CLINICAL STUDY PROTOCOL

Phase 1/2 study of APR-246 in Combination with Pembrolizumab in Subjects with Solid Tumor Malignancies

Protocol No.:	A20-11195
IND No.:	124841
Sponsor:	Aprea Therapeutics, Inc. [redacted]
Responsible Medical Officer:	Eyal C. Attar, M.D.
Medical Monitor:	[redacted]
Protocol Version:	1.0
Replaces:	n/a
Date:	13 March 2020

CONFIDENTIAL

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INVESTIGATOR'S STATEMENT

1. I have carefully read this protocol entitled "Phase 1/2 Study of APR-246 in Combination with Pembrolizumab in Subjects with Solid Tumor Malignancies" 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/Independent Ethics Committee (IRB/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, Food and Drug Administration (FDA) requirements as specified in Title 21 Code of Federal Regulations, 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 Harmonisation (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based on the conduct of the study will be released without prior consent from the Sponsor unless this requirement is superseded by the FDA, a Competent Authority of the European Union or another Regulatory Authority.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 1.0: 13 March 2020

Name: _____ Telephone: _____

_____ _____

Address: _____

_____

Signature: _____ Date: _____

Sponsor/Representative:

Signature: _____ Date: _____

[redacted]

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CLINICAL STUDY SYNOPSIS

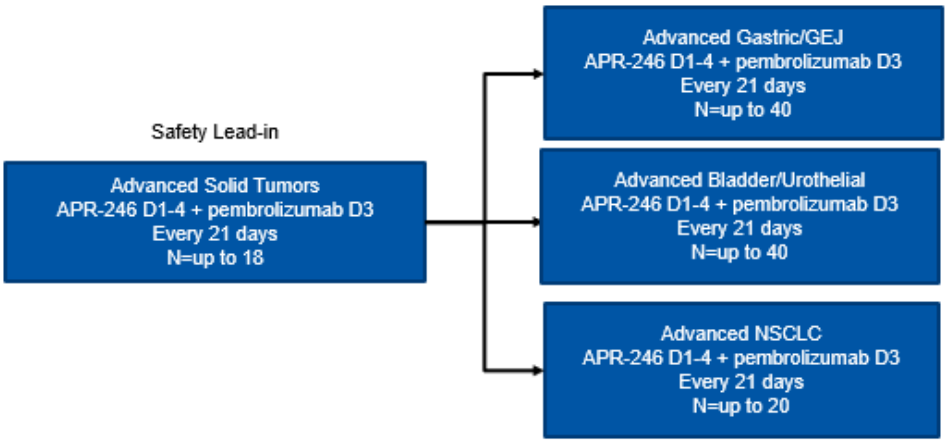
Title	Phase 1/2 Study of APR-246 in Combination with Pembrolizumab in Subjects with Solid Tumor Malignancies
Sponsor	Aprea Therapeutics, Inc.
Monitor/ Contract Research Organization (CRO)	[redacted]
Number of Study Centers	Multicenter
Clinical Phase	1/2
Sample Size	Up to 18 subjects in safety lead-in portion, up to 100 subjects in phase 2 expansion portion
Investigational Agent	APR-246
Study Design	This clinical trial is a phase 1/2, open-label, study to determine the safety and preliminary efficacy of APR-246 in combination with pembrolizumab in subjects with solid tumor malignancies. The study will include a safety lead-in portion followed by a phase 2 expansion portion in specific disease groups (Figure 1). 

Figure 1. Clinical Trial Schema

During the safety lead-in portion of the study, subjects with advanced solid tumor malignancies will receive pembrolizumab in combination with APR-246, as outlined in

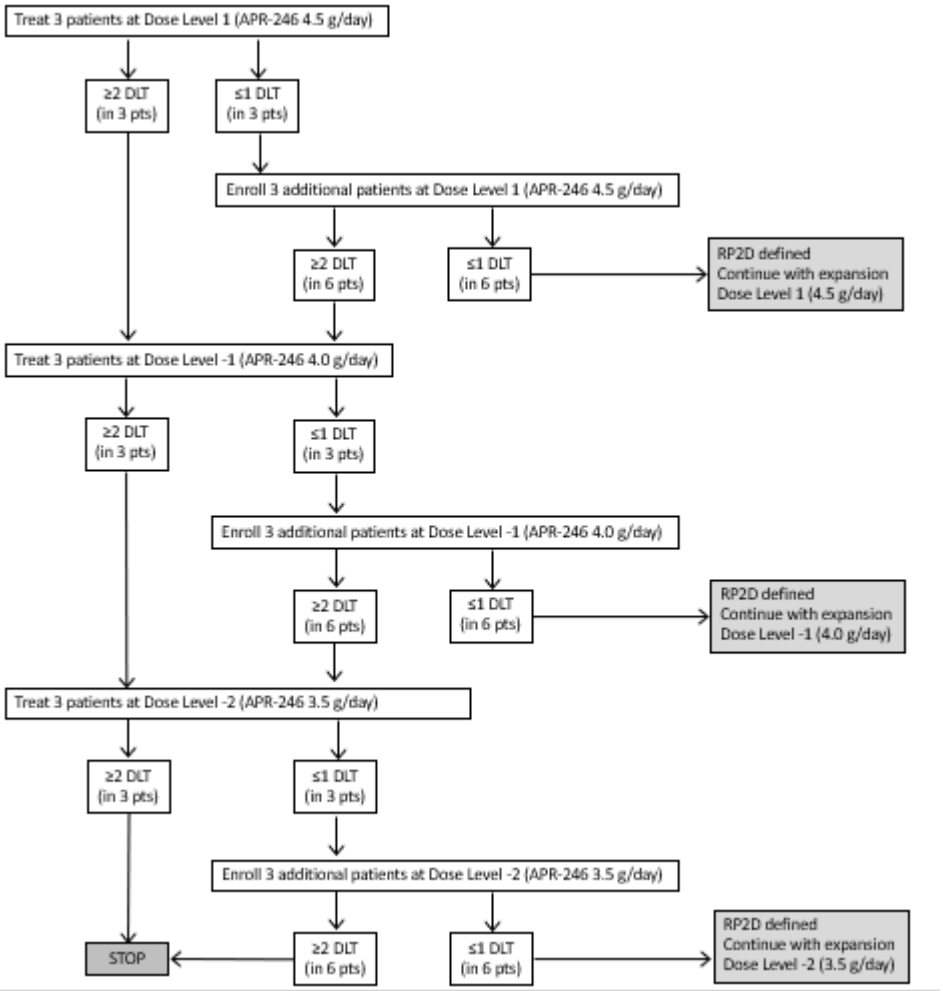


Figure 2.

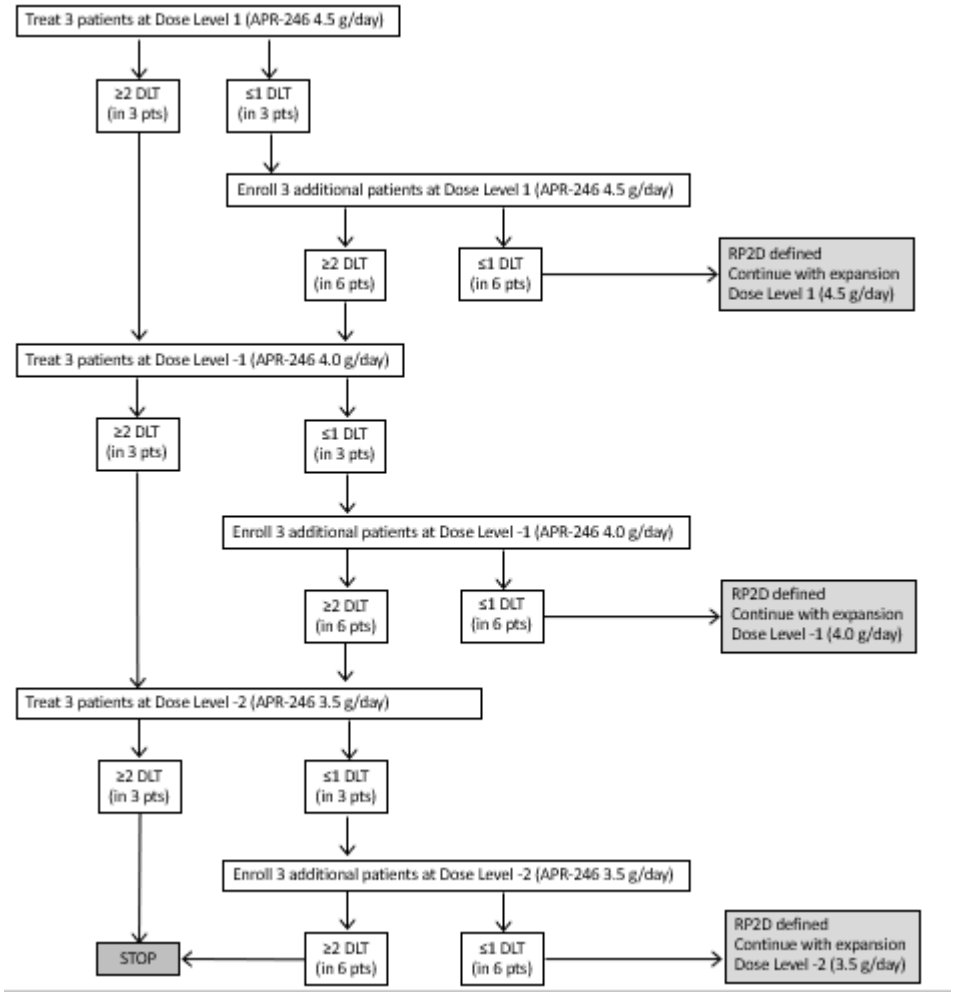


Figure 2. APR-246 Dose-Finding Study Design

Dosing will begin at the full dose of both drugs due to the well-established safety profiles of APR-246 and pembrolizumab, the non-overlapping mechanisms of action, and the desire to treat patients at doses shown to be effective in previous studies. Lower dose levels of APR-246 will be explored as necessary depending on observed toxicity.

The initial cohort of subjects will receive APR-246 at 4.5 g/day administered as an intravenous (IV) infusion on Days 1–4 in combination with pembrolizumab at the approved dose of 200 mg on Day 3 of each 21-day cycle. This cohort will enroll up to a maximum of 6 subjects. Dose-limiting toxicity (DLT) will be assessed after three subjects have been enrolled in each respective cohort and the last enrolled subject has completed the 3-week safety assessment period (i.e., one cycle of combination regimen). A subject that discontinues therapy during Cycle 1 without DLT is considered evaluable for the purpose of safety only if at least 75% of scheduled doses of APR-246 were administered in the first cycle. At the first dose level of 4.5 g/day of APR-246, if ≤ 1 subject out of 3 experiences a DLT, 3 additional subjects

	will be enrolled. If ≤ 1 subject out of 6 experiences DLT, the dose level (4.5 g/day of APR-246) will be deemed the recommended phase 2 dose (RP2D) for that cohort. If ≥ 2 subjects out of the total 3 – 6 subjects in the cohort experience DLT, the study will continue enrollment at Dose Level -1 (4.0 g/day of APR-246). If ≤ 1 subject out of 6 experiences DLT at this dose level, the dose level (4.0 g/day of APR-246) will be deemed the RP2D for that cohort. If ≥ 2 subjects out of the total 3 – 6 subjects at that dose level experience DLT, the study will continue enrollment at Dose Level -2 (3.5 g/day of APR-246). If ≤ 1 subject out of 6 experiences DLT at this dose level, the dose level (3.5 g/day of APR-246) will be deemed the RP2D for that cohort. If ≥ 2 subjects out of the total 3 – 6 subjects at this dose level experience DLT, the trial will be halted and the Data Review Team (DRT) will consider potential future dosing modifications. No dose reductions in pembrolizumab are planned. The phase 2 portion will begin once the RP2D of APR-246 in combination with pembrolizumab has been determined and will assess the anti-tumor activity of this combination. Up to 100 subjects will be enrolled in three cohorts according to their underlying disease, as outlined in Figure 1.
Study Objectives	**Primary objectives:** 1. To evaluate the safety and tolerability of APR-246 in combination with pembrolizumab in subjects with solid tumors. 2. To determine and confirm the maximum tolerated dose (MTD) for APR-246 in combination with 200 mg IV, every 3 weeks (Q3W) pembrolizumab in subjects with solid tumor malignancies. **Secondary objectives:** 1. Overall response rate (ORR) (Complete Response [CR] + Partial Response [PR]) of APR-246 in combination with pembrolizumab in subjects with solid tumor malignancies by Response Evaluation Criteria in Solid Tumors (RECIST) 1.1. 2. Progression-free survival (PFS) by RECIST 1.1. 3. Overall survival (OS). 4. Duration of response (DOR) by RECIST 1.1. 5. Disease control rate (DCR: CR + PR + stable disease [SD]) by RECIST 1.1 6. Durable SD rate (durable SD [SD ≥23 weeks]) by RECIST 1.1 7. Clinical benefit rate (CBR: CR, PR + durable SD) by RECIST 1.1. 8. The pharmacokinetic (PK) profile of APR-246 during combination treatment with pembrolizumab. **Exploratory objectives:** 1. To investigate the relationship between candidate efficacy biomarkers and anti-tumor activity of APR-246 in combination with pembrolizumab based on analyses of blood, serum, and/or tumor tissue.
Study Endpoints	**Primary endpoints:** 1. Occurrence of DLTs, classified and graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI-CTCAE, version 5.0).

	2. Frequency of treatment-emergent adverse events (TEAEs), and serious adverse events (SAEs) related to APR-246 in combination with pembrolizumab. 3. RP2D of APR-246, defined as the dose of APR-246 at which < 2 out of 6 subjects experience DLT during the 3-week safety assessment period after the start of APR-246 administration in combination with pembrolizumab using dose de-escalation of APR-246. **Secondary endpoints:** 1. ORR, defined as the proportion of subjects who have BOR of CR or PR measured by RECIST 1.1 (see APPENDIX V). ORR is measured from the start date of the treatment period until date of death from any cause. Subjects who are lost to follow-up and the subjects who are alive at the date of data cutoff will be censored at the date the subject was last known alive. 2. PFS, defined as the time from the first study dose date to the date of first documentation of confirmed disease progression or death (whichever occurs first). 3. OS, measured from the date of initiating study treatment to the date of death. 4. DOR, defined as the duration of CR or PR. 5. DCR, defined as the proportion of subjects who have BOR of CR or PR or SD (duration of SD ≥ 5 weeks). 6. Durable SD rate, defined as the proportion of subjects whose BOR is SD and the duration of SD is ≥ 23weeks. 7. CBR, defined as the proportion of subjects who have BOR of CR or PR or durable SD (duration of SD ≥ 23 weeks). 8. PK parameters: maximum concentration (C_{max}), area under curve (AUC), volume of distribution (V_d) and clearance (CL) of APR-246 when administered concurrently with pembrolizumab. **Exploratory endpoints:** Exploratory molecular analyses may include but are not limited to: gene mutations by next generation sequencing (NGS, including TP53), mRNA expression, multiplex immunohistochemistry and transcriptomics in frozen biopsies before and on therapy, multiplex flow cytometry on peripheral blood mononuclear cells (PBMCs) and multiplex cytokines in serum. **Analysis Sets:** *Safety Analysis Set* will include all subjects who receive any amount of study drug. This will be the analysis set for all safety evaluations. *MTD Analysis Set* will include all subjects who complete Cycle 1 of treatment in the safety lead-in portion of the study or discontinue early due to DLT. This will be the analysis set to determine MTD. *PK Analysis Set*: All the subjects who receive at least 1 dose of APR-246 and have evaluable concentration data.

	Pharmacodynamic Analysis Set will include all subjects who receive at least 1 dose of study drug (APR-246 or pembrolizumab) and have evaluable pharmacodynamic data. *PK/Pharmacodynamic Analysis Set*: All the subjects who receive at least 1 dose of study drug (APR-246 or pembrolizumab) and have evaluable pharmacodynamic and concentration data.
Eligibility Criteria	**<u>Inclusion Criteria</u>**: 1. Signed informed consent form (ICF) and ability to comply with protocol requirements. 2. Known tumor TP53 mutation status from recent or archival sample. 3. Histologically and/or cytologically confirmed solid tumor malignancy: a. Safety lead-in portion: i. Patients with histologically and/or cytologically confirmed diagnosis of advanced non-central nervous system (CNS) primary tumors that have progressed after first line treatment, who are intolerant to first line treatment, or who are unable to receive first line treatment, and for whom pembrolizumab, or pembrolizumab-based therapy, is considered appropriate in the opinion of the investigator. Primary CNS tumors are excluded. Patients with clinically stable, known metastatic tumors to the CNS are eligible. CNS radiography is not required in the absence of suspicion for clinical involvement. b. Phase 2 Expansion portion: i. Patients with histologically and/or cytologically confirmed diagnosis of advanced gastric or gastroesophageal junction (GEJ) tumors that have progressed after first line treatment, who are intolerant to first line treatment, or who are unable to receive first line treatment. Patients with clinically stable, known metastatic tumors to the CNS are eligible with Medical Monitor approval. CNS radiography is not required in the absence of suspicion for clinical involvement. Prior treatment with anti-PD-1/anti-PD-L1 therapy is prohibited. ii. Patients with histologically and/or cytologically confirmed diagnosis of advanced bladder/urothelial tumors that have progressed after first line treatment, or who are intolerant to first line treatment, or who are unable to receive first line treatment with cisplatin-based chemotherapy. Patients with clinically stable, known metastatic tumors to the CNS are eligible with Medical Monitor approval. CNS radiography is not required in the absence of suspicion for clinical involvement. Prior treatment with anti-PD-1/anti-PD-L1 therapy is prohibited. iii. Patients with histologically and/or cytologically confirmed diagnosis of advanced non-small cell lung cancer (NSCLC)

previously treated with anti-PD-1 or anti-PD-L1 therapy. Patients with clinically stable, known metastatic tumors to the CNS are eligible with Medical Monitor approval. CNS radiography is not required in the absence of suspicion for clinical involvement.

4. Adequate organ function as defined by the following laboratory values:
   a. Creatinine clearance > 30 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, tumor involvement, hemolysis or considered an effect of regular blood transfusions,
   c. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) < 3 × ULN, unless due to involvement by the underlying malignancy.
5. Age ≥ 18 years at the time of signing the ICF.
6. Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1 or 2 (APPENDIX II).
7. Projected life expectancy of ≥ 12 weeks.
8. In the expansion portion, measurable disease meeting the following criteria:
   a. At least 1 lesion of ≥10 mm in the longest diameter (LD) for a non-lymph node or ≥15 mm in the short-axis diameter for a lymph node that is serially measurable according to RECIST 1.1.
   b. Lesions that have had external beam radiotherapy or loco-regional therapies such as radiofrequency ablation must show subsequent evidence of substantial size increase (ex. 20% increase in LD) to be deemed a target lesion.
9. Negative serum or urine pregnancy test prior to study treatment initiation in female subjects of childbearing potential.
10. Women of childbearing potential and men with female partners of childbearing potential must be willing to use an effective form of contraception such as latex condom, hormonal birth control, intrauterine device or double barrier method (APPENDIX III) during chemotherapy treatment and for at least six months thereafter.

Exclusion Criteria:

1. Known history of untreated human immunodeficiency virus (HIV)/HIV with a detectable viral load or active hepatitis B or active hepatitis C infection.
2. Any of the following cardiac abnormalities:
   a. Myocardial infarction within six months prior to registration;
   b. New York Heart Association Class III or IV (APPENDIX IV) heart failure or known left ventricular ejection fraction (LVEF) < 40%;
   c. A history of familial long QT syndrome;
   d. Symptomatic atrial or ventricular arrhythmias not controlled by medications;
   e. QTcF ≥ 470 msec, unless due to underlying bundle branch block and/or pacemaker and with approval of the Medical Monitor.
3. Concomitant malignancies or previous malignancies with less than a 1-year

	disease-free interval at the time of signing 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. Patients with controlled, advanced prostate cancer are permitted. 4. Pregnancy or lactation. 5. Active uncontrolled systemic infection. 6. An autoimmune condition requiring ≥ 10 mg (or equivalent corticosteroid) prednisone daily, or any other systemic immunosuppressive treatment within 28 days of first dose of study therapy. 7. Known history of active tuberculosis. 8. Current (non-infectious) pneumonitis, or a history of pneumonitis that required steroids. 9. A live vaccine administered within 30 days of the first dose of study treatment. 10. Receipt of any investigational product within 14 days or 5 half-lives prior to study treatment initiation, whichever is shortest. 11. Prior intolerance to pembrolizumab or other anti-PD-1/PD-L1 agents.
Treatment Plan	This is a phase 1/2, open-label, safety and cohort expansion study to determine the safety and preliminary efficacy of APR-246 in combination with anti-PD1 monoclonal antibody (mAb) pembrolizumab in subjects with solid tumor malignancies including gastric/GEJ cancer, bladder/urothelial cancer, and NSCLC. The study will include a safety lead-in portion comprised of subjects with advanced non-CNS-primary solid tumors, followed by an expansion portion with separate cohorts for subjects with gastric/GEJ cancer, urothelial cancer and NSCLC. The safety lead-in portion of the study will follow a 3+3 dose de-escalation design. APR-246 will be given as a 6 h IV infusion once daily on Days 1–4 in 21-day cycles, with pembrolizumab administered as a 30-minute IV infusion at a standard dose of 200 mg on Day 3 before the Day 3 infusion of APR-246. Each cohort will consist of 3-6 subjects. The RP2D of APR-246 is the dose at which ≤ 1 of 6 subjects in a cohort has a DLT. Given the minimal overlap of adverse events (AEs) between APR-246 and pembrolizumab, and the absence of presumed drug-drug interactions (DDIs) based on disparate metabolism, the initial starting dose of APR-246 is 4.5 g/day. A Dose Level -1 cohort will use APR-246 at 4.0 g/day and a Dose Level -2 cohort will use APR-246 at 3.5 g/day. No reduction in pembrolizumab dose is planned. A DRT consisting of the Medical Monitor, Site Principal Investigators, 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 RP2D. In the expansion portion of the study, the DRT will evaluate safety and tolerability after 5 subjects have completed 1 cycle of treatment in each cohort. All accumulated safety data will be discussed during DRMs. The expansion portion will begin once the RP2D of APR-246 in combination with pembrolizumab has been determined.

	Subjects will be enrolled and stratified into one of three cohorts: • Patients with histologically and/or cytologically confirmed diagnosis of advanced gastric or GEJ tumors that have progressed after first line treatment, who are intolerant to first line treatment, or who are unable to receive first line treatment. Patients with clinically stable, known metastatic tumors to the CNS are eligible with Medical Monitor approval. CNS radiography is not required in the absence of suspicion for clinical involvement. Prior treatment with anti-PD-1/anti-PD-L1 therapy is prohibited. • Patients with histologically and/or cytologically confirmed diagnosis of advanced bladder/urothelial tumors that have progressed after first line treatment, or who are intolerant to first line treatment, or who are unable to receive first line treatment with cisplatin-based chemotherapy. Patients with clinically stable, known metastatic tumors to the CNS are eligible with Medical Monitor approval. CNS radiography is not required in the absence of suspicion for clinical involvement. Prior treatment with anti-PD-1/anti-PD-L1 therapy is prohibited is prohibited. • Patients with histologically and/or cytologically confirmed diagnosis of advanced NSCLC previously treated with anti-PD-1 or anti-PD-L1 therapy. Patients with clinically stable, known metastatic tumors to the CNS are eligible with Medical Monitor approval. CNS radiography is not required in the absence of suspicion for clinical involvement.
Duration of Follow-Up	Subjects will be followed as per the Study Calendar. After a subject is removed or withdrawn from study treatment, the subject will be followed for up to 1 year or until death, whichever occurs first. Off-treatment data on OS will be updated every 6 months or until death, whichever occurs 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(s). Subjects who respond and discontinue study treatment for reasons other than progressive disease (PD) should have response assessments and survival should be collected every 2 months until disease progression or death, whichever occurs first. After disease progression, data for survival should be collected every 6 months until death. *Criteria for Removal from Study Treatment* Study treatment can continue for subjects receiving clinical benefit, unless one or more withdrawal criteria are met, or at the subject's discretion, or if the study is terminated. 1. Study Treatment Discontinuation Study treatment must be discontinued if: • There is evidence of disease progression. 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 subject becomes pregnant. • A subject is significantly non-compliant with the requirements of the protocol. • A subject has an adverse experience that would, in the investigator's judgment, make continued participation in the study an unacceptable risk. • The subject starts new treatment for their underlying disease. 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 must 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. 4. Subject Withdrawal from Study A subject may voluntarily withdraw from study treatment or withdraw consent from the study at any time. The investigator may also, at his or her discretion, discontinue a subject from participating in the study 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 phase 1/2, open-label, safety and efficacy study to determine the safety and preliminary efficacy of APR-246 in combination with pembrolizumab in subjects with solid tumor malignancies. In the safety lead-in part of study (phase 1), the safety and the RP2D of APR-246 will be investigated. The RP2D of APR-246 will be defined as the dose at which < 2 out of 6 subjects experience DLT during the 3-week safety assessment period after the start of APR-246 administration in combination with pembrolizumab using dose de-escalation of APR-246. In the expansion part of the study (phase 2), the combination therapy at the RP2D will be given to patients in the 3 cohorts. Both safety and efficacy will be further investigated. *Determination of Sample Size* In the safety lead-in portion of the study, up to 18 DLT-evaluable patients will be enrolled to determine RP2D. The dose expansion consists of 3 cohorts. The ORRs to pembrolizumab for the patients with the 3 types of cancer, who were relapsed/refractory R/R to previous chemotherapies, were 21.1% and 22,7% for urothelial cancer and gastric/GEJ adenocarcinoma, respectively. The ORR for patients with advanced NSCLC who have previously been treated with anti-PD-1/PD-L1 therapy is expected to be negligible

	(i.e. <= 20%). As such, the expected response rate to the combination therapy across indications is 20% to 30%. In order to increase the estimate precision, at least 20 evaluable patients will be included in each of the 3 cohorts. *Analysis Populations* Safety population: Subjects will be evaluable for safety if they receive at least one dose of APR-246. The safety population will be used to summarize exposure and safety parameters. DLT-evaluable population: In the safety lead-in portion of the study, subjects who either experience a DLT during first the 3 weeks (Cycle 1) of the study treatment or receive ≥ 75% of scheduled Cycle 1 dose of APR-246 in combination with pembrolizumab and do not experience a DLT. Any individual subject who is not evaluable for DLT will be replaced by a new subject through additional subject enrollment. Efficacy evaluable (EE) population: All subjects who complete at least one treatment cycle of APR-246 in combination with pembrolizumab and who have at least one post-treatment clinical response assessment. Subjects who fail to complete one treatment cycle will also be considered EE if they show clear evidence of clinically significant disease progression. The EE population will be the secondary analysis population for efficacy. PK population: Subjects will be evaluable for PK if at least one post-dose sample for PK evaluation has been obtained. PK/Pharmacodynamic population: All the subjects who receive at least 1 dose of study drug (APR-246 pembrolizumab) and have evaluable pharmacodynamic and concentration data. *Statistical analysis* All the statistical analysis will be detailed in the statistical analysis plan. Generally, all the continuous variables will be summarized using n (number of subjects with available data), mean, standard deviation (StDev), median, and range (minimum and maximum). Categorical data will be summarized by frequency and percentage. Time to event data will be analyzed using Kaplan-Meier method, and the number and percentage of events, or censored, the first quartile at 25%, median with 95% confidence, and third quartile at 75% of survival time will be presented. The survival rate at the 3, 6, 9, and 12 months, and then 2, 3 and 5 years will be presented. All the study data will be listed. Safety data including AEs, vital signs, laboratory data, electrocardiogram (ECG), physical exam, and ECOG performance status will be summarized in the safety population. AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and graded using NCI-CTCAE v5.0 severity grade. The efficacy endpoints in the safety lead-in portion will be listed only. In the expansion phase, all the rates (ORR, DCR, durable SD, CBR) will be summarized with 95% exact confidence intervals (Clopper and Pearson) by cohorts. The time to event

	data (DOR, PFS, and OS) will be analyzed using Kaplan-Meier method and the corresponding survival plots will be produced. PK/safety relationships will be explored graphically and investigated by model-based analysis if appropriate. *Pharmacokinetic Analysis* The DDI between APR-246 and pembrolizumab will be evaluated using population PK (popPK) methods. The existing popPK model for APR-246 will be used to compare the observed plasma concentrations of APR-246 in presence of pembrolizumab with the predicted plasma concentrations assuming no DDI (visual predictive check). Additionally, the PK analysis dataset from this study will be integrated with the existing popPK dataset and the effect of pembrolizumab on APR-246 PK parameters will be tested as covariate in the model. *Pharmacodynamic Analysis* The effect of APR-246-pembrolizumab combination therapy on soluble, tissue, genetic and/or imaging biomarkers will be summarized using descriptive statistics using the PK/pharmacodynamic analysis set. PK/pharmacodynamic relationships will be explored graphically and may be investigated by model-based analyses.

LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	Adverse event
ADR	Adverse drug reaction
AML	Acute myeloid leukemia
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area under curve
AUC_{ss}	Steady-state area under curve
BUN	Blood urea nitrogen
CBR	Clinical benefit rate
CFR	Code of Federal Regulations
CHF	Congestive heart failure
CI	Confidence interval
CL	Clearance
CLL	Chronic lymphocytic leukemia
C_{max}	Maximum concentration
C_{min}	Minimum concentration
CNS	Central nervous system
CR	Complete response
CRO	Contract Research Organization
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CYP450	Cytochrome p450
DCR	Disease control rate
DDI	Drug-drug interaction
DLT	Dose-limiting toxicity
DOR	Duration of response
DRM	Data Review Meeting
DRT	Data Review Team

eCRF	Electronic case report form
EC	European Commission
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EE	Efficacy evaluable
EOI	End of infusion
ER	Endoplasmic reticulum
FDA	Food and Drug Administration
FFPE	Formalin-fixed paraffin-embedded
GEJ	Gastroesophageal junction
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HGSOC	High grade serous ovarian cancer
HIV	Human immunodeficiency virus
IB	Investigator's Brochure
ICF	Informed consent form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
IV	Intravenous
LBM	Lean body mass
LD	Longest diameter
LVEF	Left ventricular ejection fraction
mAb	Monoclonal antibody
MQ	2-methylene-quinuclidin-3-one
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose
NCI	National Cancer Institute
NSCLC	Non-small-cell lung cancer

NGS	Next-generation sequencing
OS	Overall survival
ORR	Overall response rate
PBMC	Peripheral blood mononuclear cell
PD	Progressive disease
PD-1	Programmed cell death protein 1
PD-L1	Programmed death-ligand 1
PET	Positron-emission tomography
PFS	Progression-free survival
PK	Pharmacokinetic(s)
PLD	Pegylated liposomal doxorubicin
PO	Orally *(per os)*
PopPK	Population pharmacokinetics
PR	Partial response
QTcF	Corrected QT interval by Fridericia
Q3W	Every 3 weeks
RECIST	Response Evaluation Criteria in Solid Tumors
RNA	Ribonucleic acid
RP2D	Recommended phase 2 dose
R/R	Relapsed/refractory
SAE	Serious adverse event
SD	Stable disease
StDev	Standard deviation
TEAE	Treatment-emergent adverse event
tid	Three times daily (*ter in die*)
TrxR1	Thioredoxin reductase 1
$T_{1/2}$	Terminal half-life
ULN	Upper limit of normal
USPI	United States Prescribing Information
V_d	Volume of distribution

1.0 GENERAL INFORMATION

1.1 Protocol Number and Title of the Study

Protocol No. A20-11195
Protocol Title: Phase 1/2 Study of APR-246 in Combination with Pembrolizumab in Subjects with Solid Tumor Malignancies

1.2 Sponsor

Aprea Therapeutics, Inc.
[redacted]

1.3 Monitor

[redacted]

1.4 Signature Authorization

The protocol will be signed by Aprea Therapeutics.

2.0 BACKGROUND INFORMATION

2.1 APR-246

[redacted]

2.2 APR-246 Preclinical Studies

2.2.1 Pharmacology and Mode of Action

[redacted]

2.2.3 Pharmacokinetics and Metabolism in Animals

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

2.3 APR-246 Clinical Studies

[redacted]

2.3.1 Phase 1/2 Study in Solid and Hematological Malignancies

[redacted]

[redacted]

[redacted]

[redacted]

2.3.2 Phase 1/1b Study in Refractory Hematologic Malignancies

In [redacted]

[redacted]

2.3.3 Phase 1/2 Study in TP53-Mutant Advanced Ovarian Cancer

[redacted]

[redacted]

2.3.4 Phase 1b/2 Study in TP53-Mutant Myeloid Neoplasms

[redacted]

2.3.5 APR-246 CNS Safety Overview

[redacted]

2.3.6 APR-246 Cardiac Safety Overview

[redacted]

[redacted]

2.4 Pembrolizumab

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control[5]. The normal function of PD-1, expressed on the cell surface of activated T cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which negatively regulates antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The observed correlation of clinical prognosis with PD-L expression in multiple cancers suggests that the PD-1/PD-L1 pathway is involved in tumor immune evasion and is thus an attractive target for therapeutic intervention.

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype which blocks the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Both pembrolizumab and the PD-1 inhibitor nivolumab contain the S228P stabilizing mutation and have no antibody-dependent cell-mediated cytotoxicity or complement-dependent cytotoxicity activity. Pembrolizumab enhances T lymphocyte immune responses in cultured blood cells from healthy human donors, cancer patients, and primates. Pembrolizumab also modulates levels of interleukin-2, tumor necrosis factor alpha, interferon gamma, and other cytokines. Importantly, pembrolizumab potentiates existing immune responses only in the presence of antigen and does not nonspecifically activate T cells.

Pembrolizumab is approved by the Food and Drug Administration (FDA) for melanoma, non-small cell lung cancer (NSCLC), bladder cancer, renal cancer, gastric/gastroesophageal junction (GEJ) adenocarcinoma, and other malignancies. Across indications, the approved dose and schedule of pembrolizumab is 200 mg IV over 30-minute infusion every 3 weeks. Warnings and precautions include immune-mediate pneumonitis, colitis, hepatitis, endocrinopathies, skin reactions, and infusion-related reactions. The most common adverse reactions (reported in ≥ 20% of patients) were: fatigue, musculoskeletal pain, decreased appetite, pruritus, diarrhea, nausea, rash, pyrexia, cough, dyspnea, constipation, pain, and abdominal pain. In combination with chemotherapy, adverse drug reactions (ADRs) included fatigue/asthenia, nausea, constipation, diarrhea, decreased appetite, rash, vomiting, cough, dyspnea, pyrexia, alopecia, and peripheral neuropathy. In combination

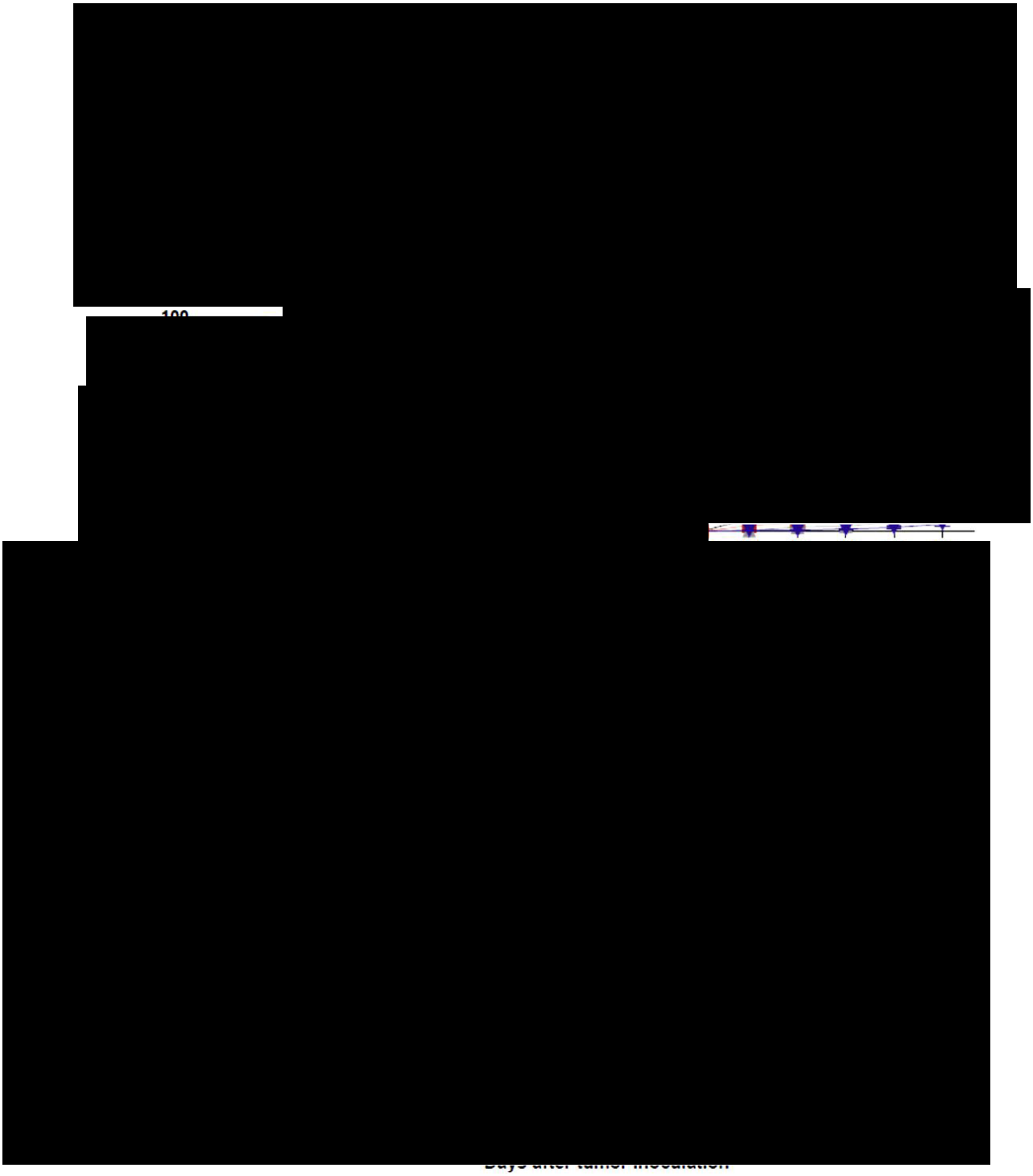
with axitinib, ADRs included diarrhea, fatigue/asthenia, hypertension, hepatotoxicity, hypothyroidism, decreased appetite, palmar-plantar erythrodysesthesia, nausea, stomatitis/mucosal inflammation, dysphonia, rash, cough, and constipation. Table 1 in the United States Prescribing Information (USPI)[6] provides recommended dose reductions of pembrolizumab in the setting of ADRs.

The PK of pembrolizumab was characterized using a population PK (popPK) analysis with concentration data collected from 2,993 patients with various cancers who received pembrolizumab doses of 1 to 10 mg/kg every 2 weeks, 2 to 10 mg/kg every 3 weeks, or 200 mg every 3 weeks. Steady-state concentrations of pembrolizumab were reached by 16 weeks of repeated dosing with an every 3-week (Q3W) regimen and the systemic accumulation was 2.1-fold. The C_{max}, minimum concentration (C_{min}), and AUC at steady state (AUC_{ss}) of pembrolizumab increased dose proportionally in the dose range of 2 to 10 mg/kg every 3 weeks.

Pembrolizumab $CL_{\%}$ is approximately 23% lower [geometric mean, 195 mL/day (40%)] at steady state than that after the first dose [252 mL/day (37%)]; this decrease in CL with time is not considered clinically important. The terminal half-life ($t_{1/2}$) is 22 days (32%).

Regarding specific populations, the following factors had no clinically important effect on the CL of pembrolizumab: age (range: 15 to 94 years), sex, race (89% White), renal impairment (eGFR ≥ 15 mL/min/1.73 m^2), mild hepatic impairment (total bilirubin ≤ upper limit of normal (ULN) and aspartate aminotransferase (AST) > ULN or total bilirubin between 1 and 1.5 times ULN and any AST), or tumor burden. The impact of moderate or severe hepatic impairment on the PK of pembrolizumab is unknown.

2.5 Rationale for Combination of APR-246 in with Pembrolizumab



2.6 Rationale for Dose and Schedule of APR-246 in Combination with Pembrolizumab

[redacted]

[redacted]

utilized.

2.7 Potential Risks and Benefits

2.7.1 Potential Risks

[redacted]

[redacted]

2.8 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 Case Report Forms (eCRFs) will be completed for every subject.
3. Requirements for institutional ethics review as set forth by the appropriate Institutional Review Board/Independent Ethics Committee (IRB/IEC), Title 21 Code of Federal Regulations (CFR) Part 56, 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 Harmonisation (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.

2.9 Subject Population

This study will enroll adult male and female subjects of age ≥ 18 years with documented histologic diagnosis of solid tumor malignancies. Tumor TP53 mutation status is not required for enrollment.

3.0 TRIAL OBJECTIVES AND PURPOSE

3.1 Primary objective:

1. To evaluate the safety and tolerability of APR-246 in combination with pembrolizumab in subjects with solid tumors.
2. To determine and confirm the maximum tolerated dose (MTD) for APR-246 in combination with 200 mg IV, Q3W pembrolizumab in subjects with solid tumor malignancies.

3.2 Secondary objectives:

1. ORR of APR-246 in combination with pembrolizumab in subjects with solid tumor malignancies by Response Evaluation Criteria in Solid Tumors (RECIST) 1.1.
2. Progression-free survival (PFS) by RECIST 1.1.
3. Overall survival (OS).
4. Duration of response (DOR) by RECIST 1.1.
5. Disease control rate (DCR: CR + PR + stable disease [SD]) by RECIST 1.1
6. Durable SD rate (durable SD [SD ≥23 weeks]) by RECIST 1.1
7. Clinical benefit rate (CBR: CR, PR + durable SD) by RECIST 1.1.
8. The PK profile of APR-246 during combination treatment with pembrolizumab.

3.3 Exploratory objectives:

1. To investigate the relationship between candidate efficacy biomarkers and anti-tumor activity of APR-246 in combination with pembrolizumab based on analyses of blood, serum, and/or tumor tissue.

4.0 TRIAL DESIGN

4.1 Overview of Trial Design

This is a phase 1/2, open-label, study to determine the safety and preliminary efficacy of APR-246 in combination with pembrolizumab in subjects with solid tumor malignancies.

In the safety lead-in part of study (phase 1), the safety and the RP2D of APR-246 will be investigated. The RP2D of APR-246 will be defined as the dose at which < 2 out of 6 subjects experience DLT during the 3-week safety assessment period after the start of APR-246 administration in combination with pembrolizumab using dose de-escalation of APR-246.

In the expansion part of the study (phase 2), both safety and efficacy for the combination therapy will be investigated in the 3 cohorts.

Study treatment may be administered on an outpatient basis. No investigational or commercial anti-tumor agents or therapies other than those described below may be administered with the intent to treat the patient's underlying malignancy.

The study will include a safety lead-in dose-finding portion followed by expansion portion. During the safety lead-in portion of the study, subjects with (non-CNS primary) solid tumor malignancies will receive pembrolizumab in combination with APR-246 in a 3 + 3 dose de-escalation design.

Dosing will begin at the full dose of both drugs due to the well-established safety profiles of APR-246 and pembrolizumab, the non-overlapping mechanisms of action, and the intent to treat patients at doses shown to be effective in previous studies. Lower dose levels of APR-246 will be explored as necessary depending on observed AEs.

The initial cohort of subjects will receive APR-246 at 4.5 g/day administered as an IV infusion on Days 1–4 in combination with pembrolizumab at the approved dose of 200 mg on Day 3 of each 21-day cycle. This cohort will enroll up to a maximum or 6 subjects. DLT will be assessed after 3 subjects have been enrolled in each respective cohort and the last enrolled subject has completed the 3-week safety assessment period (i.e., one cycle of combination regimen). A subject that discontinues therapy during Cycle 1 without DLT is considered evaluable for the purpose of safety only if at least 75% of scheduled doses of APR-246 were administered in the first cycle. At the first dose level of 4.5 g/day of APR-246, if ≤ 1 subject out of 3 experiences a DLT, 3 additional subjects will be enrolled. If ≤ 1 subject out of 6 experiences DLT, the dose level (4.5 g/day of APR-246) will be deemed the RP2D for that cohort. If ≥ 2 subjects out of the total 3 – 6 subjects in the cohort experience DLT, the study will continue enrollment at Dose Level -1 (4.0 g/day of APR-246) (see Table 1). If ≤ 1 subject out of 6 experiences DLT at this dose level, the dose level (4.0 g/day of APR-246) will be deemed the RP2D for that cohort. If ≥ 2 subjects out of 3 – 6 subjects at that Dose Level -1 experience DLT, the study will continue enrollment at Dose Level -2 (3.5 g/day of APR-246). If ≤ 1 subject out of 6 experiences DLT at this dose level, the dose level (3.5 g/day of APR-246) will be deemed the RP2D for that cohort. If ≥ 2 subjects out of 3 – 6 subjects at this dose level

experience DLT, the trial will be halted and the Data Review Team (DRT) will consider potential future dosing modifications. No dose reductions in pembrolizumab are planned.

Table 1. APR-246 Dose Levels

Dose Modification	APR-246 Dose
Starting Dose Level (DL)	APR-246 4.5 g/day 1.5 g (for first 45 minutes) + 3.0 g (for 5 hours 15 minutes)
Dose Level Reduction -1 (DL-1)	APR-246 4.0 g/day 1.33 g (for first 45 minutes) + 2.67 g (for 5 hours 15 minutes)
Dose Level Reduction -2 (DL-2)	APR-246 3.5 g/day 1.17 g (for first 45 minutes) + 2.33 g (for 5 hours 15 minutes)

Once the RP2D of APR-246 in combination with pembrolizumab has been determined, the expansion portion will begin in order to assess the anti-tumor activity of this combination in specific disease types. Up to 100 subjects will be enrolled in three cohorts of subjects with specific solid tumor malignancies as outlined in the schema.

Throughout the study, subjects may study continue treatment as long as toxicity remains acceptable and the subject has not withdrawn consent. Response and progressive disease (PD) will be assessed based on RECIST 1.1 response criteria (APPENDIX V) as outlined in the Study Calendar.

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-246

[redacted]

[redacted]

4.3.2 Pembrolizumab

Chemical Name: humanized X PD-1-mAb (H409A11) IgG4.

Formulation, preparation, storage and stability: Please see pembrolizumab USPI[6].

Route of Administration: Pembrolizumab is administered by IV infusion over 30 minutes (±5 minutes) at a dose of 200 mg every 3 weeks.

4.4 Duration of Therapy

Subjects may remain on study treatment to the end of the trial while deriving clinical benefit, unless unacceptable toxicity, progression, death or subject withdrawal. Subjects may remain on protocol therapy 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.

4.6 Drug Accountability/Disposition of Clinical Trial 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 patient 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 Registration

Prior to registration and any study-specific evaluations being performed, all subjects must have given written informed consent for the study and must have completed the pre-study evaluations (see Section 7.1). Subjects must meet all of the eligibility requirements listed in Section 5.0. Subjects will be registered on the study by using the [redacted] Oncology Interactive Web Response System automated subject registration system.

5.0 SELECTION AND WITHDRAWAL OF SUBJECTS

5.1 Inclusion Criteria:

1. Signed informed consent form (ICF) and ability to comply with protocol requirements.
2. Known tumor TP53 mutation status from recent or archival sample.
3. Histologically and/or cytologically confirmed solid tumor malignancy:
    a. Safety lead-in portion:
        i. Patients with histologically and/or cytologically confirmed diagnosis of advanced non-CNS primary tumors that have progressed after first line treatment, who are intolerant to first line treatment, or who are unable to receive first line treatment, and for whom pembrolizumab, or pembrolizumab-based therapy, is considered appropriate in the opinion of the investigator. Primary CNS tumors are excluded. Patients with clinically stable, known metastatic tumors to the CNS are eligible. CNS radiography is not required in the absence of suspicion for clinical involvement.
    b. Phase 2 Expansion portion:
        i. Patients with histologically and/or cytologically confirmed diagnosis of advanced gastric or GEJ tumors that have progressed after first line treatment, who are intolerant to first line treatment, or who are unable to receive first line treatment. Patients with clinically stable, known metastatic tumors to the CNS are eligible with Medical Monitor approval. CNS radiography is not required in the absence of suspicion for clinical involvement. Prior treatment with anti-PD-1/anti-PD-L1 therapy is prohibited.
        ii. Patients with histologically and/or cytologically confirmed diagnosis of advanced bladder/urothelial tumors that have progressed after first line treatment, or who are intolerant to first line treatment, or who are unable to receive first line treatment with cisplatin-based chemotherapy. Patients with clinically stable, known metastatic tumors to the CNS are eligible with Medical Monitor approval. CNS radiography is not required in the absence of suspicion for clinical involvement. Prior treatment with anti-PD-1/anti-PD-L1 therapy is prohibited is prohibited.
        iii. Patients with histologically and/or cytologically confirmed diagnosis of advanced NSCLC previously treated with anti-PD-1 or anti-PD-L1 therapy. Patients with clinically stable, known metastatic tumors to the CNS are eligible with Medical Monitor approval. CNS radiography is not required in the absence of suspicion for clinical involvement.
4. Adequate organ function as defined by the following laboratory values:
    a. Creatinine clearance > 30 mL/min (by Cockcroft-Gault method, APPENDIX I),
    b. Total serum bilirubin < 1.5 × ULN unless due to Gilbert's syndrome, tumor involvement, hemolysis or considered an effect of regular blood transfusions,

c. Alanine aminotransferase (ALT) and AST < 3 × ULN, unless due to involvement by the underlying malignancy.

5. Age ≥ 18 years at the time of signing the ICF.
6. Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1 or 2 (APPENDIX II).
7. Projected life expectancy of ≥ 12 weeks.
8. In the expansion portion, measurable disease meeting the following criteria:
    a. At least 1 lesion of ≥10 mm in the longest diameter (LD) for a non-lymph node or ≥15 mm in the short-axis diameter for a lymph node that is serially measurable according to RECIST 1.1.
    b. Lesions that have had external beam radiotherapy or loco-regional therapies such as radiofrequency ablation must show subsequent evidence of substantial size increase (ex. 20% increase in LD) to be deemed a target lesion.
9. Negative serum or urine pregnancy test prior to study treatment initiation in female subjects of childbearing potential.
10. Women of childbearing potential and men with female partners of childbearing potential must be willing to use an effective form of contraception such as latex condom, hormonal birth control, intrauterine device or double barrier method (APPENDIX III) during chemotherapy treatment and for at least six months thereafter.

5.2 Exclusion Criteria

1. Known history of untreated human immunodeficiency virus (HIV)/HIV with a detectable viral load or active hepatitis B or active hepatitis C infection.
2. Any of the following cardiac abnormalities:
    a. Myocardial infarction within six months prior to registration;
    b. New York Heart Association Class III or IV (APPENDIX IV) heart failure or known left ventricular ejection fraction (LVEF) < 40%;
    c. A history of familial long QT syndrome;
    d. Symptomatic atrial or ventricular arrhythmias not controlled by medications;
    e. QTcF ≥ 470 msec, unless due to underlying bundle branch block and/or pacemaker and with approval of the Medical Monitor.
3. Concomitant malignancies or previous malignancies with less than a 1-year disease-free interval at the time of signing 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. Patients with controlled, advanced prostate cancer are permitted.
4. Pregnancy or lactation.
5. Active uncontrolled systemic infection.
6. An autoimmune condition requiring ≥ 10 mg (or equivalent corticosteroid) prednisone daily, or any other systemic immunosuppressive treatment within 28 days of first dose of study therapy.
7. Known history of active tuberculosis.

8. Current (non-infectious) pneumonitis, or a history of pneumonitis that required steroids.
9. A live vaccine administered within 30 days of the first dose of study treatment.
10. Receipt of any investigational product within 14 days or 5 half-lives prior to study treatment initiation, whichever is shortest.
11. Prior intolerance to pembrolizumab or other immune-oncology agents.

5.3 Inclusion of Women, Minorities and Children

Both men and women 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.

5.4 Withdrawal Criteria

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 patient at any time if this is considered to be in the patient's best interest.

5.4.1 Study Treatment Discontinuation

Study treatment must be discontinued if:

- There is evidence of disease progression. 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 subject becomes pregnant.
- A subject is significantly non-compliant with the requirements of the protocol.
- A subject has an adverse experience that would, in the investigator's judgment, make continued participation in the study an unacceptable risk.
- The subject starts new treatment for their underlying disease.

5.4.2 Study Discontinuation

Subject is discontinued from the study if:

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

5.4.3 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 7.3.

5.5 Noncompliance

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

6.0 TREATMENT OF SUBJECTS

6.1 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.

APR-246
APR-246 is administered as a 6-hour IV infusion daily on Days 1–4 of each 21-day cycle. APR-246 fixed dose is 4.5 g. APR-246 is administered in a 2-step infusion:

- Step 1: Loading dose of 1.5 g for the first 45 minutes (± 2 min)
- Step 2: Maintenance dose of 3 g over 5 hours 15 minutes (± 30 min)

The dose of APR-246 may be reduced (please consult Section 4.1 and Table 1) or treatment interrupted if the subject develops AEs.

APR-246 vials are to be stored at 2 – 8°C (35.6 – 46.4°F). At the pharmacies and at the study centers, the prepared APR-246 study product (diluted in sodium chloride solution) is to be stored at no greater than 25ºC. The infusion should be completed within 24 hours from the time of preparation.

Detailed instructions on vial concentration, preparation and dispensing can be found in the Pharmacy Binder. The infusion timing, including start/stop times and the time of rate change, must be recorded.

Pembrolizumab

Pembrolizumab at a dose of 200 mg is to be administered on Day 3 ±1 day of each cycle before the Day 3 dose of APR-246.

6.1.1 Dose-Limiting Toxicity

A DLT is defined as any of the following:

- Any of the hematological or nonhematological toxicities noted in the table below considered to be at least possibly related to APR-246 occurring during the 3-week safety assessment period after the start of APR 246 administration in combination with pembrolizumab (Cycle 1 Day 1).
- Failure to administer ≥75% of the planned dosage of APR-246 as a result of treatment-related toxicity during Cycle 1, unless reversible CNS-related effects previously described for APR-246.
- Subjects who discontinue treatment due to treatment-related toxicity.
- Greater than 4-week delay in starting Cycle 2 because of a treatment-related toxicity, even if the toxicity does not meet DLT criteria.

Dose-Limiting Toxicities	
Toxicity Category	**Toxicity CTCAE Grade**
Hematologic	Grade 4 neutropenia for ≥ 7 days
	Grade 3 or Grade 4 febrile neutropenia [a]
	Thrombocytopenia <25,000/mm^3 associated with bleeding and/or that requires platelet transfusion
Other nonhematologic toxicity	Any other Grade 4 or a Grade 5 toxicity Grade 3 toxicities lasting > 3 days excluding: Nausea, vomiting, fatigue and diarrhea controlled by medical intervention within 72 hours. Grade 3 rash in the absence of desquamation, no mucosal involvement, does not require steroids, and resolves to Grade 1 by the next scheduled dose of pembrolizumab.
	Grade 3 hypertension not able to be controlled by medication
	Grade 3 or above gastrointestinal perforation
	Grade 3 or above wound dehiscence requiring medical or surgical intervention
	Any grade thromboembolic event
	Any Grade 3 non-hematologic laboratory value if: Medical intervention is required to treat the subject, or the abnormality leads to hospitalization

ANC = absolute neutrophil count, CTCAE = Common Terminology Criteria for Adverse Events v5.0.
[a] Febrile neutropenia Grade 3 or Grade 4:
Grade 3 is defined as ANC < 1000/mm^3 with a single temperature of >38.3 °C (101 °F) or a sustained temperature of ≥ 38 °C (100.4 °F) for more than one hour.
Grade 4 is defined as ANC < 1000/mm^3 with a single temperature of >38.3 °C (101 °F) or a sustained temperature of ≥ 38 °C (100.4 °F) for more than one hour, with life-threatening consequences and urgent intervention indicated

Only toxicities with a clear alternative explanation (e.g., due to disease progression) or transient (≤ 72 hours) abnormal laboratory values without associated clinically significant signs or symptoms based on investigator determination can be deemed a non-DLT.

All subjects enrolled in the safety lead-in portion will be assessed for DLTs during the 3-week DLT assessment period of Cycle 1. Subjects who discontinue study treatment prior to completing Cycle 1 of study treatment for any reason other than DLT will be replaced.

Additionally, AEs 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.

6.1.2 Recommended Phase 2 Dose (RP2D)

The RP2D of APR-246 will be defined as the dose at which < 2 out of 6 subjects experience DLT during the 3-week safety assessment period after administration of APR-246 in combination with pembrolizumab.

A DRT consisting of the Medical Monitor, Site Principal Investigators, and other clinical research personnel that the Sponsor may deem appropriate, will hold Data Review Meetings (DRM) on an interim basis at a frequency dependent upon study accrual. At these meetings, the DRT will review AEs and DLTs and make recommendations regarding the RP2D. In the expansion portion of the study, the DRT will evaluate safety and tolerability after 5 subjects have completed 1 cycle of treatment in each cohort. All accumulated safety data will be discussed during DRMs.

6.2 Dose Interruptions/Withholding

Study treatment may be withheld from a subject based on the investigator's decision in the event of intercurrent illness, AE, administrative reasons, or other reasons. If the subject's condition subsequently improves, or the situation that resulted in withholding study drug rectifies itself, the investigator may resume dosing as soon as possible, unless the delay is more than 4 weeks.

Dosing should be delayed for any DLT-equivalent toxicity and possible National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) > Grade 2 AEs considered related to study medication. At the investigator's discretion, dosing may recommence when the toxicity has resolved to Grade 2 or less. Immune-related reactions or allergy should resolve to ≤ Grade 1.

Treatment will be discontinued if a treatment-emergent AE (TEAE) has not resolved (to acceptable grade) after ≤ 3 weeks.

6.3 Supportive Management

6.3.1 Cardiac Monitoring

This section outlines the requirements for proceeding with treatment with APR-246, and the protocol rules for APR-246 dose modification due to AEs.

At screening, 12-lead ECGs should be collected in triplicate to confirm QT interval does not exceed 470 msec. QT interval must be calculated from a mean of all three ECG readings using Fridericia's correction ($QTcF = QT/RR^{0.33}$).

During Cycle 1, ECG should be collected in triplicate prior to infusion of APR-246 and at the end of infusion (EOI) of APR-246 (6 hours after start of infusion, ± 30 min) on Days 1–4. QTcF must be calculated from a mean of all three ECG readings to confirm it does not exceed 470 msec.

If a pre-dose ECG shows QTcF ≥ 470 msec, the QTc reading should be confirmed by manual assessment using Fridericia's correction ($QTcF = QT/RR^{0.33}$). Serum concentrations of

electrolytes should be monitored and corrected, if necessary. Additionally, concomitant medication should be reviewed and adjusted, if necessary. ECG may be repeated at any time, including the same day. APR-246 may only be administered when QTcF has returned to < 470 msec. If APR-246 is given on the same day, procedures outlined in the Schedule of Study Evaluations (Table 4) must be followed. If APR-246 cannot be administered on the same day, that dose must be omitted from the cycle.

If there is a significant change in QTcF, defined as either: a) increase > 60 msec from baseline (or pre-dose), or b) increase to an absolute value ≥ 501 msec, i.e. consistent with NCI-CTCAE Grade 3 QTc prolongation, QTc prolongation must be confirmed by a manual assessment of the ECG, and using Fridericia's correction ($QTcF = QT/RR^{0.33}$). If confirmed, the therapy should be interrupted until a cause (electrolyte disorders or an effect of a concomitant medication) has been identified and addressed, and QTcF has returned to < 470 msec. If all other causes for clinically significant QT interval prolongation are excluded, APR-246 must be permanently discontinued.

During subsequent cycles, ECG should be collected in triplicate prior to infusion of APR-246 on Day 1 of each cycle. QTcF must be calculated from a mean of all three ECG readings to confirm it does not exceed 449 msec. If pre-dose QTcF is 450-469 msec, APR-246 may be administered, and additional triplicate ECG should be performed at the EOI (6 hours after start of infusion, ± 30 min). If post-dose ECG shows a significant change in QTcF, defined as either: a) increase > 60 msec from baseline (or pre-dose), or b) increase to an absolute value ≥ 501 msec, i.e. consistent with NCI-CTCAE Grade 3 QTc prolongation, QTc prolongation must be confirmed by a manual assessment of the ECG, and using Fridericia's correction ($QTcF = QT/RR^{0.33}$). If confirmed, the therapy should be interrupted until a cause (electrolyte disorders or an effect of a concomitant medication) has been identified and addressed, and QTcF has returned to < 470 msec. If all other causes for clinically significant QT interval prolongation are excluded, APR-246 must be permanently discontinued. If QTcF is unchanged or there is no significant change, additional ECG is not required during that cycle.

If repeated QTcF measurements show a stable QTcF < 450 msec, or if QTcF remains stable within the interval of 450 – 469 msec with no significant change at the EOI during several cycles of treatment, reducing the number of ECGs performed in the study may be discussed with the Medical Monitor.

If a subject starts treatment with another medication known to prolong QT interval at any time during the study therapy, an additional pre- and post-dose (6 hours after start of infusion, ± 30 min) ECG should be performed on the next treatment day.

6.3.2 Management of CNS Adverse Events

If, during the administration period of APR-246, a subject experiences an AE that could be considered to originate from the CNS (e.g. dizziness, vertigo, nausea), medical management

is recommended as per institutional standard of care. Prompt discussion with the Medical Monitor is recommended.

Treatment with prochlorperazine 10 mg orally three times daily (tid) PRN has been reported to effectively treat CNS AE. Prochlorperazine may also be used prophylactically. When prochlorperazine is used prophylactically, it is recommended that treatment start the day prior to the Day 1 and continue at 10 mg tid through Day 4 as needed. It is important to record the dose and time of prochlorperazine administration in the eCRF.

In addition, dose modifications have been successfully used to manage CNS AEs occurring during the infusion (Table 2). For any clinical AE Grade ≥ 3, the infusion should be interrupted and if the AE resolves to CTCAE ≤ Grade 1 within 2 hours, the infusion may be resumed at the same infusion rate. If similar symptoms recur during re-challenge the infusion should be interrupted.

If an AE lasts longer than 2 hours, then the APR-246 infusion should be discontinued for that day, the remaining drug should be discarded.

Table 2. Management of CNS Adverse Events (Dizziness, Dyskinesia and Ataxia)

Worst toxicity	Dose Modifications for APR-246
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, stop infusion and ↓ 1 dose level for subsequent dose
Grade ≥ 3	Stop infusion and give medical therapy. If resolved (to ≤ Grade 1) with medical therapy, infusion may continue at the investigator's discretion. ↓ 1 dose level for subsequent dose
Grade 4	Permanently discontinue subject from APR-246.

6.3.3 Management of Nausea and Vomiting

For subjects who experience nausea and/or vomiting in association with APR-246, treatment with anti-emetics is recommended per institutional guidelines. Use of medications that prolong the cardiac QT interval is discouraged. For subjects who receive medications known to cause QT prolongation, the QT must be monitored via ECG before and after the APR-246 infusion.

- If QTc > 501 msec is observed in a subject concomitantly treated with another QT interval prolonging drug, this drug should be stopped and treatment with APR-246 may be restarted when QTc < 470 msec (refer to Section6.3.1).

A list of suggested rescue medications is provided below in Table 3.

Table 3. Medications for Managements of Nausea and Vomiting

Drug	Dosage	QT Prolongation[a]
Ondansetron	8 mg orally (PO) administered 30 minutes before the start of infusion or per label	Yes
Dolasetron	100 mg PO administered within one hour before start of infusion or per label	Yes
Palonosetron	0.5 mg PO administered approximately one hour prior to the start of infusion or per label	No
Prochlorperazine	10 mg PO three times daily. Continue until the end of Day 4 of the cycle. When used prophylactically in subsequent cycles, start the day prior to Day 1 administration of APR-246	No

[a] Please refer to Section 6.3.1 for details on concurrent administration of medications known to cause QTc interval prolongation.

6.3.4 Management of Infusion Reactions

6.3.4.1 APR-246

If a subject experiences an infusion reaction considered related to APR-246, the infusion should be immediately interrupted and appropriate medical care (e.g., epinephrine, oxygen, H1 and H2 antagonists, and/or corticosteroids) administered[9].

6.3.4.2 Pembrolizumab

Pembrolizumab is to be administered only under the care of a physician experienced in the use of pembrolizumab in treating subjects with solid tumor malignancies.

Dose Modification and Toxicity Management for Immune-related AEs Associated with Pembrolizumab

AEs associated with pembrolizumab exposure may represent an immunologic etiology. These immune-related AEs may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than one body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. In clinical studies, most AEs were reversible and could be managed with interruptions of pembrolizumab, administration of corticosteroids and/or other supportive care. For suspected AEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Based on the severity of AEs, withhold or permanently discontinue pembrolizumab and administer corticosteroids. Dose modification and toxicity management guidelines for ARs associated with pembrolizumab are provided in the USPI[6].

Dose Modification and Toxicity Management of Infusion Reactions Related to Pembrolizumab

Pembrolizumab may cause severe or life-threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines on pembrolizumab associated infusion reaction are provided in the USPI[6].

6.4 Concomitant Treatment

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

Subjects may continue their baseline medication(s) as long as they are not prohibited. Prohibited mediations include systemic immunosuppressive treatment [ex. prednisone ≥ 10 mg/day (or equivalent corticosteroid)], live vaccines and investigational anti-tumor products. Palliative and supportive care (e.g., anti-emetics, bisphosphonates) for disease-related symptoms may be utilized according to institutional practices. AEs will be treated as clinically indicated. All concomitant medications should be recorded in the eCRF.

If the subject develops an acute infusion reaction (≥ Grade 2), the infusion must be interrupted until the reaction is resolved to ≤ Grade 1. Premedication (e.g., systemic corticosteroids) may be used as required.

6.5 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] designee.

7.0 STUDY EVALUATIONS

7.1 Schedule of Study Evaluations

Study evaluations are summarized in Table 4 and described in Sections 7.2 through 7.4.

Table 4. Study Calendar

Study Calendar Evaluation [a]	Screening [a] (28 days)	Cycle 1 (21 days)						Cycle 2 and Subsequent Cycles				End of Treatment [r]	Long-Term Follow-up [s]
		D1	D2	D3	D4	D8 [b, e]	D15 [b, e]	D1[c]	D2	D3	D4		
Informed consent	×												
Medical history [d]	×												
Physical examination [e]	×	×						×				×	
Height	×												
Weight	×	×						×				×	
Vital signs [e]	×	×	×	×	×			×	×	×	×	×	
ECOG PS	×	×						×				×	
APR-246 [f]		×	×	×	×			×	×	×	×		
Pembrolizumab [g]				×						×			
Disease assessment [h]	×							× [h]				×	
Hematology [i]	×	×				×	×	×				×	
Serum chemistry [j]	×	×				×	×	×				×	
Pregnancy test [k]	×												
ECG [l]	×	×[l]	×[l]	×[l]	×[l]			×[l]					
APR-246 PK sample [m]		×	×	×	×			×	×	×	×		
Archival tissue collection [n]	×												
Fresh tissue biopsy [o]	×							×					
Blood for biomarkers [p]	×	×						×				×	
Blood for PBMC [q]	×	×						×				×	
Clinical toxicity assessment		Starting at the time of study treatment initiation through 30 days after last dose [t]											
Concomitant medications	Reviewed throughout study												
Survival												×	×

Footnotes to Study Calendar

a. All screening/baseline evaluations 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 ±3 days applies to this study visit.

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

d. 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.

e. 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 prior to APR-246 infusion, 2 hours into infusion and at EOI (±30 minutes at all time points).

f. APR-246 is administered IV on Days 1–4 of each 21-day cycle.

g. Pembrolizumab at a dose of 200 mg is to be administered on Day 3 ±1 day of each cycle before the Day 3 dose of APR-246.

h. Disease assessment should be conducted every 9 weeks (±3 days) after initiating study treatment, prior to initiation of each odd treatment cycle starting at Week 9, then every 6 weeks through the first year and then every 9 weeks, thereafter. Magnetic resonance imaging (MRI) may be used instead of computed tomography (CT) for head, neck, abdomen, and pelvis; however, the chest must be assessed using CT. Chest disease may not be followed using chest x-ray. Positron-emission tomography (PET) scanning should not be used, but PET/CT may be used if CT component is with contrast enhancement and of diagnostic quality. The same modality (CT, MRI, and/or PET) that is used for baseline disease assessment should be used throughout the study.

i. Hematology must include complete blood count with differential.

j. Serum chemistry must include sodium, potassium, chloride, CO_2, blood urea nitrogen (BUN), creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase, Thyroid Stimulating Hormone (TSH) and free T_4.

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

l. Standard 12-lead ECGs in triplicate at screening, on Days 1–4 of Cycle 1 and on Days 1 of each subsequent cycle with subject in a semi-recumbent position. Please consult Table 6 for ECG collection schedule.

m. Please consult Section 8.5 for PK collection schedule.

n. Tumor block or 20 unstained, 4-µm thick, paraffine-embedded tumor slides from an archival tissue sample, if available.

o. Optional: Percutaneous or endoscopic biopsy of a tumor lesion will be only performed at baseline (within 2 weeks prior to treatment initiation) and at Cycle 2 Day 1 (±5 days). Biopsy samples will be formalin-fixed, paraffin embedded and snap frozen.

p. Blood for serum analysis is collected on specific 5-mL dry tubes at screening, prior to treatment initiation at Cycle 1 Day 1, and at the time of second tumor biopsy/or prior to Cycle 2 Day 1 treatment if no biopsy is done.

q. Blood for PBMC isolation is collected in four 10-mL CPT tubes (total, 40 mL) at screening, prior to treatment initiation at Cycle 1 Day 1, and at the time of second tumor biopsy/or prior to Cycle 2 Day 1 treatment, if no biopsy is done. Additional PBMCs will be collected prior to Cycle 5 Day 1 treatment and 30 days after treatment discontinuation.

r. Subjects discontinuing treatment should complete their end of treatment visit 30 days after their last dose of APR-246. Physical exam, vital signs, clinical toxicity assessment, hematology, serum chemistry and disease assessment should be performed, if feasible.

s. Long-term follow up can be done remotely (e.g. via telephone, via local practitioner or via review of medical records). Assuming there is no withdrawal of consent, subjects who stop study treatment for any reason (e.g. toxicity, PD) will continue long-term follow-up (see Section 7.4). 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.

t. AE description, grade and start date and resolution date should be documented.

7.1.1 Screening

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

- Signed written informed consent
- Medical history
- Physical examination
- Height
- 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, CO_2, BUN, creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase, TSH and free T_4
- Creatinine clearance (Cockcroft-Gault method; APPENDIX I)
- 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.
- Concomitant medication review
- Optional: Archival tissue collection
- Optional: Fresh tissue biopsy
- Blood for biomarkers
- Blood for PBMC
- Baseline disease assessment at a minimum must include imaging of chest, abdomen and pelvis (CT, MRI, and/or PET) for subjects with gastric/GEJ adenocarcinoma, urothelial cancer and non-small cell lung cancer. The same modality should be used throughout the study.

7.2 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 the biobanking program and will be asked to sign the mandatory section in the main study consent form related to biobank samples. 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.

7.2.1 Cycle 1

7.2.1.1 Cycle 1 Days 1–4

Day 1 examinations marked * do not need to be repeated if already performed within 3 days prior to Day 1 Cycle 1.

- Clinical toxicity assessment
- Concomitant medications review
- Physical examination: Day 1*
- Weight: Day 1*
- ECOG performance status (Day 1*; APPENDIX II)
- Vital signs, including blood pressure, heart rate, respiration rate and temperature, collected 2 hours into APR-246 infusion (± 30 min) and at EOI (± 30 min)
- Hematology (Day 1*), including complete blood count with white blood cell differential
- Serum chemistry (Day 1*), including sodium, potassium, chloride, CO_2, BUN, creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase, TSH and free T_4
- Blood for biomarkers (Day 1*)
- Blood for PBMC (Day 1*)
- ECG: standard 12-lead ECG with subject in a semi-recumbent position; pre-dose (prior to the APR-246 PK blood draw before the infusion) and at the EOI, per Table 6
- APR-246 administration: Days 1–4
- Blood sample for APR-246 pharmacokinetics: on Days 1, 2, 3 and 4, per Table 5
- Pembrolizumab at a dose of 200 mg is to be administered on Day 3 ±1 day of each cycle before the Day 3 dose of APR-246

7.2.1.2 Cycle 1 Days 8 and 15 (±3 Days)

- Clinical toxicity assessment
- Concomitant medications review
- Hematology, including complete blood count with white blood cell differential
- Serum chemistry, including sodium, potassium, chloride, CO_2, BUN, creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase, TSH and free T_4

7.2.2 Cycle 2 and Onwards (Cycle 3+) Day 1 (±3 Days)

These tests should be performed prior to first dose

- Clinical toxicity assessment
- Concomitant medications review
- Physical examination: Day 1*

- Weight
- ECOG performance status (Day 1; APPENDIX II)
- Vital signs
- APR-246 administration: Days 1–4
- Hematology (Day 1*), including complete blood count with white blood cell differential
- Serum chemistry (Day 1*), including sodium, potassium, chloride, CO_2, BUN, creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase, TSH and free T_4
- Blood for biomarkers (Cycle 2)
- Blood for PBMC (Cycle 2 and Cycle 5)
- Fresh tissue biopsy
- ECG: standard 12-lead ECGs with subject in semi-recumbent position before APR-246 infusion on Day 1 see Table 6
- Cycle 3 and onwards, prior to each odd cycle: disease assessment

7.2.2.1 Cycle 2 and Onwards Days 2–4

- Clinical toxicity assessment
- Concomitant medications review
- Vital signs, including blood pressure, heart rate, respiration rate and temperature, collected 2 hours into APR-246 infusion (± 30 min) and at EOI (± 30 min)
- Pembrolizumab at a dose of 200 mg is to be administered on Day 3 ±1 day of each cycle before the Day 3 dose of APR-246
- APR-246 administration: Days 1–4
- *Only Cycle 3*: Blood sample for APR-246 pharmacokinetics: on Days 1, 2, 3 and 4, per Table 5

7.3 End of Treatment Visit

This visit should take place 30 days after the last dose of study treatment, if treatment is stopped early for any reasons.

- Physical examination
- Weight
- Vital signs
- Disease assessment
- ECOG performance status (APPENDIX II)
- Hematology, including complete blood count with white blood cell differential
- Serum chemistry, including sodium, potassium, chloride, CO_2, BUN, creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase, TSH and free T_4
- Blood for biomarkers

- Blood for PBMC
- Clinical toxicity assessment up to 30 days after the last dose
- Survival follow-up

7.4 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). After a subject is removed or withdrawn from the study, the subject will be followed for 1 year or until death, whichever occurs first. Off-treatment data on OS will be updated every 6 months or until death, whichever occurs 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(s). Subjects who respond and discontinue study treatment for reasons other than PD should have response assessments and survival should be collected every 2 months until disease progression or death, whichever occurs first. After disease progression, data for survival should be collected every 6 months until death.

8.0 STUDY ASSESSMENTS

8.1 Safety Assessments

8.1.1 Safety Analysis

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

8.1.2 Reporting of Adverse Events

8.1.2.1 Adverse Events

An AE is any untoward medical occurrence in a subject or clinical investigation 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 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.

All AEs (except Grade 1 and 2 laboratory abnormalities that do not require an intervention), 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. 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.

8.1.2.2 Attribution Definitions

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 of chemicals. Information on the drug withdrawal may be lacking or unclear.

Unlikely related: A clinical event, including laboratory test abnormality, with a 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.

8.1.2.3 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 current IB; 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.

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

8.1.2.4 Serious Adverse Event (SAE)

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.

8.1.2.5 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 8.1.2.6.

8.1.2.6 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, the Sponsor's monitoring CRO must be notified immediately (within 24 hours of becoming aware of the event) by email, fax or telephone. Notification by email is preferred. The email address, fax and telephone numbers listed below may be used during both business and non-business hours. During non-business hours a recorded message will provide the telephone caller with the contact information for the on-call monitor.

All SAEs require that a Serious Adverse Event Report Form be completed and forwarded either via facsimile or as a PDF via email to Sponsor's monitoring CRO at the fax number or email listed below within 24 hours of becoming aware of the event.

SAEs will be reported to: [redacted]

8.1.2.7 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 the [redacted] and the Sponsor's Medical Officer.

DRT consisting of the Medical Monitor, Site Principal Investigators, 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 and DLTs and make recommendations regarding the RP2D. In the expansion portion of the study, the DRT will evaluate safety and tolerability after 5 subjects have completed 1 cycle of treatment in each cohort. All accumulated safety data will be discussed during DRMs.

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.

8.2 Efficacy Assessments

Tumor assessments will be performed by the investigators based on RECIST 1.1, which will also guide treatment decisions. All scans for tumor assessments performed during the study should be archived in accordance with the standard local practice. The scans must be accessible in the event of a Sponsor request to submit them for central review.

Tumor assessments will be carried out every 9 weeks (±3 days) after initiating study treatment, prior to initiation of each odd treatment cycle starting at Week 9, then every 6 weeks through the first year and then every 9 weeks, thereafter. CT/MRI scans of chest, abdomen, and pelvis and of other known sites of disease will be obtained at screening (within 28 days prior to Cycle 1 Day 1), at all tumor assessment time points, and as indicated clinically. Color photographs containing a millimeter scale must be taken of all skin lesions being used as target lesions. Historical standard of care scans that are performed with scanning parameters consistent with the requirements for this protocol within 28 days prior to dosing are acceptable. Subjects with squamous cell carcinoma of the head and neck must also have head and neck scans performed.

MRI may be used instead of CT for head, neck, abdomen, and pelvis; however, the chest must be assessed using CT. Chest disease may not be followed using chest x-ray. PET scanning should not be used, but PET/CT may be used if CT component is with contrast enhancement and of diagnostic quality.

A brain scan (CT with contrast or MRI pre- and post-gadolinium) must be performed at screening to assess potential CNS disease and/or metastases for patients with predilections to develop CNS metastases. For subjects with previously treated eligible brain metastases, a brain scan must be performed at all tumor assessment time points. For all subjects, a follow-up brain scan must be performed to confirm CR within 1 week of response confirmation, or if clinically indicated.

The tumor assessment schedule should not be affected by interruptions in study treatment.

Subjects taken off study treatment without disease progression will also undergo tumor assessments per the Study Calendar (Table 4) until disease progression is documented or another anticancer therapy is initiated.

The same method of assessment must be used at all time points as was used at screening. Throughout the study it is critical that the same imaging methodology be applied, and contrast be consistently provided unless IV contrast becomes medically contraindicated during the course of treatment or the dose of contrast needs to be adjusted based on the subject's health status.

In order for SD to be considered the BOR, it must occur ≥ 5 weeks following the first dose of study drug.

The first radiological assessment of tumor response status will be performed at Week 9 (±3 days), unless there is clinical indication warranting earlier radiologic imaging. If imaging at Week 9 shows SD, treatment will be continued and tumor assessments will be conducted at the next regularly scheduled imaging time point, i.e., every 6 weeks through the first year, then every 9 weeks, thereafter. Responses (PR or CR) should be confirmed no less than 4 weeks after the initial response, but generally at the next scheduled tumor assessment time point.

If the time point tumor assessment is PD, treatment may be continued and tumor assessments be repeated at least 4 weeks later, but generally at the next scheduled tumor assessment time point in order to confirm PD. If repeat imaging shows a reduction in the tumor burden compared to the initial tumor assessment demonstrating PD, treatment may be continued as per treatment schedule. If repeat imaging confirms PD, subjects will be considered for discontinuation from study treatment. In determining the tumor time point response, investigators should consider all target lesions as well as non-target lesions and new lesions. The decision to continue study treatment after the first evidence of PD is at the investigator's discretion based on the clinical status of the subject. However, subjects may continue to receive study treatment in the setting of PD if they are deriving clinical benefit in the opinion of the investigator.

Subjects may continue receiving study treatment while waiting for confirmation of PD if they are clinically stable as defined by the following criteria:

- Absence of signs and symptoms (including worsening of laboratory values) indicating disease progression
- No decline in ECOG performance status
- Absence of rapid progression of disease
- Absence of progressive tumor at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention

If PD is confirmed and the subject is experiencing clinical benefit, the site may continue study treatment.

8.2.1 Objective Response Rate

ORR is defined as the proportion of subjects who have BOR of CR or PR as measured by RECIST 1.1 (see APPENDIX V).

ORR is measured from the start date of the treatment period until date of death from any cause. Subjects who are lost to follow-up and the subjects who are alive at the date of data cutoff will be censored at the date the subject was last known alive.

8.2.2 Disease Control Rate

DCR is defined as the proportion of subjects who have BOR of CR or PR or SD (duration of SD ≥ 5 weeks).

8.2.3 Duration of Response

DOR is defined as the duration of CR or PR.

8.2.4 Durable Stable Disease Rate

Durable SD rate is defined as the proportion of subjects whose BOR is SD and the duration of SD is ≥ 23 weeks.

8.2.5 Clinical Benefit Rate

CBR is defined as the proportion of subjects who have BOR of CR or PR or durable SD (duration of SD ≥ 23 weeks).

8.3 Progression-Free Survival (PFS)

PFS is defined as the time from the first study dose date to the date of first documentation of confirmed disease progression or death (whichever occurs first).

8.4 Overall Survival

OS is measured from the date of initiating study treatment to the date of death.

8.5 Pharmacokinetics

Plasma concentrations of APR-246 will be measured in Cycle 1 and again in Cycle 3, at the timepoints provided in Table 5.

Table 5. PK Blood Sampling Timepoints for APR-246 in Cycle 1 and Cycle 3

APR-246 plasma collection timepoints [a]	**D1**	**D2**	**D3**	**D4**
Prior to APR-246 infusion	×	×	×	×
At the end of APR-246 infusion	×		×	
1 hour after the end of APR-246 infusion	×		×	
3 hours after the end of APR-246 infusion	×			

[a] A window of ±15 min applies to each time point.

The data will be evaluated using population pharmacokinetics (see section below).

8.6 Electrocardiographic Assessment

Table 6 describes the routine ECG requirements from screening through Cycle 4:

Table 6. ECG Assessment Requirements

Time Point	ECG, number	Timing
Baseline/Screening	Triplicate	Must be within 28 days of C1D1
Cycle 1, Days 1-4	Triplicate	Pre-dose; Post dose (at the end of infusion; ± 10 min)
Cycles 2+, Day 1	Triplicate	Pre-dose

If repeated QTcF measurements show a stable QTcF < 450 msec, or if QTcF remains stable within the interval of 450 – 469 msec with no significant change at the EOI during several cycles of treatment, reducing the number of ECGs performed in the study may be discussed with the Medical Monitor.

If a subject starts treatment with another medication known to prolong QT interval at any time during the study therapy, an additional pre- and post-dose (6 hours after start of infusion, ± 30 min) ECG should be performed on the next treatment day.

Please consult Section 6.3.1 for additional requirements for proceeding with treatment with APR-246.

8.7 Correlative Studies

Blood and serum biomarkers

Peripheral blood samples will be collected at specified time points for all enrolled subjects. Peripheral blood mononuclear cells (PBMCs) will be isolated and stored for future flow cytometry analyses. Serum samples will be stored for future cytokine analyses.

Archived, formalin-fixed paraffin-embedded (FFPE) tissue

Optional archived FFPE tumor tissue samples will be collected from all subjects for potential assessment of mutations and other genetic alterations and/or proteins including PD-1/PD-L1 status and other relevant biomarkers (e.g., tumor infiltrating lymphocytes, T cell repertoire, ribonucleic acid [RNA] signature profiles) which may be important in the development and progression of cancer as well as for potential use in diagnostic development. Appropriate technology/methodologies will be used based on the amount of tumor tissue available.

Fresh tissue

For all tumor types, optional fresh tumor biopsies will be collected at baseline and on treatment to examine markers of target engagement, relevant pharmacodynamic biomarkers, and potential markers of response. Fresh biopsies should be limited to readily

accessible tumor lesions (e.g., skin, peripheral lymph nodes, liver metastases that can be readily accessed using CT guidance). Subjects should have the biopsy before administration of the first dose of study drug and at a time point 3 weeks after the first dose (if they have recovered adequately from the biopsy taken prior to starting therapy).

Instructions for the processing, storage, and shipping of samples will be provided in the Laboratory Manual.

Pharmacokinetic-Pharmacodynamic Analyses:

Data from both safety lead-in and exploratory portions of the study will be used to explore PK/PD relationships for effects of APR-246 in combination with pembrolizumab on ORR, other efficacy-related parameters including PFS and OS, AEs/dose reductions, and Phase 2 blood and tumor biomarkers. Exploratory/graphical analyses will be conducted for PK/PD evaluations and may be followed by model-based analyses.

9.0 STATISTICS

9.1 Sample Size

In the safety lead-in portion of the study, up to 18 DLT-evaluable patients will be enrolled to determine the RP2D.

The dose expansion consists of 3 cohorts. The ORRs to pembrolizumab for the patients with the 3 types of cancer, who were R/R to previous chemotherapies, were 21.1% and 22,7% for urothelial cancer and gastric/GEJ adenocarcinoma, respectively. The ORR for patients with advanced NSCLC who have previously been treated with anti-PD-1/PD-L1 therapy is expected to be negligible (i.e. <= 20%). As such, the expected response rate to the combination therapy across indications is 20% to 30%. In order to increase the estimate precision, at least 20 evaluable patients will be included in each of the 3 cohorts.

Table 7 summarizes the 95% CI of response rates of 20% and 30% by the sample size. For example, if the sample size is 20 patients, at least 2 responders are needed to be over the lower boundary of 95% CI of 20% response rate.

Table 7. 2-sided 95% Confidence Interval for the ORR of 20% and 30% (20, 30 and 40 sample sizes)

	95% CI for 20% ORR	**95% CI for 30% ORR**
N=20	(5.7%, 43.7%)	(11.9%, 54.3%)
N=30	(7.7 %, 38.6%)	(14.7 %, 49.4%)
N=40	(9.1 %, 35,6%)	(16.6 %, 46.5%)

9.2 Analysis Populations

Safety population: Subjects will be evaluable for safety if they receive at least one dose of APR-246. The safety population will be used to summarize exposure and safety parameters.

DLT-evaluable population: All subjects who either experience a DLT during first the 3 weeks (Cycle 1) of the study treatment or receive ≥ 75 % of scheduled Cycle 1 dose of APR-246 in combination with pembrolizumab and do not experience a DLT. Any individual subject who is not evaluable for DLT will be replaced by a new subject through additional subject enrollment.

Efficacy evaluable (EE) population: All subjects who complete at least one treatment cycle of APR-246 in combination with pembrolizumab and who have at least one post-treatment clinical response assessment. Subjects who fail to complete one treatment cycle will also be considered EE if they show clear evidence of clinically significant disease progression. The EE population will be the secondary analysis population for efficacy.

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

PK/Pharmacodynamic population: All subjects who receive at least 1 dose of study drug (APR-246 pembrolizumab) and have evaluable pharmacodynamic and concentration data.

9.3 Data Summaries

Generally, all the continuous variables will be summarized using n (number of subjects with available data), mean, standard deviation (StDev), median, and range (minimum and maximum). Categorical data will be summarized by frequency and percentage. Time to event data will be analyzed using Kaplan-Meier method, and the number and percentage of events, or censored, the first quartile at 25%, median with 95% confidence, and third quartile at 75% of survival time will be presented. The survival rate at the 3, 6, 9, and 12 months, and then 2, 3 and 5 years will be presented. All the study data will be listed.

The following general study data, demographic, baseline characteristics and disease history will be summarized:

- Subject disposition
- Protocol deviation
- Demographics
- Cancer disease diagnosis and history
- Prior cancer treatments
- Medical history
- Prior/concomitant medications

Safety data including AEs, vital signs, laboratory data, ECG, physical exam, ad ECOG performance status will be summarized in the safety population. AEs will be coded using MedDRA and graded using NCI-CTCAE v5.0 severity grade.

The efficacy endpoints in the safety lead-in portion will be listed only. In the expansion phase, all the rates will be summarized with 95% exact CI (Clopper and Pearson) by cohorts. The time to event data will be analyzed using Kaplan-Meier method and the corresponding survival plots will be produced.

To assess any DDI between APR-246 and pembrolizumab, the primary PK parameters of APR-246 will be compared graphically and via descriptive statistics with historical data using the PK analysis set. If warranted, additional analyses may be performed. A popPK model for APR-246 will be developed. The model will be parameterized in terms of CL and V_d.

The effect of APR-246-pembrolizumab combination therapy on soluble, tissue, genetic and/or imaging biomarkers will be summarized using descriptive statistics using the PK/pharmacodynamic analysis set. PK/pharmacodynamic relationships will be explored graphically and may be investigated by model-based analyses.

Descriptive statistics/results from exploratory molecular analyses will be written and may include but are not limited to: TP53 variant allele frequency by next-generation sequencing (NGS), p53 immunohistochemistry, mutations in other genes by NGS, RNA expression.

9.4 Pharmacokinetic Analysis

Pharmacokinetic Analysis

The DDI between APR-246 and pembrolizumab will be evaluated using popPK methods. The existing popPK model for APR-246 will be used to compare the observed plasma concentrations of APR-246 in presence of pembrolizumab with the predicted plasma concentrations assuming no DDI (visual predictive check). Additionally, the PK analysis dataset from this study will be integrated with the existing popPK dataset and the effect of pembrolizumab on APR-246 PK parameters will be tested as covariate in the model.

Pharmacodynamic Analysis

The effect of APR-246-pembrolizumab combination therapy on soluble, tissue, genetic and/or imaging biomarkers will be summarized using descriptive statistics using the PK/pharmacodynamic analysis set. PK/pharmacodynamic relationships will be explored graphically and may be investigated by model-based analyses.

10.0 QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

10.1 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.

10.2 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.

10.3 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.

10.4 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.

11.0 ETHICAL CONSIDERATIONS

11.1 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 ICF 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.

11.2 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.

11.3 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 U.S. 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.

12.0 DATA HANDLING AND RECORD KEEPING

12.1 Data to be Entered Directly in the electronic Case Report Form

The eCRF will be the source record.

12.2 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.

12.3 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
Highly 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
Highly 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 a highly 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 highly effective method of contraception should be used.
● **Sexual abstinence** Sexual abstinence is considered a highly 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 70kg man.
1 MET = 3.5 mL O_2/min/kg body weight.

APPENDIX V - Tumor Measurement Based on RECIST 1.1

See the international criteria proposed by the RECIST Committee, version 1.1[10] for additional details on RECIST1.1.

Measurable disease will be defined as the presence of at least one measurable lesion that can be accurately measured in at least one dimension with the LD a minimum size of:

- > 10mm by CT scan (CT scan slice thickness no greater than 5 mm)
- 10mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable).
- 20 mm by chest x-ray.

For malignant lymph nodes to be considered pathologically enlarged and measurable, a lymph node must be ≥ 15mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5mm). At baseline and in follow-up, only the short axis will be measured and followed.

All other lesions, including small lesions (LD < 10 mm or pathological lymph nodes with ≥ 10 to < 15 mm short axis) as well as truly non-measurable lesions, will be considered non-measurable. Lesions considered truly non-measurable include: leptomeningeal disease; ascites; pleural/pericardial effusion; inflammatory breast disease; lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

All measurements should be recorded in metric notation, using calipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging based evaluation should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam. Clinical lesions will only be considered measurable when they are superficial and ≥10 mm diameter as assessed using calipers (e.g. skin nodules). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesions is recommended.

Baseline Documentation of Target and Non-Target Lesions

All measurable lesions up to a maximum of 5 lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline.

Target lesions should be selected on the basis of their size (lesions with the longer diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements.

A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

All other lesions (or sites of disease) including pathological lymph nodes should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required, and these lesions should be followed as "present" or "absent", or in rare cases "unequivocal progression".

Evaluation of Target Lesions using RECIST 1.1 Criteria
NOTE: In addition to the information below, also see section 4.3.2 in the international criteria proposed by the RECIST Committee, version 1.1[10] for special notes on the assessment of target lesions.

Complete response (CR) − Disappearance of all target lesions. Any pathological lymph node (LN) (whether target or non-target) must have decreased in short axis to < 10 mm.

Partial response (PR) − At least a 30% decrease in the sum of the LD of the target lesions taking as reference the baseline sum LD.

Progressive Disease (PD) − At least a 20% increase in the sum of the LD of the target lesions taking as reference the smallest sum LD recorded since the treatment started including baseline if that is the smallest on study. In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5mm. The appearance of one or more new lesions also constitutes PD.

Stable disease (SD) − Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD since the treatment started.

Evaluation of Non-Target Lesions using RECIST 1.1 Criteria
Complete response (CR) − Disappearance of all non-target lesions and normalization of tumor marker levels. All LN must be non-pathological in size (< 10 mm short axis).

Non-complete response (non-CR)/non-progression (non-PD) − Persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal limits.

Progressive disease (PD) − Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions.

Evaluation of Best Overall Response using RECIST 1.1 Criteria
The best overall response is the best response recorded from the start of the study treatment until the end of treatment provided the confirmation criteria are met. To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat studies that should be performed > 4 weeks after the criteria for response are first met. If a CR/PR cannot be confirmed the original "response" should be considered stable disease. The best overall response will be defined according to the following table:

Overall Response First Time Point	**Overall Response Subsequent Time Point**	**BEST Overall Response**
CR	CR	CR
CR	PR	SD, PD, or PR[1]
CR	SD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	NE[2]	SD provided minimum criteria for SD duration met, otherwise, NE[2]
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
PR	NE[2]	SD provided minimum criteria for SD duration met, otherwise, NE[2]
NE	NE[2]	NE[2]

[1] If a CR is truly met at first time point, then any disease seen at a subsequent time point, even disease meeting PR criteria relative to baseline, makes the disease PD at that point (since disease must have

reappeared after CR). Best response would depend on whether minimum duration for SD was met. However, sometimes 'CR' may be claimed when subsequent scans suggest small lesions were likely still present and in fact the subject had PR, not CR at the first time point. Under these circumstances, the original CR should be changed to PR and the best response is PR.

[2] NE=inevaluable