CL/F and Vd/F). The PK of the study drugs and metabolites will be summarized using descriptive statistics (mean, standard deviation, CV% mean, geometric mean, CV% geometric mean). The concentration data for APR-548 will be evaluated using popPK analysis in combination with data from other studies.

During the dose escalation part of the safety run-in and Cycle 1 of the combination treatment, PK analysis of APR-548 and its metabolites will be conducted on an ongoing basis to allow review of the PK data prior to the dose escalation decision. Prediction of APR-548 for human PK will be updated on an ongoing basis to ensure that the exposure levels at the next dosing cohort do not exceed the pre-specified average maximum levels. Furthermore, PK variability will also be taken into account to ensure that the exposure levels in an individuum will not exceed the pre-specified individual maximum levels. Exploratory exposure/safety analysis will be conducted on an ongoing basis for APR-548 and the metabolites to detect any relationship of safety signals with exposure levels and to guide the dose selection for the next cohort. If additional preclinical data indicate that more differentiated safety margins should be applied for one or more APR-548 metabolites, the protocol will be amended accordingly.

8.8 Exploratory analyses

Descriptive statistics/results from exploratory analyses will be written. Results for PD biomarkers will include marker changes in different blood cell populations; results for response prediction and monitoring may include but are not limited to *TP53* VAF by NGS, mutations in other genes by NGS, RNA expression.

9.0 QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

9.1 Monitoring of the Study and Regulatory Compliance

The project manager, or designee, will make an initiation site visit to each institution to review the protocol and its requirements with the investigator(s), inspect the drug storage area, fully inform the investigator of his/her responsibilities and the procedures for assuring adequate and correct documentation. During the initiation site visit the eCRFs will be reviewed. Other pertinent study materials will also be reviewed with the investigator's

research staff. During the course of the study, the monitor will make regular site visits in order to review protocol compliance, examine eCRFs and individual subject's medical records and assure that the study is being conducted according to pertinent regulatory requirements. All eCRF entries will be verified with source documentation. The review of medical records will be done in a manner to assure that subject confidentiality is maintained.

9.2 Curricula Vitae and Financial Disclosure of Investigators

All Principal Investigators will be required to provide a current signed and dated curriculum vitae, a completed FDA Form 1572 and a financial disclosure statement to Sponsor's monitoring CRO. All Sub-Investigators will be required to provide a current curriculum vitae and a financial disclosure statement to Sponsor's monitoring CRO.

9.3 Protocol Modifications

No modification of the protocol should be implemented without the prior written approval of the Sponsor or the Sponsor's representative. Any such changes which may affect a subject's treatment or informed consent, especially those increasing potential risks, must receive prior approval by the IRB/IEC. The exception to this is where modifications are necessary to eliminate an immediate hazard to trial subjects, or when the change involves only logistical or administrative aspects of the trial (e.g., change in monitor, change in telephone number). Other administrative revisions which may impact the clinical portion of a study will be duly reported to the IRB/IEC by the Principal Investigator.

9.4 Publication Policy

The publication of the results of the study will be subject to the terms and conditions of the clinical trial agreement between the Sponsor and Investigators. Sponsor approval is required for publication of any data from this trial.

10.0 ETHICAL CONSIDERATIONS

10.1 Informed Consent

The investigator will obtain written informed consent from each subject, or their authorized representative, participating in the study. The form must be signed, witnessed, and dated. The informed consent form will contain all the Essential Elements of Informed Consent set forth in 21 CFR, Part 50, the European Union Directive 2001/20/EC and its associated Detailed Guidances, European Union GCP Directive 2005/28/EC, the ICH Guideline for GCP, Section 4.8, and the terms of the Declaration of Helsinki (2013). Copies of the signed document should be given to the subject and filed in the investigator's study file, as well as the subject's medical record if in conformance with the institution's Standard Operating Procedures.

10.2 Institutional Review Board/Independent Ethics Committee

The study will not be initiated without approval of the appropriate IRB/IEC and compliance with all administrative requirements of the governing body of the institution. This protocol, consent procedures, and any amendments must be approved by the IRB/IEC in compliance with current regulations of the FDA and the European Union as applicable and in accordance with ICH/GCPs. A letter of approval will be sent to the Sponsor prior to initiation of the study and when any subsequent modifications are made. The IRB/IEC will be kept informed by the investigator, Sponsor's monitoring CRO or the Sponsor, as required by national regulations, as to the progress of the study as well as to any serious and unexpected AEs.

10.3 Subject Privacy

In order to maintain subject confidentiality, all eCRFs, study reports and communications relating to the study will identify subjects by initials and assigned subject numbers; subjects should not be identified by name. In accordance with local, national or federal regulations, the investigator will allow the Sponsor or designee personnel access to all pertinent medical records in order to verify the data gathered on the eCRFs and to audit the data collection process. Regulatory agencies such as the US FDA may also request access to all study records, including source documentation for inspection. Clinical information will not be released without the written permission of the subject as outlined in the subject consent form.

11.0 DATA HANDLING AND RECORDKEEPING

11.1 Data to be Entered Directly in the Case Report Form

The eCRF will be the source record.

11.2 Recording of Data

Data collected during the study will be entered in the subject's eCRF by the investigational site staff. The staff will keep records of the subject's visit in the files considered as source documents for the site, e.g., hospital chart, research chart. The investigator will be responsible for the recording of all data on the eCRF and for submitting the data to the Sponsor or their designee in a timely manner. Should any value be significantly different from normal, the investigator will comment in the appropriate sections provided in the eCRF.

The investigator will provide access to his/her original records to permit a representative from the Sponsor to verify the proper transcription of data.

11.3 Study Records

U.S. Federal laws require that an investigator maintain all study records for the indication under investigation for two years following the date a Product Licensing Application is approved or, if no application is to be filed or if the application is not approved for such indication, until two years after the investigation is discontinued and the FDA is notified.

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APPENDIX I - Cockcroft-Gault Equation

Males:

$$\text{Creatinine CL (mL/min)} = \frac{\text{Weight (kg)} \times (140 - \text{Age})}{72 \times \text{serum creatinine (mg/dL)}}$$

Females:

$$\text{Creatinine CL (mL/min)} = \frac{\text{Weight (kg)} \times (140 - \text{Age})}{72 \times \text{serum creatinine (mg/dL)}} \times 0.85$$

APPENDIX II - ECOG Performance Status

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

APPENDIX III - Acceptable Contraceptive Methods

- Male or female condom with or without spermicide
- Cervical cap, diaphragm or sponge with spermicide

Effective Contraceptive Methods That Are User Dependent [a]
Failure rate of <1% per year when used consistently and correctly.

- Combined (estrogen- and progesterone-containing) hormonal contraception [b]
  - Oral
  - Intravaginal
  - Transdermal
  - Injectable
- Progestogen-only hormonal contraception [b]
  - Oral
  - Injectable

Effective Methods That Have Low User Dependency
Failure rate of <1% per year when used consistently and correctly.

- Progesterone-only contraceptive implant [b, c]
- Intrauterine hormone-releasing system (IUS) [b]
- Intrauterine device (IUD)
- Bilateral tubal occlusion
- **Vasectomized partner**

A vasectomized partner is an effective contraception method provided that the partner is the sole male sexual partner of the female of childbearing potential and the absence of sperm has been confirmed. If not, an additional effective method of contraception should be used.

- **Sexual abstinence**

Sexual abstinence is considered an effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.)

Notes:
Use should be consistent with local regulations regarding the use of contraceptive methods for participants of clinical studies.
a) Typical use failure rates are lower than perfect-use failure rates (i.e., when used consistently and correctly).
b) If hormonal contraception efficacy is potentially decreased due to interaction with study treatment, condoms must be used in addition to the hormonal contraception during the treatment period and for at least 120 days, (corresponding to time needed to eliminate study treatment plus 30 days for study treatments with genotoxic potential) after the last dose of the study treatment.
c) If locally required, in accordance with Clinical Trial Facilitation Group (CTFG) guidelines, acceptable hormonal contraceptives are limited to those which inhibit ovulation.

APPENDIX IV - New York Heart Association (NYHA) Classification

NYHA Grading		**MET***
Class I	No limitations. Ordinary physical activity does not cause undue fatigue, dyspnea or palpitations (asymptomatic LV dysfunction)	>7
Class II	Slight limitation of physical activity. Ordinary physical activity results in fatigue, palpitation, dyspnea or angina pectoris (mild Congestive Heart Failure (CHF)).	5
Class III	Marked limitation of physical activity. Less than ordinary physical activity leads to symptoms (moderate CHF)	2 – 3
Class IV	Unable to carry on any physical activity without discomfort. Symptoms of CHF present at rest (severe CHF).	1.6

*MET (metabolic equivalent) is defined as the resting VO_2 for a 40-year-old 70-kg man.
1 MET = 3.5 mL O_2/min/kg body weight.

APPENDIX V - Response Criteria for Subjects with MDS and CMML According to IWG 2006 Criteria[39]

Altering Disease Natural History	
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 × 10^9/L Neutrophils ≥1.0 × 10^9/L Blasts 0%
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 cytopenia, 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
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 × 10^9/L)	Absolute increase of ≥30 × 10^9/L for subjects starting with >20 × 10^9/L Increase from <20 × 10^9/L to >20 × 10^9/L and by at least 100%
Neutrophil response (HI-N) (Pretreatment <1.0 × 10^9/L)	At least 100% increase and an absolute increase of >0.5 × 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
HI	
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 VI - 2016 WHO Classification for CMML and MDS[40] 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 × 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 × 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 × 10^9/L; and absolute neutrophil count <1.8 × 10^9/L.
To be classified as dysplasia, >10% of any cell lineage must be dysplastic. PB monocytes must be <1 × 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 need 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.

APPENDIX VII – *TP53* Sequence Variant Interpretation Algorithm

Inclusion of subjects in the study is based on *TP53* sequencing performed in a laboratory at each participating site according to established local routines. A study-specific variant interpretation algorithm will be used to discriminate between eligible and non-eligible *TP53* sequence variants.

In order to select subjects with high unmet medical need due to poor prognosis, the study will enroll subjects that have any *TP53* mutation which is not pre-defined as 'benign' or 'likely benign'. The operational definition of 'eligible *TP53* mutation' in this clinical study therefore includes variants classified as pathogenic, likely pathogenic and variant of uncertain significance (VUS) in a specified database (UMD-TP53). Subjects harboring at least one such *TP53* sequence variant are eligible for inclusion, while subjects who only have variant(s) classified as benign or likely benign are not eligible. Thus, also *TP53* VUS are eligible, avoiding exclusion of subjects with possible pathogenic *TP53* variants presently classified as VUS.

The basic parameters for *TP53* sequencing are listed in Table 17.

Table 17. Basic parameters for *TP53* sequencing and interpretation.

Sequencing method[41,42]	• Any NGS method (amplicon, hybrid capture) that has been validated locally for sequencing *TP53* • Covers TP53 exons 2-11
Data processing[41,42]	Use HVGS nomenclature: http://varnomen.hgvs.org Reference sequences • genomic: hg19 or HG38 (do not use hg18) • cDNA: NM_000546.5 • Protein: NP_000537.3
Data interpretation[42,43] 5-tier classification of functional impact on p53: Pathogenic, likely pathogenic, VUS, likely benign, benign	Pathogenic, likely pathogenic or variant of uncertain significance (VUS): • Frameshift mutations • Nonsense mutations • Donor / acceptor splice sites +/- 2 • Synonymous mutations with splice impact • Missense with functional impact → Eligible for inclusion in the clinical study Benign or likely benign:

	• Synonymous mutations without functional impact • Missense without functional impact* → Not eligible for inclusion in the clinical study *A list derived from the UMD-TP53 database[43] is provided as common reference for variant interpretation, see Table 18 and Table 19.

The *TP53* sequence variant interpretation algorithm is shown below.

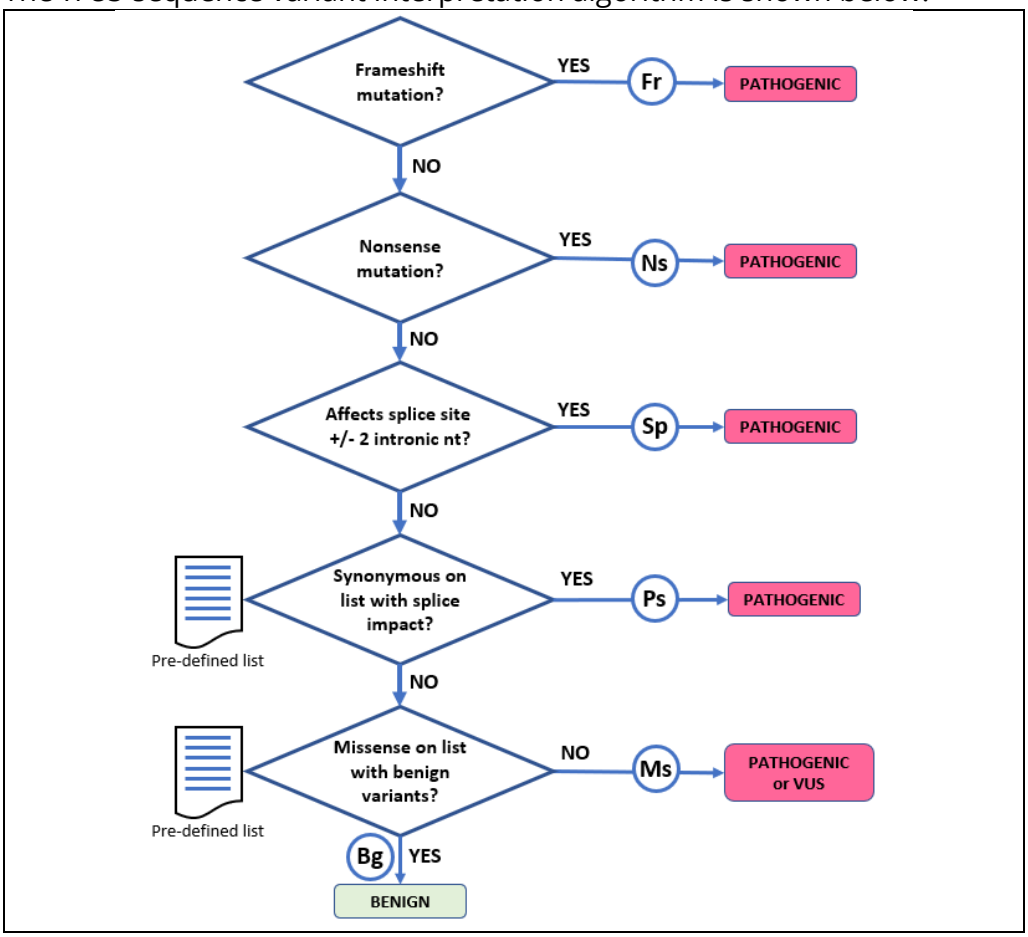

Figure 4. Algorithm for *TP53* variant interpretation.

Abbreviations for pathogenicity criteria: Fr, Ns, Sp, Ps, Ms (Bg = benign). VUS = Variant of unknown significance.

Thus, the following criteria (for variant classification or variant type) define each variant according to the type of event:

Fr - Frameshift, whether they are in or out of frame
Ns - Nonsense
Sp - Splice
Ps - Pathogenic synonymous (according to the list in Table 18)
Ms - Missense (not in the list of benign variants in Table 19)
Bg - Benign (relevant to report if multiple variants present in the same subject)

The pre-defined lists are presented below, for pathogenic synonymous, Ps (Table 18) and benign missense, Bg (Table 19).

Table 18. Listing of synonymous variants with pathogenic splice impact

cDNA_Variant_ NM_000546.5	NG_017013.2_Variant	HG19_Variant	HG38_Variant	TP53_alpha_ NP_000537.3
c.375G>A	NG_017013.2:g.16557 G>A	chr17:g.7579312G>A	chr17:g.7675994G>A	p.T125=
c.375G>T	NG_017013.2:g.16557 G>T	chr17:g.7579312G>T	chr17:g.7675994G>T	p.T125=
c.375G>C	NG_017013.2:g.16557 G>C	chr17:g.7579312G>C	chr17:g.7675994G>C	p.T125=
c.672G>A	NG_017013.2:g.17692 G>A	chr17:g.7578177G>A	chr17:g.7674859G>A	p.E224=
c.993G>A	NG_017013.2:g.19016 G>A	chr17:g.7576853G>A	chr17:g.7673535G>A	p.Q331=

Table 19. Listing of benign or likely benign *TP53* variants to be used for exclusion

cDNA_Variant_ NM_000546.5	NG_017013.2_Variant	HG19_Variant	HG38_Variant	*TP53*_alpha_ NP_000537.3
c.31G>C	NG_017013.2:g.15987G>C	chr17:g.7579882G>C	chr17:g.7676564G>C	p.E11Q
c.91G>A	NG_017013.2:g.16164G>A	chr17:g.7579705G>A	chr17:g.7676387G>A	p.V31I
c.139C>T	NG_017013.2:g.16321C>T	chr17:g.7579548C>T	chr17:g.7676230C>T	p.P47S
c.145G>C	NG_017013.2:g.16327G>C	chr17:g.7579542G>C	chr17:g.7676224G>C	p.D49H
c.173C>G	NG_017013.2:g.16355C>G	chr17:g.7579514C>G	chr17:g.7676196C>G	p.P58R
c.215C>G	NG_017013.2:g.16397C>G	chr17:g.7579472C>G	chr17:g.7676154C>G	p.P72R
c.217G>A	NG_017013.2:g.16399G>A	chr17:g.7579470G>A	chr17:g.7676152G>A	p.V73M
c.248C>T	NG_017013.2:g.16430C>T	chr17:g.7579439C>T	chr17:g.7676121C>T	p.A83V
c.319T>C	NG_017013.2:g.16501T>C	chr17:g.7579368T>C	chr17:g.7676050T>C	p.Y107H
c.329G>A	NG_017013.2:g.16511G>A	chr17:g.7579358G>A	chr17:g.7676040G>A	p.R110H
c.460G>A	NG_017013.2:g.17399G>A	chr17:g.7578470G>A	chr17:g.7675152G>A	p.G154S
c.566C>T	NG_017013.2:g.17586C>T	chr17:g.7578283C>T	chr17:g.7674965C>T	p.A189V
c.665C>T	NG_017013.2:g.17685C>T	chr17:g.7578184C>T	chr17:g.7674866C>T	p.P222L
c.704A>G	NG_017013.2:g.18292A>G	chr17:g.7577577A>G	chr17:g.7674259A>G	p.N235S
c.847C>T	NG_017013.2:g.18778C>T	chr17:g.7577091C>T	chr17:g.7673773C>T	p.R283C
c.869G>A	NG_017013.2:g.18800G>A	chr17:g.7577069G>A	chr17:g.7673751G>A	p.R290H
c.883C>T	NG_017013.2:g.18814C>T	chr17:g.7577055C>T	chr17:g.7673737C>T	p.P295S
c.935C>G	NG_017013.2:g.18958C>G	chr17:g.7576911C>G	chr17:g.7673593C>G	p.T312S
c.1015G>A	NG_017013.2:g.21857G>A	chr17:g.7574012G>A	chr17:g.7670694G>A	p.E339K

c.1073A>T	NG_017013.2:g.21915A>T	chr17:g.7573954A>T	chr17:g.7670636A>T	p.E358V
c.1079G>C	NG_017013.2:g.21921G>C	chr17:g.7573948G>C	chr17:g.7670630G>C	p.G360A
c.1096T>G	NG_017013.2:g.21938T>G	chr17:g.7573931T>G	chr17:g.7670613T>G	p.S366A

Examples of results:

HG19_Variant	cDNA_Variant _NM_000546.5	TP53_alpha_NP_000537 .3	Criterion
chr17:g.7578406G>A	c.524G>A	p.R175H	Ms

Missense variant hot spot

HG19_Variant	cDNA_Variant _NM_000546.5	TP53_alpha_NP_000537 .3	Criterion
chr17:g.7578212C>T	c.637C>T	p.R213*	Ns

Nonsense variant hot spot

HG19_Variant	cDNA_Variant _NM_000546.5	TP53_alpha_NP_000537 .3	Criterion
chr17:g.7578223_7578222 del	c.626_627del	p.(R209Kfs*6)	Fr

Indel variant hot spot (deletion)

HG19_Variant	cDNA_Variant _NM_000546.5	TP53_alpha_NP_000537 .3	Criterion
chr17:g.7579471dup	c.216dup	p.(V73Rfs*76)	Fr

Indel variant hot spot (duplication)

HG19_Variant	cDNA_Variant _NM_000546.5	TP53_alpha_NP_000537 .3	Criterion
chr17:g.7574005_7574004ins A	c.1022_1023ins A	p.(F341Lfs*6)	Fr

Indel variant hot spot (Insertion)

HG19_Variant	cDNA_Variant _NM_000546.5	TP53_alpha_NP_000537 .3	Criterion
chr17:g.7578555G>A	c.376-1G>A	p.?	Sp

Splice site variant

HG19_Variant	cDNA_Variant _NM_000546.5	TP53_alpha_NP_000537 .3	Criterion
chr17:g.7579312G>A	c.375G>A	p.T125=	Ps

Pathogenic synonymous variant

HG19_Variant	cDNA_Variant _NM_000546.5	TP53_alpha_NP_000537 .3	Criterion

chr17:g.7579472C>G	c.215C>G	p.P72R	Bg
chr17:g.7578210A>G	c.639A>G	p.R213=	Bg

Non-pathogenic common SNPs

HG19_Variant	cDNA_Variant _NM_000546.5	TP53_alpha_NP_000537 .3	Criterion
chr17:g.7578561C>T	c.376-7C>T	p.(=)	Bg

Intronic mutation