MD Anderson IND Sponsor Cover Sheet	
Protocol ID	2020-0210
Protocol Title	A phase I-II, open label study evaluating the safety and efficacy of alisertib and pembrolizumab in patients with Rb-deficient head and neck squamous cell carcinomas
Protocol Phase	I-II
Protocol Version	06
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IND #	151006

PROTOCOL SUMMARY

Study Title: A phase I-II, open label study evaluating the safety and efficacy of alisertib and pembrolizumab in patients with Rb-deficient head and neck squamous cell carcinomas

Phase: I-II

Objectives

The primary objective of this study is to:

- Phase I: To determine the recommend phase II dose of the combination of alisertib and pembrolizumab.
- Phase II: To determine the overall response rate (ORR) of patients with immunotherapy-refractory recurrent or metastatic Rb-deficient head and neck squamous cell carcinoma (HNSCC) treated with the combination of pembrolizumab and alisertib.

The secondary objectives of this study are to:

- To evaluate the safety of the combination of pembrolizumab and alisertib in patients with solid tumors.
- To determine the overall survival and progression free survival (PFS) in HNSCC patients treated with the combination of pembrolizumab and alisertib.
- To determine the relationship between pharmacokinetics, pharmacodynamics, baseline immune and tumor biomarkers and clinical responses in patients treated with alisertib and pembrolizumab.
- To determine correlations between clinical responses and the effect of the treatment on human papilloma virus (HPV)-reactive T cells in HPV+ cancers.
- To determine correlations between clinical responses and tumor infiltrating lymphocyte function and T cell repertoire.

Patient Population
Specific inclusion and exclusion criteria are detailed in Section 3.2.

Number of Patients
24-40

Study Design and Methodology
This is a phase I-II, open label study evaluating the safety and efficacy of alisertib and pembrolizumab in adult cancer patients. The phase I cohort will test the safety of the combination of these drugs with non-overlapping toxicities in solid tumor patients who have exhausted standard of care therapy options. Patients will receive full dose αPD-1 antibody pembrolizumab (200mg IV every 3 weeks) in combination with the Aurora A kinase inhibitor alisertib. Alisertib will be started at 40mg po BID x 7 days every 3 weeks) and then escalated in subsequent cohorts as follows:

Dose Level	Alisertib Dose
-1	30 mg po BID x 7 days every 21 days
1	40 mg po BID x 7 days every 21 days
2	50 mg po BID x 7 days every 21 days

Patients who cannot swallow tablets may use an oral solution of alisertib with greater bioavailability. An equivalent dose of the oral solution can be administered with dosing details in section 1.7.

In the phase II study, patients with Rb-deficient HNSCC with recurrent/metastatic disease who are immunotherapy-refractory will be treated with the combination. Tumors will be measured every 6 weeks and evaluated using the Response Evaluation Criteria In Solid Tumors (RECIST) 1.1 guidelines.[1]

We will obtain tumor biopsies at baseline and at 6 weeks in patients in phase II portion of the study after the first interim analysis (i.e., if the efficacy of the combination passes the futility metric after 14 patients are treated). We will obtain blood every 6 weeks for correlative studies on all patients in the phase II study. Pharmacokinetic (PK) analysis of alisertib will be conducted in the first 12 patients treated at the recommended phase II dose on days 1 and 7 of course 1.

Duration of Study
Patients will remain on study until progression of cancer, unacceptable toxicity, or withdrawal of consent.

Treatments Administered
Pembrolizumab and alisertib

Efficacy Data Collected
The following evaluations will be conducted to assess the efficacy of alisertib:

- Tumor size measured by CT or MRI using RECIST 1.1 every 6 weeks.
- Progression free survival.
- Overall survival.

Pharmacokinetic/Pharmacodynamic/Pharmacogenomic/CorrelativeStudies Collected
The following studies will be conducted to assess the pharmacokinetics/pharmacodynamics/ pharmacogenomics of alisertib:

- To confirm adequate drug exposure and target inhibition and based on findings from prior studies[2-4] we will draw blood for pharmacokinetic (PK) analysis of alisertib in the first 12 patients treated at the recommended phase II dose on days 1 and 7 of course 1 at 1, 2, 3, 8, and 10 h after the morning dose of alisertib and calculate C_{max}, T ½ , and AUC values.
- To measure target inhibition, pharmacodynamics (PD) studies will be done in pre- and post- treatment tumor. Specifically, we will use immunohistochemistry (IHC) to quantify the number of cancer cells that stain for phosphorylation of Histone H3 (Ser10)[5] and mitotic protein monoclonal 2 (MPM2).[6]

Safety Data Collected
The following evaluations will be conducted to assess the safety of alisertib:

- Continuous serious adverse event (SAE) monitoring from date of consent until 60 days after the last dose of study drug. Continuous adverse event (AE) monitoring (as per NCI-CTCAE classification, version 5.0) from first dose of study drug until 60 days after the last dose of study drug. Blood chemistry, hematology, vital signs, and physical examination.

Statistical Procedures
Bayesian optimal interval design will be applied for the phase I dose escalation part of the study.[7-9] The target toxicity rate for the maximum tolerated dose (MTD) is 0.3. The maximum sample size is 12. We will enroll and treat patients in cohorts of size 2. To guide dose-escalation decisions, if the

observed dose-limiting toxicity (DLT) rate at the current dose is ≤ 0.236, the next cohort of patients will be treated at the next higher dose level; if it is ≥ 0.359, the next cohort of patients will be treated at the next lower dose level; otherwise the next cohort of patients will be treated at the same dose. We have performed simulation studies and the design will have at least 60% probability selecting the correct dose under various scenarios ranging from low to high toxicity profiles.

In the phase II cohort of immunotherapy-refractory patients, any responses will be of interest. We will use the Bayesian Optimal Phase 2 (BOP2) design. Design.[10] The assumptions are that the null response rate is 5% and the target response rate is 20%. Fourteen patients will be enrolled initially. If there are no responses, then the trial will be terminated due to lack of efficacy. If any responses are observed, an additional 14 patients will be enrolled. The treatment is considered efficacious if there are at least 4 responders in 28 patients and inefficacious otherwise. The design will have 82% power if the response rate is at least 0.2 with a one-sided 5% type I error.

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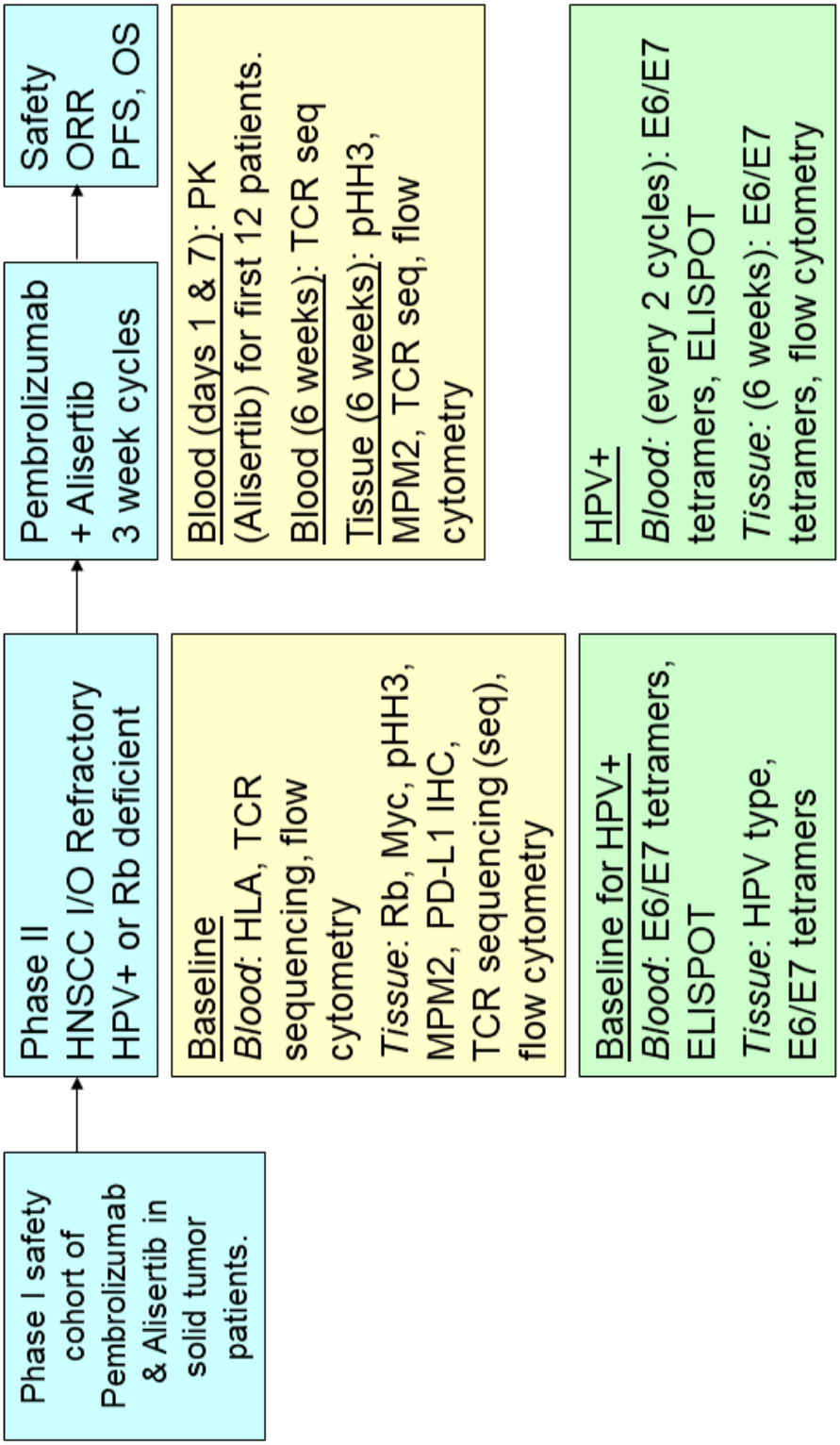
Study Flow Diagram



Schedule of Events

		Cycle 1	Cycle 1	Cycle 2	Subsequent Cycles[11]	
Assessments[1]	Screening Day -22 to Day -1	C1D1	C1D7	C2D1	D1	At PD or Termination[10]
Physical Exam and PS[2]	X	X	X	X	X	X
Vitals	X	X	X	X	X	X
Hematology[3]	X	X	X	X	X	X
Chemistry[4, 9]	X	X	X	X	X	X
TSH and free T4[5]	X				X	
EKG[6]	X					X
Pharmacokinetics on selected patients		X	X			
Serum or urine pregnancy test in WOCBP	X	X		X	X	
Imaging with tumor measurements[7]	X				X	
AE assessment		X	X	X	X	X
Optional biopsies[8]	X				X[8]	
Correlative blood studies[8]	X				X	
Blood tests for HIV, hepatitis B, and hepatitis C	X					

[1] Assessments may be done within 24-48 hours of dosing with drug administration
[2] Symptom directed
[3] CBC with differential.
[4] Chemistry: electrolyte panel, LFTs and kidney function tests, direct bilirubin, LDH, ALP, BUN, total protein, calcium, phosphate, magnesium, glucose.
[5] Thyroid function tests every two cycles.
[6] Single, 12-lead EKG will be collected at screening (within 22 days of first dose), end of treatment (i.e., at PD or termination).
[7]Every two cycles, up to 3 days prior to day 1 of the subsequent cycles.
[8]Tumor biopsies at baseline (up to 22 days prior to C1D1) and at 6 weeks (± 3 days) in patients in phase II portion of the study after the first interim analysis. Any biopsies performed will exclude biopsies of lesions in proximity to cardio-pulmonary, visceral, or vital neurovascular structures that can be considered high risk procedures. Blood every 6 weeks (± 3 days) for correlative studies as described in section 3.10.2.
[9]Add serum osmolarity, lactic acid levels and calculate the anion gap and osmolar gap for those using the Alisertib oral solution.
[10]Patients withdrawn from the study will be continuously monitored for survival through chart review and/or phone call every 2-3 months.
[11]Patients who remain on study for ≥ 10 cycles without progression may opt for tumor measurements every 3 cycles rather than every 2 cycles.

LIST OF ABBREVIATIONS

Abbreviation	Definition
°C	degrees Celsius
µM	Micromolar
20S	20S proteasome subunit
AE	adverse event
ANC	absolute neutrophil count
Bc1-2	B-cell lymphoma-2; a gene that inhibits apoptosis
BSA	body surface area
CAM	cell adhesion molecules
cm	Centimeter
CR	complete response
CTCAE	(NCI) Common Terminology Criteria for Adverse Events
ctDNA	circulating tumor DNA
CTEP	Cancer Therapy Evaluation Program
dL	Deciliter
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
ECT	enteric-coated tablet
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HNSCC	head and neck squamous cell carcinoma
HPV	human papilloma virus
ht	Height
IκB	I kappa B kinase; cytokine response kinase that activates transcription factor NF-kappa b at serine 32 and 36
ICAM-1	intercellular adhesion molecule 1
ICH	International Conference on Harmonisation
IEC	independent ethics committee
IND	Investigational New Drug
IRB	institutional review board
IV	Intravenous
IκBα	I kappa B alpha-associated protein kinase

Abbreviation	Definition
kg	Kilogram
Ki	inhibitory constant
lbs	Pounds
m^2	square meters
mg	Milligram
min	Minute
mL	Milliliter
mm^3	cubic millimeters
mmol	Millimole
MTD	maximum tolerated dose
NCI	National Cancer Institute
NF-κB	nuclear factor-κB
ng	Nanogram
nM	Nanomole
OS	oral solution
p21	p21(ras) farnesyl-protein transferase
p27	cyclin-dependent kinase inhibitor
p53	tumor suppressor protein with molecular weight of 53 kDa
PIC	powder-in-capsule
SAE	serious adverse event
SCLC	small cell lung cancer
US	United States
USP	United States Pharmacopeia
VCAM-1	vascular cell adhesion molecule 1
w/w	weight-to-weight ratio
wt	Weight

1. INTRODUCTION AND STUDY RATIONALE

1.1 Overview of the Diseases

The drug combination will be tested in head and neck squamous cell carcinoma (HNSCC) which is the seventh leading cause of cancer-related deaths globally; killing more than 375,000 individuals yearly.[11] Most HNSCC is caused by tobacco or the human papillomavirus (HPV). The incidence of HPV-driven HNSCC is increasing in the US and worldwide.[12-14] HPV+ HNSCC affects predominantly young (aged <55 years) people who suffer chronic adverse effects from therapy for decades – making less toxic therapy (i.e., therapy de-intensification) a research priority.[15]

The majority of HNSCC patients are diagnosed with locally advanced disease and treated with a combination of surgery, radiotherapy, and/or chemotherapy. Most of those who recur following initial definitive therapy receive palliative systemic therapy.

Comprehensive genomic characterization of HNSCC has not translated into clinical advances, largely because of a lack of druggable, driver oncogenes.[16-21] The lack of a biomarker-selected, molecular targeted therapies for HNSCC, permanent toxicity resulting from current therapy, and poor prognosis for those who progress after definitive therapy demonstrate an unmet clinical need for HNSCC.

Following progression after first-line, cisplatin-based chemotherapy, the standard of care for metastatic or recurrent HNSCC is αPD1 immune checkpoint therapy (ICT) with pembrolizumab or nivolumab. This standard is based on three pivotal studies that demonstrated response rates of 14-18%, six-month progression-free survival rates of 23%, and a one-year survival rate of 36%.[22-25] Although ICT has a striking effect in some HNSCC patients,[26] the majority (~85%) still experience progression. Response rates to non-ICT, standard of care therapies in platinum-resistant patients is 5.8%.[25] First-line pembrolizumab in HNSCC has a response rate of 17%.[27] Pembrolizumab was approved on June 10, 2019 by the federal drug administration (FDA) in combination with platinum and 5-fluorouracil (5-FU) for all recurrent and metastatic HNSCC patients and as a single agent for those whose tumors express PD L1 (Combined Positive Score [CPS] ≥1) as front line therapy.

1.2 Alisertib

Alisertib (International Nonproprietary Name, also known as MLN8237, TAK823) is a selective small molecule inhibitor of Aurora A kinase that is being developed for the treatment of advanced malignancies. Aurora A kinase is expressed in all actively dividing cells; it is localized to centrosomes and the proximal mitotic spindle during mitosis, where it

functions in a diverse set of mitotic processes. The chemical name for alisertib is sodium 4-([9-chloro-7-(2-fluoro-6-methoxyphenyl)-5H-pyrimido[5,4-d][2]benzazepin-2-yl]amino)-2-methoxybenzoate hydrate. Alisertib has demonstrated activity against a broad range of nonclinical tumor models grown in vitro and in vivo, as described in the following. Alisertib is also expected to be toxic to proliferating normal tissues, such as the bone marrow and gastrointestinal (GI) epithelium, because any cell that is in mitosis, where Aurora A kinase is expressed and active, should be susceptible to the effects of an Aurora A kinase inhibitor.

1.3 Nonclinical Summary

1.3.1 Nonclinical Pharmacology

Alisertib is a selective, potent, adenosine triphosphate (ATP)-competitive, and reversible inhibitor of Aurora A kinase. Alisertib inhibited proliferation of a wide variety of tumor cell lines grown in culture and induced phenotypes consistent with Aurora A kinase inhibition, including mitotic spindle defects, mitotic delay, and apoptosis.

Alisertib demonstrated antitumor activity when orally administered daily for approximately 21 days in a broad spectrum of nonclinical xenograft tumor models including human solid and hematologic tumors. The maximally efficacious dose (ED) for each model varied between 10 and 30 mg/kg if given once daily (QD) and 20 mg/kg if given twice daily (BID). Less frequent dosing was also efficacious, demonstrating that continuous dosing is not necessary for antitumor activity. A single oral (PO) dose of alisertib resulted in inhibition of activated Aurora A kinase and an increase in mitotic cells, demonstrating that mitotic index (MI) can be used as a pharmacodynamic marker of alisertib in some in vivo settings. The pharmacokinetic (PK)/pharmacodynamic studies in mice bearing HCT-116 tumor xenografts suggest that the maximum pharmacodynamic effect (mitotic accumulation) and efficacy are achieved at steady state plasma concentrations of 1 μM. The oral dose of alisertib at 30 mg/kg QD resulted in plasma concentrations of 1 μM for 8 to 12 hours postdose. Plasma concentrations of alisertib associated with saturating levels of pharmacodynamic and antitumor activity (1 μM) were exceeded at the recommended phase 2 dose (RP2D) of alisertib in patients (50 mg BID).

Alisertib can be combined with standard of care agents and results in synergistic or additive antitumor effects in multiple tumor models. (see IB for additional information) [Combination treatment with alisertib and docetaxel resulted in additive or synergistic effects during the dosing period, with prolonged tumor growth delay in multiple solid tumor xenograft models after terminating treatment. These effects were also observed in alternative intermittent dosing schedules. In diffuse large B-cell lymphoma (DLBCL) xenograft

models, combination treatment with alisertib and rituximab resulted in synergistic, additive, or subadditive effects depending on the dose and model; however, prolonged tumor growth delays were observed in every case after terminating treatment, and in some cases, complete cures were maintained. Combination treatment with paclitaxel or carboplatin in small cell lung cancer (SCLC) xenograft models, and with paclitaxel in a human breast cancer model, generally resulted in additive or synergistic antitumor activity.]

1.3.2 Nonclinical Pharmacokinetics

Alisertib was orally bioavailable in all animal species tested with limited distribution to red blood cells. Alisertib was highly bound to the plasma protein of all 5 species studied, with percent binding of 97.5% in humans. M1 (acyl glucuronide) and M2 (*O*-demethylated) metabolites of alisertib were also highly bound to human plasma protein.

Alisertib had low to moderate intrinsic hepatic clearance (CL_{int}), as determined in vitro in rat, monkey, dog, and human liver S9 fractions. On the basis of the results from the radiolabeled human absorption, distribution, metabolism, and excretion (ADME) study and the in vitro investigations, alisertib was metabolized by both oxidation and glucuronidation pathways. In human liver microsomes (HLMs), alisertib was metabolized by multiple cytochrome P450 (CYP) (CYP1A2, 2C8, 2C9, and 3A4) and uridine diphosphate-glucuronosyltransferase (UGT) (UGT1A1, 1A3, and 1A8) isozymes. CYP3A4 was the major (86.2%) CYP isozyme contributing to the oxidative metabolism of alisertib. Therefore, moderate and strong inhibitors of CYP3A4 and clinically significant enzyme inducers may alter the systemic exposure of alisertib.

The major metabolic route of [^{14}C]alisertib in rats in vivo was acyl glucuronidation, with >77.9% of the dose metabolized by this pathway. In rats, unchanged alisertib was the predominant circulating component in plasma over a 24-hour period. With repeated dosing of alisertib, the metabolite profiles in rat and dog plasma on Days 1 and 7 were similar, suggesting the lack of accumulation of metabolites. After PO administration of [^{14}C]alisertib to bile duct-cannulated rats, radioactivity was primarily eliminated in the bile.

Following a single oral dose of [^{14}C]alisertib to human subjects, alisertib was the major circulating drug-related component in plasma, and the average percentage of alisertib was 48% (n = 3) of the total plasma radioactivity (TRA). Two major circulating oxidative metabolites were *O*-demethyl (M2) and hydroxy (M3) metabolite with average percentages of 35% and 6% of TRA, respectively. Acyl glucuronide (M1) and an isomeric glucuronide conjugate were also observed, and each represented ≤10% of the total plasma radioactivity.

The total percentages of the 2 combined isomeric forms of the M1 metabolite averaged 12% of plasma radioactivity.

Urinary excretion was a minor route of clearance of total radioactivity with an average of <3% of the total administered dose recovered in urine over 14-days post-dose. Fecal excretion was the major route of clearance of total radioactivity in humans with an average of 88% of the total dose recovered in feces over 14 days post dose. Unchanged alisertib was one of the major drug-related components. Among the metabolites, M3 was one of the major drug-related components in feces. Overall, metabolites resulting from phase 1 oxidative pathways contributed to approximately 60% of the administered dose in human subjects. Therefore, cytochrome P450-mediated oxidation (particularly CYP3A4, as inferred from follow-up reaction phenotyping studies in vitro) is the most important clearance mechanism for alisertib. These findings suggest that there is a potential for increased systemic exposure of alisertib when co-administered with inhibitors of CYP3A4.

Alisertib was not an inducer of CYP1A2, 2B6, and 3A4/5 isozyme activities at concentrations up to 20 µM. Alisertib was a weak inhibitor of CYP2B6 and CYP2C8 in HLMs. However, alisertib is unlikely to inhibit the 5 major CYP isozymes, 1A2, 2C9, 2C19, 2D6, and 3A4/5 and was not a time-dependent inhibitor of CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, or 3A4/5. Additionally, alisertib metabolites M1 and M2 were not potent reversible inhibitors of CYP enzymes and therefore, it is unlikely that alisertib, M1, or M2 will cause drug-drug interactions (DDIs) by inhibiting major CYP isozymes.

If studies in combination with irinotecan, erlotinib or paclitaxel [Potential interaction of alisertib with other anticancer agents (irinotecan, erlotinib and paclitaxel) was investigated in liver microsomes. Alisertib inhibited the formation of 7 ethyl-10-hydroxycamptothecin (SN38), the active metabolite of irinotecan, and 7 ethyl 10-hydroxycamptothecine-glucuronide (SN38 Glu), a metabolite of SN38, in HLMs. In mouse liver microsomes (MLMs) and HLMs, erlotinib hydrochloride inhibited the UGT isozyme-mediated metabolism of alisertib.[28] However, erlotinib hydrochloride did not inhibit the CYP isozyme mediated metabolism of alisertib. In addition, alisertib inhibited both CYP2C8- and CYP3A4/5-mediated metabolism of paclitaxel in HLMs with half-maximal inhibitory concentration (IC_{50}) values of 16.3 and 72.9 µM, respectively.]

Alisertib demonstrated no efflux transporter activity. Alisertib was not a human P-glycoprotein (P-gp) substrate, but inhibited the P-gp-mediated efflux of paclitaxel in Caco-2 cells with an IC_{50} of 4.0 µM. The potential for inhibition of intestinal P-gp efflux by higher intestinal luminal concentrations of alisertib cannot be ruled out. Therefore, orally co-

administered narrow therapeutic range substrates of P-gp (eg, digoxin) should be administered with caution. On the basis of in vitro results described in the IB and considering the high permeability of alisertib, alisertib is not likely to be involved in other transporter-based DDIs.

1.3.3 Safety Pharmacology and Toxicology

Safety pharmacology studies with alisertib did not identify significant adverse effects in the central nervous system (CNS) and cardiovascular systems at tolerated doses. Alisertib exhibited minimal activity against human ether-à-go-go related gene (hERG) current (IC_{50} and K_i >100 μM), but did exhibit in vitro activity against the gamma-aminobutyric acid A alpha 1 (GABAAα1) benzodiazepine binding site (K_i = 290 nM), and related effects were observed at non-tolerated doses/exposures in safety pharmacology studies.

The dose-limiting toxicities (DLTs) for alisertib in both rats and dogs after repeat daily oral dosing for 2 cycles (each cycle consisted of 7 consecutive days separated by a 14-day dose holiday) or for 6 cycles (each cycle consisted of 21 consecutive days of dosing separated by a 7-day dose holiday) were consistent with inhibition of Aurora A kinase by alisertib. Principal findings in toxicology studies in rats and dogs included GI signs, panleukopenia, decreased reticulocyte counts, and increased mitotic figures and apoptosis (single cell necrosis) in tissues with a high basal cellular replication rate. No off-target effects were seen in the Good Laboratory Practices (GLP) compliant toxicology studies and in general the on-target findings were reversible following cessation of dosing.

Alisertib was negative for mutagenicity in the Ames assay and equivocal for clastogenicity in a rat bone marrow micronucleus assay.

1.4 Summary of Effects in Humans

As of 29 March 2016, the following company-sponsored alisertib studies were in progress or completed: 10 single-agent phase 1 studies (C14001, C14002, C14003, C14010, C14013, C14014, C14017, C14019, TB-MA010030, and TB-MA010033); 2 single-agent, phase 1, DDI studies (C14015 and C14020); 3 single-agent, phase 2 studies (C14004, C14005, and C14006); 1 single-agent, phase 1/2 study (C14007); 1 single–agent, phase 3 study (C14012); and 5 combination studies (C14008 [phase 1/2] with paclitaxel, C14009 [phase 1] with docetaxel, C14011 [phase1] with either rituximab, or rituximab + vincristine, C14018 with paclitaxel [phase 2], and C14022 with paclitaxel in Asian patients [phase 1]).

Three dosage forms of alisertib have been developed for clinical studies: powder in capsule (PIC), enteric-coated tablet (ECT), and oral solution. The initial phase 1 (C14001 through

C14003) and phase 2 (C14004 through C14006) studies employed the PIC formulation. Studies C14001 and C14003 were amended to evaluate the ECT formulation; C14001 evaluated the relative bioavailability (BA) of the PIC and ECT formulations in solid tumor patients, and C14003 evaluated the clinical safety and PK of the ECT formulation in patients with hematologic malignancies. Relative BA of the PIC, ECT, and oral solution has also been investigated in Study C14010.[29] The ECT formulation is being used in more recent studies (C14007 through C14009, C14011 through C14015, C14017, TB-MA010030 and TB-MA010033) and will be used in future studies.

Study Summary of Company Sponsored Studies

Study Number	Study Description	Enrollment Status
C14001/2	Phase 1 dose escalation study in solid tumor.	Complete
C14003	Phase 1 dose escalation study in advanced hematologic malignancies with a peripheral T cell lymphoma and diverse subtype expansion cohort.	Complete
C14004	Phase 2 study in aggressive non-Hodgkin lymphoma.	Complete
C14005	Phase 2 study in acute myelogenous leukemia or high-grade myelodysplastic syndrome.	Complete
C14006	Phase 2 study in epithelial ovarian, fallopian tube, or primary peritoneal carcinoma.	Complete
C14007	Phase 1/2 study to confirm the MTD in nonhematologic malignancies; phase 2 evaluation in lung, breast, head and neck, or gastroesophageal malignancies.	Complete
C14008	Phase 1/2 study to determine the MTD of alisertib combined with weekly paclitaxel; phase 2 evaluation of efficacy of combination in patients with relapsed ovarian cancer (open label, randomized).	Complete
C14009	Phase 1 study to determine the MTD of alisertib combined with docetaxel in patients with solid tumors, including prostate cancer, appropriate for docetaxel treatment	Complete
C14010	Phase 1 study evaluating the bioavailability of alisertib oral solution versus PIC formulation and the effect of food on alisertib PK (ECT formulation).	Complete
C14011	Phase 1 study evaluating patients with relapsed/refractory aggressive B-cell lymphoma treated with alisertib combined with rituximab and vincristine.	Complete
C14012	Phase 3 study in patients with relapsed or refractory peripheral T-cell lymphoma (international, multicenter).	Complete
C14013	Phase 1 dose escalation and PK study of alisertib in East Asian patients with advanced solid tumor or lymphomas.	Complete
C14014	Phase 1 study investigating the mass balance, PK, and metabolism of radio-labelled alisertib in patients with either advanced solid tumors or lymphomas.	Complete
C14015	Phase 1 study evaluating the effect of either esomeprazole or rifampin on the PK of alisertib and evaluation of the effects of alisertib on the QTc interval in patients with either advanced solid tumors or lymphomas.	Complete
C14017	Phase 1 study to investigate the effect of food on the PK of alisertib in patients with either advanced solid tumors or lymphomas.	Complete
C14018	A randomized, double-blind, placebo-controlled, phase 2 study of alisertib in combination with paclitaxel versus placebo in combination with paclitaxel as second-line therapy for small cell lung cancer (SCLC)	Complete

Study Summary of Company Sponsored Studies

Study Number	Study Description	Enrollment Status
C14019	A phase 1 study evaluating the PK of alisertib (MLN8237) in adult patients with advanced solid tumors or relapsed/refractory lymphoma with varying degrees of hepatic function	Ongoing
C14020	A phase 1 study evaluating the effect of itraconazole on the PK of alisertib in adult patients with advanced solid tumors or relapsed/refractory lymphoma	Complete
C14022	Phase 1 dose escalation and dose expansion study of alisertib in combination with a modified weekly paclitaxel in previously treated Asian patients with advanced solid tumors.	Ongoing
TB-MA010030	Phase 1, open-label, multicenter, dose-escalation study in patients with advanced solid tumors conducted in Japan.	Complete
TB-MA010033	Phase 1, open-label, multicenter, dose-escalation study in patients with relapsed or refractory non-Hodgkin lymphoma conducted in Japan.	Complete

ECT: enteric coated tablet; MTD: maximum tolerated dose; PIC: powder in capsule; PK: pharmacokinetics.

TB-MA010030 and TB-MA010033 were conducted in Japan and Studies C14013 and C14022 were conducted in East Asia. They are company-sponsored studies, but were not conducted under the US FDA IND (75,473). Patients in these studies were treated with the ECT formulation of alisertib BID for 7 consecutive days (Days 1-7) in a 21-day cycle.

Clinical PK data available as of 29 March 2016 are summarized below. Following oral administration of alisertib as the ECT formulation, peak concentrations of alisertib were generally achieved by 3 hours postdose. The overall peak-to-trough ratio was 2.2, and the accumulation ratio (R_{ac}) was 2.4. The terminal half-life was approximately 21 hours following 7 days of multiple dosing. Steady-state plasma exposures of alisertib increased in an approximately dose-proportional manner over the range of 5 to 200 mg/day in patients with advanced solid tumors. Pharmacokinetic steady-state conditions were approximately achieved by Day 7 following daily oral administration. The predominant route of elimination for alisertib is fecal, consistent with hepatic metabolism and biliary excretion of alisertib. Renal clearance of unchanged alisertib was negligible. Based on the results from the radio-labeled human ADME study (C14014) and the follow-up in vitro investigations, alisertib is metabolized by both oxidation and glucuronidation pathways, and multiple cytochrome P450 (CYP) isozymes (CYP3A4, 2C8, 2C9, 2C19, and 1A2) and UGT isozymes (UGT1A1, 1A3, and 1A8) are involved. Phase 1 oxidative metabolism was identified to be of quantitative importance, with an estimated contribution of CYP3A4 of approximately 60% to overall apparent oral clearance (CL/F) in humans. **Therefore, strong inhibitors of CYP3A4 and clinically significant inducers may alter systemic exposures of alisertib and should be avoided**. The PK properties of alisertib in patients with

hematologic malignancies were generally consistent with those observed in patients with nonhematologic malignancies. Based on the results of a population PK analysis in 671 adult cancer patients, the CL/F of alisertib was unaffected by age, sex, creatinine clearance, bilirubin, formulation (ECT vs PIC), body weight, body surface area (BSA), or UGT1A1 genotype (number of *28 alleles). Region (West/East) was identified as a significant covariate on alisertib relative bioavailability, which was 51.7% (95% CI = 36.5%-70.1%) higher for patients from the East region (East Asia/Japan). These results support the use of a common fixed starting dose of alisertib in the adult patient with the need to adjust dosing for the covariate of region (Japan or East Asia vs. West). The analysis results support achievement of comparable systemic exposures of alisertib following administration of doses of 50 mg BID to patients in the Western region and alisertib doses of 30 mg BID to patients in East Asia/Japan.

The results of metabolite profiling from the mass balance study (C14014) indicated the presence of 2 metabolites M1 (alisertib acyl glucuronide) and M2 (*O*-desmethyl) in circulation, as products of parallel primary pathways of alisertib biotransformation via glucuronidation and oxidative metabolism, respectively. Accordingly, plasma samples from Study C14015 were used to measure concentrations of M1 and M2 in addition to the parent drug to characterize the PK of these metabolites. Systemic exposures of the alisertib metabolites M1 and M2 following 50 mg BID multiple dose administration of alisertib were 44% and 42% of parent drug exposure, respectively.

The effect of a high-fat meal on the single-dose PK of alisertib administered as 5 × 10-mg ECT was evaluated in Study C14017. The geometric mean $AUC_{0\text{-inf}}$ following alisertib single dose administration under fed conditions was 115% of that under fasted conditions (90% CI: 96%, 139%), with a substantial overlap in the range of individual values of $AUC_{0\text{-inf}}$ when dosed in the fed versus fasted states. Based on the relatively minor (15%) increase in alisertib geometric mean $AUC_{0\text{-inf}}$ upon administration with a high-fat meal in relation to overall PK variability (45% coefficient of variation [CV] in $AUC_{0\text{-inf}}$), it is concluded that food does not produce a clinically relevant effect on alisertib PK. These results support the conclusion **that alisertib can be administered without regard for the timing of meals.**

The relative BA of the oral solution in reference to PIC in 14 patients was estimated to be 1.26 in reference to the PIC (90% CI: 1.09, 1.47). The rate of absorption of alisertib from the oral solution was higher than that from the PIC formulation, based on a shorter median time to first maximum plasma concentration ([T_{max}] 1 hour for the oral solution compared with 2

hours for the PIC). The geometric mean ratio of dose-normalized C_{max} of oral solution versus PIC was 1.90 (90% CI: 1.52, 2.37).]

Co-administration of alisertib with esomeprazole, a proton pump inhibitor (PPI) resulted in a 28% increase in total systemic exposure ($AUC_{0\text{-}inf}$) of alisertib, supporting the conclusion **that gastric acid-reducing agents be avoided in patients receiving alisertib.**

Co-administration of alisertib with rifampin, a strong metabolic enzyme inducer resulted in a 47% decrease in total systemic exposure ($AUC_{0\text{-}inf}$) of alisertib, supporting the conclusion that **chronic use of concomitant strong inducers of pregnane x-receptor (PXR)-inducible enzymes be avoided in patients receiving alisertib.**

Alisertib (50 mg) in single doses and after multiple doses BID for 7 days was not associated with any clinically relevant changes or findings in the electrocardiogram (ECG), did not prolong the QT interval, and can be concluded not to affect cardiac repolarization dynamics. No alisertib concentration-effect relationship was identified, consistent with findings from the statistical analyses in supporting a **lack of effect on the QT interval.**

Pharmacodynamic evaluations have been performed in patient skin and tumor biopsies using assays that reflect Aurora A kinase inhibition. The data collected to date from these biopsies provide evidence that a dominant mechanism of alisertib is inhibition of Aurora A kinase in both skin and tumor, and these effects are generally dose- and/or exposure-dependent.

Adverse events (AEs) observed to date (as of 29 March 2016) are generally reversible, dose-dependent, and are consistent with the pharmacologic profile of alisertib as an anti-mitotic agent with predominant effects in proliferative tissues. The more commonly observed (≥ 30% incidence) treatment-emergent AEs (TEAEs) all grades from pooled data across the alisertib single-agent studies include: neutropenia (48%), anemia (47%), fatigue (47%), diarrhea (44%), alopecia (36%), stomatitis (32%), nausea (31%), and thrombocytopenia (30%).

Central nervous system (CNS) effects, including transient dose-dependent somnolence and/or confusion, have also been observed. These CNS effects may not be associated with Aurora A kinase inhibition but instead likely represent benzodiazepine-like effects of alisertib due to its structural similarity to benzodiazepine. At higher dose levels evaluated in early phase 1 studies, severe CNS effects were sometimes considered DLTs, although these effects abated during the planned treatment-free period. In the phase 1 studies, CNS effects appeared to be related to high peak plasma levels resulting from single daily doses of alisertib. The frequency and/or severity of benzodiazepine-associated CNS toxicities may be reduced in adult patients enrolled to recent studies that administered alisertib with divided

doses (eg, BID), a schedule designed to reduce peak plasma levels while maintaining overall AUC. CNS effects have been reversible with cessation of alisertib treatment, dose reduction, and dose modification of other sedative medications.

Studies C14001 and C14002, conducted in patients with solid tumors determined the RP2D of the PIC formulation of alisertib administered in a 21-day cycle as 50 mg BID. Similarly, Study C14003 in hematologic malignancies identified alisertib ECT 50 mg BID for 7 days in a 21-day cycle as the recommended dosing regimen to take forward into future studies. While most clinical experience supports a 7-day schedule followed by a 2-week treatment-free period, clinical experience also supports treatment schedules with up to 14 days of drug administration in a 28-day cycle, and up to 21 days of drug administration in a 35-day cycle. The clinical experience includes treatments with multiple doses and schedules as described in the IB.

Alisertib is also available in an oral solution (OS). The relative bioavailability of alisertib oral solution is ~130% in reference to PIC formulation in adult cancer patients.[29] That is, the OS is expected to achieve 30% higher alisertib exposures than that of PIC. In addition, the alisertib exposures from 50 mg ECT were comparable to that of 50 mg PIC (investigated in study C14001). Taken together, the alisertib oral solution is expected to achieve 30% higher exposures than that of ECT as well. This was the rational for the alisertib mass balance study (C14014) using 35 mg oral solution to achieve comparable exposures at 50 mg ECT in adult patients, which was the unit dose of alisertib MTD.

An investigator-initiated study (IIS) (NCT02219789) showed that in patients with endocrine resistant ER+ metastatic breast cancer a favorable safety profile and preliminary efficacy was observed with a pulse dose of alisertib in combination with standard dose of Fulvestrant consisting of 50 mg twice daily alisertib on days 1–3, 8–10, and 15–17 of a 28-day cycle. This regimen of alisertib resulted in significantly lower grade ≥3 event rates relative to those observed with the traditional dose and schedule.[30]

1.5 Risks and Benefits

As of 29 March 2016, 1292 patients have been treated with alisertib, in company-sponsored studies. Clinical safety data include experience from patients who received multiple cycles followed by treatment-free periods between each cycle, and from patients who reduced or discontinued treatment. Based on the available clinical data, drug abuse, dependency, and drug withdrawal effects were not observed.

The identified risks associated with alisertib treatment include: reversible myelosuppression including anemia; febrile neutropenia (including fatal febrile neutropenia); leukopenia;

lymphopenia; neutropenia (including fatal neutropenia); pancytopenia (including fatal pancytopenia); thrombocytopenia; GI toxicity including abdominal pain, diarrhea, dyspepsia, mucosal inflammation, nausea, oral pain, stomatitis, and vomiting; asthenia; fatigue; pyrexia; infection (including fatal infection); sepsis (including fatal sepsis); liver function test abnormal including aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, alkaline phosphatase (ALP), and gamma-glutamyltransferase (GGT); decreased appetite; dehydration; gait disturbance; sedation; somnolence; confusional state; disorientation (and associated memory loss); alopecia; rash (including bullous dermatitis); and palmar-plantar erythrodysesthesia syndrome.

While all of these toxicities are potentially associated with risk or discomfort to the patient, they are anticipated to be reversible. However, it is possible that alisertib will have other toxicities that have not been observed in or predicted from its evaluation in rats and dogs and from ongoing studies in humans.

Results from the alisertib human ADME Study C14014 indicate that oxidative metabolism is the predominant route of elimination for alisertib. Results of subsequent in vitro drug metabolism studies suggest that the oxidative metabolism of alisertib is mainly mediated via CYP3A4. These findings suggest that alisertib systemic exposures may be potentially increased when co-administered with clinically significant CYP3A inhibitors. The DDI study (C14020) with itraconazole (a strong CYP3A inhibitor) revealed that itraconazole produced an approximately 40% increase in alisertib systemic exposures. These results validate recommendations that the concomitant use of strong CYP3A inhibitors be avoided. Selection of an alternate concomitant medication with no or minimal CYP3A inhibition potential is recommended. If co-administration of strong CYP3A inhibitors is medically necessary, investigators should monitor for toxicities and follow the dose modifications for toxicity per study protocol.

To mitigate the inherent risks in clinical studies of alisertib, patients are evaluated frequently while they are receiving treatment.

The oral solution formulation of alisertib contains propylene glycol and polyethylene glycol 400 (PEG400). The concentrations of these excipients in the formulation are within the range of levels used in Food and Drug Administration (FDA)-approved drug products and are generally considered safe for use as an excipient in liquid medications. Information may be found at https://www.atsdr.cdc.gov/csem/csem.asp?csem=12&po=0. Ingestion of large amounts of propylene glycol increases the risk of metabolic lactic acidosis. Approximately 55% to 75% of propylene glycol is metabolized to pyruvate, acetate, and lactate, while 25%

to 45% remains unchanged; accumulation of lactic acid is the reason that propylene glycol toxicity commonly presents with an anion gap metabolic acidosis.[31] **Therefore, in clinical trials evaluating the oral solution, patients will be closely monitored for signs and symptoms of metabolic acidosis**. The schedule will include a treatment-free period within every treatment cycle. The risks related to propylene glycol administration (as a component of the oral solution) have not been established however it is very likely that the low dose, intermittent exposure to oral propylene glycol in patients with normal renal function poses a low risk for toxicity.

Because alisertib inhibits Aurora A kinase, it is possible that alisertib may interfere with cancer growth and cause cancer cell death. Nonclinical results indicate that alisertib is not a major substrate for efflux mechanisms that have been associated with cross-resistance between some types of anticancer agents. Thus, alisertib has potential to provide antitumor activity through a non-cross-resistant pathway. Based on laboratory studies with agents that target Aurora A kinase, it is also possible that alisertib will induce cancer cell senescence,[32] leading to a terminal outcome for cancer cells that may be initially represented by stable disease (SD) instead of immediate tumor reduction (response).

Alisertib has shown antitumor activity in the form of objective tumor responses and/or prolonged disease stabilization in a number of different tumor types, including solid tumors (SCLC, ovarian, head/neck, NSCLC, liposarcoma, colorectal, and breast) and hematological tumors (peripheral T-cell lymphoma [PTCL], multiple myeloma, follicular lymphoma, DLBCL, Burkitt's lymphoma, MCL, and acute myeloid leukaemia [AML]).

Reference Safety Information for Assessment of Expectedness of Serious Adverse Reactions

The table below contains the expected SARs for regulatory reporting purposes only along with their reported frequency. Refer to the IB for a comprehensive overview of the safety profile of alisertib, including expected nonserious adverse drug reactions (ADRs). Fatal SARs are considered unexpected and will be reported as SUSARs.

SARs Considered Expected for Safety Reporting Purposes

SARs		Number of Patients From Sponsored Clinical Trials (N=927)	
MedDRA		**All SARs**	**Life-threatening SARs**
System Organ Class	**Preferred Term**	**n (%)**	**n (%)**
Blood And Lymphatic System Disorders	Febrile Neutropenia	69 (7.4)	28 (3.0)
	Neutropenia	35 (3.8)	33 (3.6)
	Anemia	22 (2.4)	9 (1.0)
	Thrombocytopenia	16 (1.7)	13 (1.4)
	Leukopenia	10 (1.1)	9 (1.0)
	Pancytopenia	7 (0.8)	6 (0.6)
	Febrile Bone Marrow Aplasia	1 (0.1)	1 (0.1)
Gastrointestinal Disorders	Stomatitis	29 (3.1)	3 (0.3)
	Diarrhea	20 (2.2)	N/A
	Vomiting	8 (0.9)	N/A
	Nausea	5 (0.5)	N/A
	Aphthous Ulcer	3 (0.3)	N/A
	Dysphagia	3 (0.3)	N/A
	Abdominal Pain	2 (0.2)	N/A
Infections And Infestations	Pneumonia	14 (1.5)	2 (0.2)
	Sepsis	5 (0.5)	3 (0.3)
	Lung Infection	2 (0.2)	N/A
	Septic Shock	2 (0.2)	1 (0.1)
	Urinary Tract Infection	2 (0.2)	N/A
General Disorders And Administration Site Conditions	Pyrexia	7 (0.8)	N/A
	Fatigue	6 (0.6)	N/A
	Asthenia	4 (0.4)	N/A
Metabolism And Nutrition Disorders	Dehydration	10 (1.1)	N/A
	Hypokalemia	4 (0.4)	2 (0.2)
Investigations	White Blood Cell Count Decreased	4 (0.4)	2 (0.2)
	Neutrophil Count Decreased	3 (0.3)	2 (0.2)

SARs		Number of Patients From Sponsored Clinical Trials (N=927)	
MedDRA		**All SARs**	**Life-threatening SARs**
System Organ Class	**Preferred Term**	**n (%)**	**n (%)**
	Platelet Count Decreased	2 (0.2)	N/A
Nervous System Disorders	Somnolence	3 (0.3)	N/A
	Balance Disorder	1 (0.1)	N/A
	Depressed Level Of Consciousness	1 (0.1)	N/A
	Dizziness	1 (0.1)	N/A
	Sedation	1 (0.1)	N/A
Respiratory, Thoracic And Mediastinal Disorders	Dyspnea	3 (0.3)	N/A
Skin And Subcutaneous Tissue Disorders	Acute Generalized Exanthematous Pustulosis	2 (0.2)	N/A
	Palmar-Plantar Erythrodysaesthesia Syndrome	2 (0.2)	N/A
	Dermatitis Bullous	1 (0.1)	1 (0.1)
	Rash	1 (0.1)	N/A
Hepatobiliary Disorders	Hyperbilirubinemia	2 (0.2)	N/A
Psychiatric Disorders	Confusional State	3 (0.3)	N/A
Renal And Urinary Disorders	Acute Kidney Injury	4 (0.4)	N/A

Source: Clinical Database (cutoff date: 29 March 2016: with correction 29 March 2019).
MedDRA: Medical Dictionary of Regulatory Activities; n: number of subjects in the group; N: total number of subjects; SAR: serious adverse reactions.
Note: System Organ Class and Preferred Term are coded by MedDRA V21.0. Studies included in the analyses: C14001 - C14007, C14010, C14012 - C14014, C14017, C14019, TB-MA010030, TB-MA010033.

1.6 Pembrolizumab

1.6.1 Scientific Background

Pembrolizumab has been evaluated for the treatment of HNSCC as outlined in section 1.1. In KEYNOTE 012[22] patients with recurrent or metastatic HNSCC with no prior immunotherapy were treated with pembrolizumab at 200mg IV weekly with an overall response rate of 18%.[22] Long term follow up demonstrated that 85% of responses lasted ≥6 months and the overall survival at 12 months was 38%.[26] Pembrolizumab was approved on June 10, 2019 by the FDA in combination with chemotherapy for all recurrent and metastatic HNSCC patients and as a single agent for those whose tumors express PD L1 (CPS ≥1) as front line therapy based on data from KEYNOTE 048.[33] Pembrolizumab alone was non-inferior to chemotherapy plus cetuximab in the total population and resulted in an improved survival in those with CPS ≥1. The response rates to pembrolizumab alone were 17%, 19%, and 23% in the overall population, CPS ≥1, and CPS ≥20 populations respectively.[33]

1.6.2 Nonclinical Pharmacology

Pembrolizumab is a potent humanized immunoglobulin G4 monoclonal antibody with high specificity of binding to the PD-1 receptor, thus inhibiting its interaction with PD-L1 and programmed cell death ligand 2 (PD-L2). Based on preclinical in vitro data, pembrolizumab has high affinity and potent receptor blocking activity for PD-1.

1.6.3 Nonclinical Toxicity

Pembrolizumab has an acceptable preclinical safety profile. For summary of preclinical study data for pembrolizumab, refer to Keytruda ® IB.

1.6.4 Clinical Pharmacokinetics and Pharmacodynamics

Pharmacokinetics and pharmacodynamics of pembrolizumab will not be evaluated in this study.

The planned dose of pembrolizumab for this study is 200 mg Q3 weeks. Based on the totality of data generated in the Keytruda development program, 200 mg Q3 weeks is the appropriate dose of pembrolizumab for adults across all indications and regardless of tumor type. As outlined below, this dose is justified by:

- Clinical data from 8 randomized studies demonstrating flat dose- and exposure efficacy relationships from 2 mg/kg Q3 weeks to 10 mg/kg every 2 weeks (Q2 weeks),

- Clinical data showing meaningful improvement in benefit-risk including overall survival at 200 mg Q3 weeks across multiple indications, and
- Pharmacology data showing full target saturation in both systemic circulation (inferred from pharmacokinetic [PK] data) and tumor (inferred from physiologically based PK [PBPK] analysis) at 200 mg Q3 weeks.

Among the 8 randomized dose-comparison studies, 2262 participants were enrolled with melanoma and non-small cell lung cancer (NSCLC), covering different disease settings (treatment naïve, previously treated, PD-L1 enriched, and all-comers) and different treatment settings (monotherapy and in combination with chemotherapy). Five studies compared 2 mg/kg Q3 weeks versus 10 mg/kg Q3 weeks (KN001 Cohort B2, KN001 Cohort D, KN002, KN010, and KN021), and 3 studies compared 10 mg/kg Q3 weeks versus 10 mg/kg Q2 weeks (KN001 Cohort B3, KN001 Cohort F2 and KN006). All of these studies demonstrated flat dose- and exposure-response relationships across the doses studied representing an approximate 5- to 7.5-fold difference in exposure. The 2 mg/kg (or 200 mg fixed-dose) Q3 weeks provided similar responses to the highest doses studied. Subsequently, flat dose-exposure response relationships were also observed in other tumor types including head and neck cancer, bladder cancer, gastric cancer and classical Hodgkin Lymphoma, confirming 200 mg Q3 weeks as the appropriate dose independent of the tumor type. These findings are consistent with the mechanism of action of pembrolizumab, which acts by interaction with immune cells, and not via direct binding to cancer cells.

Additionally, pharmacology data clearly show target saturation at 200 mg Q3 weeks. First, PK data in KN001 evaluating target-mediated drug disposition conclusively demonstrated saturation of PD-1 in systemic circulation at doses much lower than 200 mg Q3 weeks. Second, a PBPK analysis was conducted to predict tumor PD-1 saturation over a wide range of tumor penetration and PD-1 expression. This evaluation concluded that pembrolizumab at 200 mg Q3 weeks achieves full PD-1 saturation in both blood and tumor.

Finally, population PK analysis of pembrolizumab, which characterized the influence of body weight and other participant covariates on exposure, has shown that the fixed-dosing provides similar control of PK variability as weight-based dosing, with considerable overlap in the distribution of exposures from the 200 mg Q3 weeks fixed dose and 2 mg/kg Q3 weeks dose.

1.6.5 Clinical Experience

Pembrolizumab is in clinical development as an intravenous (IV) immunotherapy for advanced malignancies. Keytruda® (pembrolizumab) is indicated for the treatment of

patients across several indications. For more details on specific indications refer to the Keytruda ® IB. For summary of ongoing clinical study data, refer to Keytruda ® IB.

1.6.6 Potential Risks of pembrolizumab

For comprehensive list of risks associated with pembrolizumab, please refer to Keytruda ® IB. Also see Adverse Events management in Section 7.5.

1.7 Study rationale and selection of drug doses

Aurora kinases are serine/threonine mitotic kinases that control centrosome maturation, spindle assembly, and chromosome segregation. Cancers with *RB1* loss are hyper dependent upon Aurora kinases for survival.[34, 35] Inhibitors of Aurora A or B selectively killed *RB1* null cancer cells.[35-37] Inactivation of the Rb/E2F pathway can lead to a hyperactivated spindle assembly checkpoint, replication stress, fork stalling, and double stranded DNA breaks.[38-40] These defects may underlie the dependence of Rb-deficient cancer cells on Aurora kinase for mitotic progression and escape from mitotic catastrophe.

The Rb pathway is altered in a significant number of HNSCCs. HPV+ cancer cells express the viral oncoprotein E7 that leads to Rb protein degradation. In the Cancer Genome Atlas (TCGA), *RB1* loss of function (LOF) occurs an about 4% of HPV negative and 6% of HPV positive HNSCC by homozygous deletion or mutation. This LOF was associated with decreased *RB1* gene expression.[17] Analysis of an independent set of 484 oral and oropharynx squamous cell carcinomas demonstrated that approximately 6% of them harbored deleterious single nucleotide variants (SNVs) in *RB1*. Additionally, deletions of the genetic locus were discovered in 34% of the HPV+ tumors.[21] Deletion or LOF mutations of *CDKN2A* (p16) occurs in about 57% of HPV negative HNSCC[17] and may lead to the loss of Rb function via activation of CDK4/6.[41]

We hypothesize that Rb-deficient cancers rely on mitotic kinases such as Aurora to maintain mitotic fidelity such that their inhibition leads to irreversible mitotic arrest, DNA damage, and apoptosis. Furthermore, we hypothesize that the resulting DNA damage will stimulate the cGAS/STING pathway, producing type I interferons, and that the resulting immunogenic cell death (ICD) will lead to host T cell engagement and increased sensitivity to ICT. This second hypothesis is crucial because cancers nearly always develop resistance to even highly effective targeted therapies, limiting their long-term benefits. In contrast, ICT results in durable responses in some patients, making it imperative to seek strategies that enhance the efficacy of ICT.

If Aurora kinase inhibition leads to ICD in Rb deficient cancers, then we expect to find that the combination of pembrolizumab and alisertib has a higher response rate and longer PFS than pembrolizumab alone. The addition of Aurora kinase inhibitors to ICT may reverse resistance in refractory patients. We will test baseline PD-L1 and tumor infiltrating lymphocytes (TIL) function because they correlate with responses to ICT.[22, 24-27, 42-44] Likewise, we will test the basal protein expression of Rb[34, 35] and Myc[45, 46] as both are linked to sensitivity to Aurora kinase inhibitors. Immunity developed against HPV viral proteins (in HPV+ HNSCC) may enhance recurrence-free survival of HPV^+ cancers[47] so comprehensive immune profiling of blood and tumor samples will be used to characterize TIL function, HPV reactivity, and the composition of the T cell repertoire.

In the safety (phase I) cohort, all patients will receive full dose pembrolizumab (200mg IV every 3 weeks) because this is a standard of care drug with known efficacy. Please see also above section 1.6.4. Alisertib will be started at 40 mg po BID x 7 days every 3 weeks and then escalated in subsequent cohorts as follows:

Dose Level	Alisertib Dose Enteric Coated Tablets (ECT)
-1	30 mg po BID x 7 days every 21 days
1	40 mg po BID x 7 days every 21 days
2	50 mg po BID x 7 days every 21 days

The relative bioavailability of ECT *vs*. oral solution has not been directly evaluated in a crossover fashion; however, the relative bioavailability of the oral solution formulation in reference to PIC has been determined to be 1.26, with an associated 90% CI of 1.09 – 1.47. That is, the oral solution is expected to achieve approximately 30% higher alisertib exposures than that of PIC and ECT formulation. Therefore, the dose levels for patients requiring alisertib administered as oral solution are as follows:

Dose Level	Alisertib Dose Oral Solution Formulation (5 mg/mL)
-1	21 mg po BID x 7 days every 21 days (4.2 mL)
1	28 mg po BID x 7 days every 21 days (5.6 mL)
2	35 mg po BID x 7 days every 21 days (7.0 mL)

2. STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of this study is to:

- Phase I: To determine the recommend phase II dose of the combination of alisertib and pembrolizumab.
- Phase II: To determine the overall response rate (ORR) of patients with immunotherapy-refractory recurrent or metastatic Rb-deficient head and neck squamous cell carcinoma (HNSCC) treated with the combination of pembrolizumab and alisertib.

2.2 Secondary Objectives

The secondary objectives of this study are to:

- To evaluate the safety of the combination of pembrolizumab and alisertib in patients with solid tumors.
- To determine the overall survival and progression free survival (PFS) in HNSCC patients treated with the combination of pembrolizumab and alisertib.
- To determine the relationship between pharmacokinetics, pharmacodynamics, baseline immune and tumor biomarkers and clinical responses in patients treated with alisertib and pembrolizumab.
- To determine correlations between clinical responses and the effect of the treatment on human papilloma virus (HPV)-reactive T cells in HPV+ cancers.
- To determine correlations between clinical responses and tumor infiltrating lymphocyte function and T cell repertoire.

3. INVESTIGATIONAL PLAN

This is an investigator-initiated study. The principal investigator is Faye Johnson. MD Anderson is the IND Sponsor.

3.1 Overall Design and Plan of the Study

This is an open label, phase I-II study of αPD-1 (pembrolizumab) and Aurora kinase (alisertib) inhibition in patients with recurrent or metastatic cancer.

In the phase I portion, we will enroll and treat patients in cohorts of size 2 with a target toxicity rate for the MTD of 0.3 (see statistical design). Patients will receive full dose αPD-1 antibody pembrolizumab (200mg IV every 3 weeks) in combination with the Aurora A kinase inhibitor alisertib. Alisertib will be started at 40 mg po BID x 7 days every 3 weeks and then escalated in subsequent cohorts as follows:

Dose Level	Alisertib Dose
-1	30 mg po BID x 7 days every 21 days
1	40 mg po BID x 7 days every 21 days
2	50 mg po BID x 7 days every 21 days

The dose levels for patients requiring alisertib administered as oral solution are as follows (see section 1.7 for dose conversion details):

Dose Level	Alisertib Dose Oral Solution Formulation (5 mg/mL)
-1	21 mg po BID x 7 days every 21 days (4.2 mL)
1	28 mg po BID x 7 days every 21 days (5.6 mL)
2	35 mg po BID x 7 days every 21 days (7.0 mL)

In the phase II study, patients with Rb-deficient HNSCC with recurrent/metastatic disease who are immunotherapy-refractory will be treated with the combination. For HPV negative HNSCC, those tumors with *RB1* loss of function mutation, *RB1* homozygous deletion, or *CDKN2A* deletion or *CDKN2A* LOF mutations will be tested for Rb loss with Rb IHC.[41, 48] Tumors will be measured every 6 weeks and evaluated using the Response Evaluation Criteria In Solid Tumors (RECIST) 1.1 guidelines.[1]

In both the phase I and phase II portions of the study, patients will receive study drugs until progression, unacceptable toxicity, or withdrawal of consent. Tumors will be measured every 6 weeks and evaluated using the Response Evaluation Criteria In Solid Tumors (RECIST) 1.1 guidelines.[1] Patients who remain on study for ≥ 10 cycles without progression may opt for tumor measurements every 3 cycles rather than every 2 cycles. To confirm adequate drug exposure and target inhibition and based on findings from prior studies[2, 3] we will draw blood for pharmacokinetic (PK) analysis of alisertib in the first 12 patients treated at the recommended phase II dose on days 1 and 7 of course 1 at 1, 2, 3, 8, and 10 h after the morning dose of alisertib and calculate C_{max}, T ½ , and AUC values.

We will obtain optional tumor biopsies at baseline and at 6 weeks in patients in phase II portion of the study after the first interim analysis (i.e., if the efficacy of the combination passes the futility metric after 14 patients are treated). We will obtain blood every 6 weeks in all patients on the phase II study for correlative studies described in section 5.5.

3.2 Selection of Patients

The total number of patients to be enrolled on this study is 24-40.

Enrollment is defined as the first day of alisertib treatment (i.e., Day 1 of Cycle 1).

3.2.1 Inclusion Criteria

Inclusion Criteria – phase II only

1. Histologically or cytological confirmed diagnosis of Rb-deficient HNSCC for which no standard curative therapy is available.
2. Rb deficient HNSCC includes CLIA-certified testing confirming one of the following:
   A. HPV positive as determined by any one of the following: p16 immunohistochemistry (IHC), HPV RNA in situ hybridization (ISH), RNAscope (mRNA ISH), DNA ISH, DNA PCR, or qRT PCR.
   B. No Rb protein expression in the tumor as determined by IHC.[41, 48]
3. Progression on prior treatment with an anti-PD-1 antibody or an anti-PD-L1 antibody.
   A. Has received at least 2 doses of a PD-1/PD-L1 checkpoint blockade therapy.
   B. Clinical or radiographical progression has been documented within 12 weeks from the last dose of PD-1/PD-L1 checkpoint blockade therapy.

Inclusion Criteria – phase I only

1. Histologically or cytological confirmed diagnosis of an invasive solid tumor malignancy, for which no standard curative or life prolonging therapy is available.

Inclusion Criteria – both phase I and phase II

1. Male or female patients ≥ 18 years of age.
2. ECOG performance status of ≤ 2 (see section 7.4).
3. Clinical laboratory values as specified below within 22 days before the first dose of study drug
   - Absolute neutrophil count (ANC) > 1500/mm³
   - Platelets > 100,000/mm³
   - Hgb > 9 g/dL. Values must be obtained without need for myeloid growth factor or platelet transfusion support within 14 days, however, erythrocyte growth factor is allowed as per published ASCO guidelines.
   - Total bilirubin ≤ 1.5 x upper limit of normal (ULN)

- SGOT (AST) and SGPT (ALT)< 2.5 x ULN. AST and/or ALT may be up to 5X ULN if with known liver mets or total 3 x ULN with direct bilirubin ≤ ULN in patients with well-documented Gilbert syndrome.
- Adequate renal function as defined by calculated creatinine clearance ≥30 ml/min (Cockroft-Gault Formula, see Section 7.5).

4. Measurable disease according to RECIST version 1.1.
5. Voluntary written consent must be given before performance of any study related procedure not part of standard of care, with the understanding that consent may be withdrawn by the patient at any time without prejudice to future medical care.
6. Patients must be able to either swallow alisertib enteric coated tablets, swallow alisertib oral solution formulation, or administer alisertib oral solution formulation via a feeding tube that terminates in the stomach.
7. Willing to provide blood and tissue for correlative research purposes.
8. Female patients who:
   - Are postmenopausal for at least 1 year before the screening visit, OR
   - Are surgically sterile, OR
   - If they are of childbearing potential, agree to practice 1 highly effective method of contraception and 1 additional effective (barrier) method at the same time, from the time of signing the informed consent through 120 days after the last dose of study drug, OR
   - Agree to practice true abstinence, when this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence [eg, calendar, ovulation, symptothermal, postovulation methods], withdrawal, spermicides only, and lactational amenorrhea are not acceptable methods of contraception. Female and male condoms should not be used together.)
9. Male patients, even if surgically sterilized (i.e., status postvasectomy), who:
   - Agree to practice effective barrier contraception during the entire study treatment period and through 120 after the last dose of study drug, OR
   - Agree to practice true abstinence, when this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence [e.g., calendar, ovulation, symptothermal, postovulation methods], withdrawal, spermicides only, and lactational amenorrhea are not acceptable methods of contraception. Female and male condoms should not be used together.)

3.2.2 Exclusion Criteria

1. Radiation therapy to more than 25% of the bone marrow. Whole pelvic radiation is considered to be over 25%.
2. Prior allogeneic bone marrow or organ transplantation.

3. Known gastrointestinal (GI) disease or GI procedures that could interfere with the oral absorption or tolerance of alisertib. Examples include, but are not limited to partial gastrectomy, history of small intestine surgery, and celiac disease.
4. Inability to swallow (or use a feeding tube to administer) oral medication or inability or unwillingness to comply with the administration requirements related to alisertib.
5. Known history of uncontrolled sleep apnea syndrome and other conditions that could result in excessive daytime sleepiness, such as severe chronic obstructive pulmonary disease; requirement for supplemental oxygen.
6. Requirement for constant administration of proton pump inhibitor, H2 antagonist, or pancreatic enzymes throughout the study. The intermittent use of H2-antagonists and antacids (including carafate) is only allowed within these guidelines:
    a. H2 antagonists until D-1 and after the dosing of alisertib is done
    b. Antacid formulations until 2 hours before doing and after 2 hours following dosing.
    c. PPI is allowed until D-5 of first alisertib dose. PPIs are prohibited throughout the study
7. Myocardial infarction within 6 months prior to enrollment or has New York Heart Association (NYHA) Class III or IV heart failure (see Section 7.3), uncontrolled angina, severe uncontrolled ventricular arrhythmias, or electrocardiographic evidence of acute ischemia or active conduction system abnormalities. Prior to study entry, any ECG abnormality at Screening has to be documented by the investigator as not medically relevant.
8. Female subject who is pregnant or breast-feeding. Confirmation that the subject is not pregnant must be established by a negative urine or serum β-human chorionic gonadotropin (β-hCG) pregnancy test result obtained during screening. Pregnancy testing is not required for post-menopausal or surgically sterilized women.
9. Female patient who intend to donate eggs (ova) during the course of this study or 120 days after receiving their last dose of study drug(s).
10. Male patients who intend to donate sperm during the course of this study or 120 days after receiving their last dose of study drug(s).
11. Other severe acute or chronic medical or psychiatric condition, including uncontrolled diabetes, malabsorption, resection of the pancreas or upper small bowel, requirement for pancreatic enzymes, any condition that would modify small bowel absorption of oral medications, or laboratory abnormality that may increase the risk associated with study participation or investigational product administration or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the patient inappropriate for enrollment in this study.
12. Diagnosed or treated for another invasive malignancy within 2 years of enrollment, with the exception of complete resection of basal cell carcinoma or squamous cell carcinoma of the skin, an *in situ* malignancy, or low-risk prostate cancer after curative therapy.
13. Has received any anti-cancer treatment including investigational agents within 21 days prior to first dose of alisertib.

14. Patients who receive gamma knife radiosurgery for brain metastases or whole brain radiation are eligible if gamma knife radiosurgery was completed > 2 weeks before treatment is started or whole brain radiation was performed > 4 weeks before treatment is started, and are clinically stable (not requiring steroids or anti-epileptic drugs).
15. Known hypersensitivity to any of the excipients of alisertib enteric coated tablets or severe reaction to any human monoclonal antibody.
16. Patients with a prior history of clinically significant metabolic acidosis (exclusion only for patients receiving alisertib oral solution).
17. Major surgery within 28 days prior to first dose of alisertib or persisting side effects that have not improved to NCI-CTCAE grade 1 or better.
18. Patients who are on (or will require) prolonged systemic corticosteroid treatment during the study except for replacement dosing for adrenal insufficiency.
19. Concurrent severe and/or uncontrolled medical conditions that would, in the investigator's judgment, contraindicate patient participation in the clinical study or require concomitant anti-cancer drugs (e.g. active or uncontrolled severe infection, chronic active hepatitis, immuno-compromised, acute or chronic pancreatitis, uncontrolled high blood pressure, interstitial lung disease).
20. Patients with active, known, diagnosed or suspected autoimmune disease. Patients suffering from vitiligo, type I diabetes mellitus, residual hypothyroidism due to autoimmune thyroiditis only requiring thyroid hormone replacement therapy, or psoriasis not requiring systemic treatment can be enrolled.
21. Patients diagnosed with active interstitial lung disease (ILD)/pneumonitis or a history of ILD/pneumonitis or another condition requiring immunosuppressive doses of systemic medication such as systemic corticosteroids or absorbed topical corticosteroids (doses ≥ 10 mg/day prednisone or equivalent) or other immunosuppressive medications within 14 days of study drug administration. Inhaled or topical corticosteroids, adrenal replacement doses, or < 10 mg daily prednisone or equivalent are permitted.
22. Administration of any live vaccine within 30 days before first dose of study drug.
23. Uncontrolled infection with human immunodeficiency virus (HIV), hepatitis B virus or hepatitis C virus, except for: Patients with HIV who have controlled infection (undetectable viral load and CD4 count above 350 cells/mm3 either spontaneously or on a stable antiviral regimen) are permitted; Patients with hepatitis B (HepBsAg+) virus who have controlled infection (serum hepatitis B virus DNA PCR that is below the limit of detection AND receiving antiviral therapy for hepatitis B) are permitted; Patients who are hepatitis C virus antibody positive (HCV Ab +) who have controlled infection (undetectable HCV RNA by PCR either spontaneously or in response to a successful prior course of anti-HCV therapy) are permitted.
24. Participating in another therapeutic clinical trial.
25. Prior immune-related adverse events (irAE) as follows:
    A. Any life-threatening irAE will <u>not</u> be eligible.
    B. Any grade irAE of the following types will <u>not</u> be eligible:
        i. Nervous system irAE
        ii. Ocular irAE
        iii. Cardiovascular irAE
        iv. Severe Cutaneous Adverse Reactions (SCAR)

v. Hematological irAE

C. Any grade endocrine irAE will be eligible if replacement therapy can compensate for the resulting deficit.
D. Grade ≥ 2 irAE of the following will not be eligible:
   i. Colitis
   ii. Hepatitis
   iii. Bullous Dermatoses
   iv. Pneumonitis
   v. Musculoskeletal
   vi. Renal
E. Grade ≥ 3 cutaneous irAE will not be eligible.
F. All irAEs must have resolved to Grade ≤ 1 at least 14 days before the planned first dose.

3.3 Study Treatments

3.3.1 Clinical Trial Materials Note:

Alisertib drug product is supplied as the ECT dosage form in 10 mg strength, with dose strength expressed as the milligrams of active drug (free acid). The key formulation excipients of the alisertib tablet formulation that aid in the in vivo absorption of the drug are the buffer (sodium bicarbonate), the surfactant (sodium lauryl sulfate), and the enteric coating. Alisertib ECT must be swallowed whole can be administered with or without food.

Alisertib drug product can be supplied as the oral solution dosage form at 5 mg/mL, with dose strength expressed as the milligrams of active drug (free acid) per milliliter of solution. The key formulation excipient of the alisertib oral solution formulation that aids in the in vivo absorption of the drug is the buffer (sodium bicarbonate).

The oral solution formulation of the drug must be given on an empty stomach, at least one hour before and two hours after food or drink except water.

Pembrolizumab is supplied as pembrolizumab 100 mg/4 mL vials (25 mg/ml) solution for IV infusion.

3.3.2 Preparation, Handling, and Storage of Drugs

Alisertib ECT are packaged in a 60-cc high-density polyethylene (HDPE) bottle with a rayon coil, induction seal, desiccant packs, and a polypropylene child-resistant cap.

Tablets should remain in the bottle provided until use. The container should be stored at the investigative site at controlled room temperature (20-25°C; 68-77°F; excursions are

permitted from 15-30C; 59-86F) and used before the retest expiry date provided by Puma Biotechnology. Containers should be kept closed during storage

The packaged and labeled study drug, alisertib oral solution, will be provided by Puma Biotechnology and will be handled at the investigative site as open-label material. The labels on the study drug will fulfill all requirements specified by governing regulations. Fifty-five milliliters of oral solution are packaged into each 2 oz amber HDPE bottle. Alisertib will be supplied as an oral solution at 5 mg/mL strength. The bottles will have a child-resistant cap.

The oral solution should remain in the bottle provided until use. The container should be stored at the investigative site at controlled room temperature (20-25°C; 68-77°F; excursions are permitted from 15-30C; 59-86F) and used before the retest expiry date provided by Puma Biotechnology. Containers should be kept closed during storage.

Alisertib is an anticancer drug, and as with other potentially toxic compounds, caution should be exercised when handling MLN8237.

The Pembrolizumab Package Insert contains specific instructions for pembrolizumab preparation and administration. The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study intervention received, and any discrepancies are reported and resolved before use of the study intervention.

Only participants enrolled in the study may receive study intervention, and only authorized site staff may supply or administer study intervention. All study interventions must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the investigator and authorized site staff.

The investigator, institution, or the head of the medical institution (where applicable) is responsible for study intervention accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).

For all study sites, the local country Sponsor personnel or designee will provide appropriate documentation that must be completed for drug accountability and return, or local discard and destruction if appropriate. Where local discard and destruction is appropriate, the investigator is responsible for ensuring that a local discard/destruction procedure is documented.

The study site is responsible for recording the lot number, manufacturer, and expiry date for any locally purchased product (if applicable) as per local guidelines unless otherwise instructed by the Sponsor.

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution, and usage of study interventions in accordance with the protocol and any applicable laws and regulations.

Drug Administration and Dosage Schedule

Alisertib Administration

Alisertib ECT will be given PO in a dosage of 30-50 mg BID for 7 Days (Days 1-7) of each 21-day treatment cycle (± 3 days).

Alisertib will be supplied as 10 mg ECT, with the dose strength expressed as milligrams of active drug (free acid). All tablets are to be ingested whole.

Alisertib drug product can be supplied as the oral solution dosage form at 5 mg/mL, with dose strength expressed as the milligrams of active drug (free acid) per milliliter of solution. The key formulation excipient of the alisertib oral solution formulation that aids in the in vivo absorption of the drug is the buffer (sodium bicarbonate).

Antiemetogenic agents may be administered at the discretion of the investigator.

Neutralizing antacids and calcium-containing supplements cannot be taken from 2 hours prior to alisertib dosing until up to 2 hours after dosing

Although not prohibited, the use of benzodiazepines for the prophylaxis or treatment of nausea or vomiting is discouraged because of the potential benzodiazepine-like effects of alisertib.

Study drug will be administered only to eligible patients under the supervision of the investigator or identified subinvestigator(s).

Pembrolizumab Administration

Pembrolizumab administration will consist of a total dose of 200 mg administered intravenously every 3 weeks (day 1 of each 21 day cycle ± 3 days).

3.3.3 Dose Modification and Delay

Toxicity will be evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), Version 5.0. These criteria are provided in the Study Manual and are available online at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

To manage excessive toxicity, reduction of the total alisertib dose can be done by reducing the daily dose administered and/or by interruption of the schedule treatment within a cycle.

Alisertib and pembrolizumab have very distinct toxicity profiles. If a DLT is clearly due to one drug, in the judgement of the principal investigator, then the non-offending agent may be continued without dose or schedule adjustment.

Definition of DLT

For this protocol, DLT will be defined as an adverse event at least possibly related to the study medication and meeting the following criteria.

Toxicity	DLT Definition as per NCI CTCAE version 5
Blood and lymphatic system disorders	Febrile neutropenic defined as fever ≥ 38.3 C (≥ 38 > 1 hour) with grade ≥ 3 neutropenia
	Grade ≥ 4 anemia (Life-threatening consequences with urgent intervention)
Investigations	Grade 3 neutrophil count decreased (ANC < 1.0 – 0.5 x 10^9/L) for ≥ 5 days, or Grade ≥ 4 (ANC < 0.5 x 10^9/L)
	Grade 3 platelet count decreased (PLT <50,000 – 25,000 /mm3) with any ≥ grade 3 bleeding, or Grade ≥ 4 platelet count decreased (PLT <25,000/mm3) with or without bleeding
Renal and urinary disorders	Acute kidney injury Grade ≥ 3 Acidosis Grade ≥ 3
Nervous system disorders	Somnolence Grade ≥ 3 (obtundation or stupor)

Gastrointestinal disorders	Mucositis oral Grade ≥ 3
Other non-hematologic**	Grade ≥ 3

* Any toxicities that caused a dose delay of >2 weeks of the intended next dose will also be considered dose-limiting

** Grade 3 nausea, vomiting, or diarrhea with maximal supportive treatment(s) resolving within 3 days will **not** be considered dose-limiting.

Toxicity will be evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), Version 5, effective November 27, 2017. These criteria are provided in the Study Manual and are available online at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

The NCI CTCAE is not adequate for monitoring the potential adverse effects of propylene glycol for patients receiving the oral solution. Because propylene glycol is the solvent that is used to administer some intravenous benzodiazepines, data exist for how to monitor for and manage the potential toxicities of hyperosmolarity and an anion gap metabolic acidosis in these patients. An osmolar gap >10 mmoles/L indicates that the serum propylene glycol concentration may high enough to cause toxicity.[49, 50] If patients receiving the oral solution have an osmolar gap >10 mmoles, then the medication will be held and an arterial blood gas sample will be obtained to evaluate for propylene glycol toxicity and a metabolic acidosis. The oral solution may be resumed if the blood pH is <7.3 and the propylene glycol levels are below 2 mg/dL.

To manage excessive toxicity, reduction of the total alisertib dose can be done by reducing the daily dose administered and/or by interruption of the schedule treatment within a cycle.

3.3.4 Criteria for Retreatment and Dose Delays

Treatment with alisertib will be repeated every 21 days. In order for a new cycle of therapy to begin, the patient must meet the following criteria:

- ANC must be ≥ 1,500/mm^3
- Platelet count must be ≥ 75,000/mm^3.
- In addition, all toxicities considered by the investigator to be related to therapy with alisertib must have resolved to ≤ Grade 1 or to the patient's baseline values before a new cycle of therapy may begin.

If the patient fails to meet the above-cited criteria for retreatment, then initiation of the next cycle of therapy can be delayed for up to 3 weeks. At the end of one week, the patient should be re-evaluated to determine whether the criteria for retreatment have been met. Should treatment need to be delayed for more than 1 week (i.e., a rest period of more than 7 days) because of incomplete recovery from treatment-related toxicity, the dose of alisertib will be reduced per Table 1. A second dose reduction to may occur should treatment need to be delayed for more than 1 week because of incomplete recovery from treatment-related toxicity on the reduced dosage. If a patient requires a third dose reduction or a treatment delay of more than 3 weeks at any dose, therapy with alisertib will be discontinued. However, pembrolizumab may be continued.

Table 1A Table of Dose Adjustments for alisertib ECT formulation

Dose Level[1]	Dose	Schedule	Cycle Length
-3	Discontinue or 11 mg of the oral solution[2,3]	PO BID	7 days of a 21 day cycle
-2	Discontinue or 20 mg[2]	PO BID	7 days of a 21 day cycle
-1	30 mg	PO BID	7 days of a 21 day cycle
1	40 mg	PO BID	7 days of a 21 day cycle
2	50 mg	PO BID	7 days of a 21 day cycle

[1]Starting dose defined by phase I results.

[2]Only two dose reductions are allowed. If the phase II starts at dose level -1, then dose level -3 may be needed.

[3]This dose is equivalent to 15 mg ECT but the 15mg ECT is not available.

Table 1B Table of Dose Adjustments for alisertib oral solution formulation

Dose Level[1]	Dose	Schedule	Cycle Length
-3	Discontinue or 11 mg (2.2 mL)[2]		
-2	Discontinue or 14 mg (2.8 mL)[2]	PO BID	7 days of a 21 day cycle
-1	21 mg (4.2 mL)	PO BID	7 days of a 21 day cycle
1	28 mg (5.6 mL)	PO BID	7 days of a 21 day cycle
2	35 mg (7.0 mL)	PO BID	7 days of a 21 day cycle

[1]Starting dose defined by phase I results.

[2]Only two dose reductions are allowed. If the phase II starts at dose level -1, then dose level -3 may be needed.

3.3.5 Dose Modifications for Hematological Toxicity

If a patient experiences any of the following hematological toxicities during the dosing period, dosing will be discontinued for the remainder of that cycle and the dose will be decreased 1 level for all subsequent cycles of treatment.

- Grade 4 neutropenia (ANC < 500 cells/mm^3) lasting more than 7 consecutive days
- Grade 4 thrombocytopenia (platelet count < 25,000/µL) lasting more than 7 consecutive days
- Platelet count less than 10,000/µL at any time
- Grade 3 neutropenia with fever or infection, or both, where fever is defined as an oral temperature greater than 38.5°C
- Grade 3 thrombocytopenia with clinically significant bleeding

3.3.6 Dose Modifications for Non-Hematological Toxicities

If a patient experiences any of the following toxicities during the dosing period, dosing will be discontinued for the remainder of that cycle and the dose will be decreased 1 level for all subsequent cycles of treatment, and treatment may resume after drug-related toxicities have resolved to ≤ Grade 1 or to baseline.

- Any Grade 3 nonhematological toxicity that is considered by the investigator to be related to study drug other than:
    - Grade 3 or greater nausea or emesis, or both, that occurs in the absence of optimal antiemetic therapy (5-hydroxytryptamine 3 [5-HT3] serotonin receptor antagonist);
    - Grade 3 or greater diarrhea that occurs in the absence of optimal supportive therapy with loperamide or a comparable anti-diarrheal;
    - Grade 3 fatigue that lasts less than 1 week

- Grade 2 non-hematological toxicities that are considered by the investigator to be related to study drug and in the opinion of the investigator require dose reduction.

In general, study drug treatment should be discontinued if a patient experiences a Grade 4 toxicity. If, in the opinion of the investigator and study sponsor it is in the patient's interest to continue therapy with alisertib, then after recovery from the toxicity or toxicities in question to ≤ Grade 1 or to baseline values, the dose of alisertib should be reduced by at least 1 dose level with subsequent cycles of therapy.

When a dose reduction of alisertib is required, no re-escalation of dose will be permitted. If a patient requires more than 2 dose reductions, therapy with alisertib will be discontinued.

Study-pause criteria

Any death or ≥ 2 grade 4 events not definitely related to underlying disease, progression of disease, or intercurrent illness, and at least possibly related to the investigational product will be considered as study-pause criteria.

3.3.7 Treatment Assignment

N/A (single-arm study)

3.3.8 Packaging and Labeling

The study drug, provided by Puma Biotechnology, will be labeled and handled at the investigative site as open-label material; packaging labels will fulfill all requirements specified by governing regulations. Alisertib will be supplied as ECT in 10 mg strength. The 60-cc HDPE bottles will have a child-resistant cap and be labeled for take-home use. Patients will receive instructions for home use of alisertib, including the requirement that Alisertib be administered as intact tablets.

Alisertib drug product can be supplied as the oral solution dosage form at 5 mg/mL, with dose strength expressed as the milligrams of active drug (free acid) per milliliter of solution. The key formulation excipient of the alisertib oral solution formulation that aids in the in vivo absorption of the drug is the buffer (sodium bicarbonate).

Please see Pembrolizumab Package Insert for specific instructions on handling and labeling.

3.4 Excluded Concomitant Medications and Procedures

The following medications and procedures are prohibited during the study drug administration portion of the study:

- Alternative therapy, including palliative radiotherapy or surgery, for treatment of the patient's malignancy while on treatment Note: Participants can receive palliative radiotherapy for painful bone lesions. Targeted external beam irradiation should not be used in the primary tumor field where assessment for tumor is indicated.

- Requirement for administration of any proton pump inhibitor. Use of any PPI in either continued or intermittent use will be prohibited during the conduct of the study and patients must discontinue any use of PPI within five days prior to the first dose of alisertib. Patients may be administered alternative agents to manage gastric acidity or reflux (eg, H2 receptor antagonists, antacids) with exceptions described below.

- Histamine-2 (H2) receptor antagonists are not permitted from the day prior (Day -1) through to the end of alisertib dosing (e.g., Day 7), except as required for premedication for a protocol-specific agent (eg, taxane).

- Enzyme inducers, such as the enzyme-inducing antiepileptic drugs phenytoin, carbamazepine or phenobarbital, or rifampin, rifabutin, rifapentine or St. John's wort within 14 days prior to the first dose of alisertib.

- Results from alisertib human absorption, distribution, metabolism, and excretion (ADME) study (C14014) indicate that oxidative metabolism is the predominant route of elimination for alisertib. Results of subsequent in vitro drug metabolism studies suggest that the oxidative metabolism of alisertib is mainly mediated via CYP3A4. These findings suggest that alisertib systemic exposures may be potentially increased when co-administered with clinically significant CYP3A inhibitors. The effect of itraconazole, a strong CYP3A inhibitor on alisertib PK is being assessed. Until these results become available, it is recommended that the concomitant use of moderate or strong CYP3A inhibitors or grapefruit juice/grapefruit-containing products be avoided. Selection of an alternate concomitant medication with no or minimal CYP3A inhibition potential is recommended. If co-administration of moderate or strong CYP3A inhibitors is

medically necessary, investigators should monitor for toxicities and follow the dose modifications for toxicity per study protocol

- Investigational agents other than alisertib and pembrolizumab.
- Live vaccines during drug administration portion of the study, and within 30 days of the last dose of study intervention. Examples of live vaccines include, but are not limited to, measles, mumps, rubella, chickenpox, yellow fever, intranasal seasonal influenza, rabies, BCG, and typhoid (oral).
- Anticancer hormonal therapy (e.g. androgen deprivation, androgen receptor blockade, anti-estrogens).
- Systemic glucocorticoids for any purpose other than: (1) to modulate symptoms of an AE that is suspected to have an immunologic etiology, (2) as required during and after PCI, (3) as needed for the prevention of emesis, (4) topical or ocular use, (5) premedication for IV contrast allergies, (6) for inhalation in the management of asthma or chronic obstructive pulmonary disease, (7) Replacement for adrenal insufficiency.
- In addition to the medications listed here, site staff should refer to the approval product labels for prohibited medications, as well as drug-drug interactions for each agent used in this study. Participants who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from treatment but continue in study for assessment of disease status and survival.

3.5 Permitted Concomitant Medications and Procedures

Myeloid growth factors to treat patients with neutropenia according to the American Society of Clinical Oncology (ASCO) Guidelines.[1] When applicable (eg, Phase 1 studies) use of myeloid growth factors should be avoided (if medically appropriate) in Cycle 1 until patients have developed a DLT or dose-limiting Grade 4 neutropenia. Antiemetic agents may be administered at the discretion of the investigator but are not commonly required as a prophylactic agent. All other manifestations of the patient's malignancy should be treated at the discretion of the investigator.

Antacids are permitted; however, they should be administered more than 2 hours before or 2 hours after administration of alisertib.

Medications with potential CNS effects are not prohibited in this study, but it is recommended that their use be minimized to avoid confusion in the interpretation of CNS effects should they occur during the course of treatment with alisertib. Because of alisertib's structural and pharmacological similarity to the benzodiazepines, concomitant therapy with benzodiazepines is discouraged but not prohibited.

In appropriate settings, such as combinations with agents known to produce frequent thrombocytopenia, restricted uses of anticoagulants should be considered.

All other medical conditions should be treated at the discretion of the investigator in accordance with local community standards of medical care.

3.6 Precautions and Restrictions

Patients should not drive, operate dangerous tools or machinery, or engage in any other potentially hazardous activity that requires full alertness and coordination if they experience sedation while enrolled in this study.

Patients are to be instructed to limit the use of alcohol while enrolled in this study. Patients should consume no more than 1 standard unit of alcohol per day during the study and for 30 days from the last dose of alisertib. A standard unit of alcohol is defined as a 12 oz beer (350 mL), 1.5 oz (45 mL) of 80-proof alcohol, or one 6-oz (175 mL) glass of wine.

It is not known what effects alisertib has on human pregnancy or development of the embryo or fetus. Therefore, male patients should avoid impregnating a female partner.

Male patients, even if surgically sterilized (i.e., status post-vasectomy) must agree to one of the following:

Practice effective barrier contraception during the entire study treatment period and through 120 days after the last dose of study drug, or completely abstain from heterosexual intercourse.

3.7 Management of Clinical Events

3.7.1 Nausea and Vomiting

Prophylactic antiemetic therapy will not be used in this study unless it becomes clear that alisertib causes acute nausea and vomiting. If prophylactic antiemetic therapy is needed, 5-HT_3 receptor antagonists (without corticosteroids) should be tried first. Because of the potential of benzodiazepines to cause sedation, the use of benzodiazepines for antiemetic prophylaxis should be reserved for patients who cannot be satisfactorily managed otherwise.

Although this study will not initially employ prophylactic antiemetics, there is no prohibition against antiemetic use in the management of a patient who develops nausea or vomiting, or both.

3.7.2 Diarrhea

Antidiarrheal medications will not be used prophylactically; however, patients will be instructed to take loperamide, 4 mg, at the occurrence of the first loose stool and then 2 mg every 2 hours until they are diarrhea-free for at least 12 hours. During the night, patients may take 4 mg of loperamide every 4 hours. Fluid intake should be maintained to avoid dehydration.

3.7.3 Central Nervous System Effects

If a patient experiences excessive sedation believed to be related to alisertib, treatment with alisertib should be interrupted. Patients whose sedation is not considered immediately life-threatening should be carefully monitored and given appropriate supportive care.

3.7.4 Management of New or Worsening Pulmonary Symptoms

If new or worsening pulmonary symptoms (eg, dyspnea) or radiological abnormalities occur in the absence of a clear diagnosis, an interruption in study intervention dosing is recommended and further diagnostic workup (including a highresolution computed tomography [CT] scan) should be performed to exclude pneumonitis. Following investigation, if no evidence of abnormality is observed on CT imaging and symptoms resolve, then study intervention can be restarted, if deemed appropriate by the investigator. If significant pulmonary abnormalities are identified, these need to be discussed with the Principal Investigator.

3.7.5 Treatment Compliance

All drug(s) will be administered to eligible patients under the supervision of the investigator or identified sub-investigator(s). The pharmacist will maintain records of drug receipt (if applicable), drug preparation, and dispensing, including the applicable lot numbers, patients' height, body weight, and body surface area (see Section 7.5), and total drug administered in milliliters and milligrams. Any discrepancy between the calculated dose and dose administered and the reason for the discrepancy must be recorded in the source documents.

3.7.6 Pembrolizumab-related Adverse Events

Adverse events associated with pembrolizumab exposure may represent an immunologic etiology. These immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than 1 body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of pembrolizumab, administration of corticosteroids and/or other supportive care. For suspected irAEs, 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 irAEs, withhold or permanently discontinue pembrolizumab and administer corticosteroids. Please see associated Section 7.6 for the management of irAEs.

Pembrolizumab may also cause severe or life-threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and general resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines on pembrolizumab are outlined in associated Section 7.6, as well.

3.8 Duration of Treatment and Patient Participation

Patients will remain on study until progression of cancer, unacceptable toxicity, or withdrawal of consent.

3.9 Termination of Treatment and/or Study Participation

Patients will be informed that they have the right to withdraw from the study at any time for any reason, without prejudice to their medical care. The investigator also has the right to withdraw patients from the study for any of the following reasons:

- Intercurrent illness
- Occurrence of an unacceptable adverse event
- A treatment cycle delay or alisertib interruption of> 2 weeks because of toxicity
- Patient request
- Protocol violations
- Non-compliance
- Administrative reasons
- Failure to return for follow-up
- General or specific changes in the patient's condition unacceptable for further treatment in the judgment of the investigator
- Progressive disease at any time

At the time of withdrawal, all study procedures outlined for the End of Study visit should be completed. The primary reason for a patient's withdrawal from the study is to be recorded in the source documents.

Patients withdrawn from the study will be continuously monitored for survival through chart review and/or phone call every 2-3 months.

3.10 Efficacy, Pharmacodynamic/Pharmacogenomic/Correlative studies, and Safety Measurements

3.10.1 Efficacy Measurements

Tumors will be measured every 6 weeks and evaluated using the Response Evaluation Criteria In Solid Tumors (RECIST) 1.1 guidelines.[1] Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with any of the protocol agents.

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ⩾20 mm (⩾2 cm) by chest x-ray or as ⩾10 mm (⩾1 cm) with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area may not be considered measurable unless there has been demonstrated progression in the lesion.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ⩾15 mm (⩾1.5 cm) in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm [0.5 cm]). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm [<1 cm] or pathological lymph nodes with ⩾10 to <15 mm [⩾1 to <1.5 cm] short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. 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, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

Response Criteria (Target Lesions)

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm (<1 cm).

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum 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 5 mm (0.5 cm). (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

Response Criteria (Non-Target Lesions)

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm [<1 cm] short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or 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. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

3.10.2 Pharmacokinetic and optional Pharmacodynamic/ Pharmacogenomic/Correlative Studies

To confirm adequate drug exposure and target inhibition and based on findings from prior studies[2, 3] we will draw blood for pharmacokinetic (PK) analysis of alisertib in the first 12 patients treated at the recommended phase II dose on days 1 and 7 of course 1 at 1, 2, 3, 8, and 10 h after the morning dose of alisertib and calculate C_{max}, T ½ , and AUC values. To measure target inhibition, pharmacodynamics (PD) studies will be done in pre- and post-treatment tumor. Specifically, we will use immunohistochemistry (IHC) to quantify the number of cancer cells that stain for phosphorylation of Histone H3 (Ser10)[5] and mitotic protein monoclonal 2 (MPM2).[6]

3.10.3 Safety Measurements

Safety parameters commonly used for evaluating investigational systemic anticancer treatments are included as safety endpoints, including, but not limited to, the incidence of, causality and outcome of AEs/SAEs; and changes in vital signs and laboratory values. Adverse events will be assessed as defined by NCI CTCAE, version 5.0.

3.11 Study Drug Administration

Alisertib will be administered PO at a dosage of 30-50 mg BID for 7 days in each treatment cycle, followed by a 14-day, treatment-free period.

Patients will be instructed to take each oral dose of alisertib with 8 ounces (1 cup, 240 mL) of water. For BID dosing, the doses must be taken at least 6 hours apart.

Alisertib will be supplied as 10 mg ECT, with the dose strength expressed as milligrams of active drug (free acid); higher strengths may be supplied depending on the observed MTD. All tablets are to be ingested whole.

Patients who have difficulty swallowing tablets will have the option to take alisertib oral solution formulation.

Alisertib drug product can be supplied as the oral solution dosage form at 5 mg/mL, with dose strength expressed as the milligrams of active drug (free acid) per milliliter of solution. The key formulation excipient of the alisertib oral solution formulation that aids in the in vivo absorption of the drug is the buffer (sodium bicarbonate).

Antiemetogenic agents may be administered at the discretion of the investigator. Although not prohibited, the use of benzodiazepines for the prophylaxis or treatment of nausea or vomiting is discouraged because of the potential benzodiazepine-like effects of alisertib.

3.12 Description of Investigational Agents

Alisertib drug product is supplied as the ECT dosage form in 10 mg strengths with dose strength expressed as the milligrams of active drug (free acid); other strengths may be supplied based on the observed MTD. The key formulation excipients of the alisertib tablet formulation that aid in the in vivo absorption of the drug are the buffer (sodium bicarbonate), the surfactant (sodium lauryl sulfate), and the enteric coating.

Alisertib drug product can be supplied as the oral solution dosage form at 5 mg/mL, with dose strength expressed as the milligrams of active drug (free acid) per milliliter of solution. The

key formulation excipient of the alisertib oral solution formulation that aids in the in vivo absorption of the drug is the buffer (sodium bicarbonate).

3.13 Preparation, Reconstitution, and Dispensation

Alisertib ECT are packaged (10 tablets to a bottle) in a 60-cc high-density polyethylene (HDPE) bottle with a child-resistant cap.

Tablets should remain in the bottle provided until use. The container should be stored at the investigative site at controlled room temperature (20-25°C; 68-77°F; excursions are permitted from 15-30C; 59-86F) and used before the retest expiry date provided by Puma Biotechnology. Containers should be kept closed during storage.

The packaged and labeled study drug, alisertib oral solution, will be provided by Puma Biotechnology and will be handled at the investigative site as open-label material. The labels on the study drug will fulfill all requirements specified by governing regulations. Fifty-five milliliters of oral solution are packaged into each 2 oz amber HDPE bottle. Alisertib will be supplied as an oral solution at 5 mg/mL strength. The bottles will have a child-resistant cap. The oral solution should remain in the bottle provided until use. The container should be stored at the investigative site at controlled room temperature (20-25°C; 68-77°F; excursions are permitted from 15-30C; 59-86F) and used before the retest expiry date provided by Puma Biotechnology. Containers should be kept closed during storage.

Alisertib is an anticancer drug, and as with other potentially toxic compounds, caution should be exercised when handling alisertib. It is recommended that gloves and protective garments be worn during preparation.

3.14 Packaging and Labeling

The packaged and labeled study drug, alisertib ECT, will be provided by Puma Biotechnology and will be handled at the investigative site as open-label material. The labels on the study drug will fulfill all requirements specified by governing regulations. Ten alisertib ECT are packaged into each 60-cc HDPE bottle. Alisertib will be supplied as ECT 10 mg strength. The bottles will have a child-resistant cap and be labeled for take-home use. Patients will receive instructions for home use of alisertib, including the requirement that alisertib be administered as intact tablets.

Alisertib drug product can be supplied as the oral solution dosage form at 5 mg/mL, with dose strength expressed as the milligrams of active drug (free acid) per milliliter of solution. The

key formulation excipient of the alisertib oral solution formulation that aids in the in vivo absorption of the drug is the buffer (sodium bicarbonate).

The packaged and labeled study drug, alisertib oral solution, will be provided by Puma Biotechnology and will be handled at the investigative site as open-label material. The labels on the study drug will fulfill all requirements specified by governing regulations. Fifty-five milliliters of oral solution are packaged into each 2 oz amber HDPE bottle. Alisertib will be supplied as an oral solution at 5 mg/mL strength. The bottles will have a child-resistant cap.

As required by local regulations, any modifications to the plan for drug supply or storage will be communicated to the investigator and detailed in the Study Manual.

3.15 Storage, Handling, and Accountability

Tablets should remain in the bottle provided until use. The container should be stored at the investigative site at controlled room temperature (20-25°C; 68-77°F; excursions are permitted from 15-30°C; 59-86°F) and used before the retest expiry date provided by Puma Biotechnology. Containers should be kept closed during storage.

The oral solution should remain in the bottle provided until use. The container should be stored at the investigative site at controlled room temperature (20-25°C; 68-77°F; excursions are permitted from 15-30C; 59-86F) and used before the retest expiry date provided by Puma Biotechnology. Containers should be kept closed during storage.

Because alisertib is an investigational agent, it should be handled with due care. In case of contact with broken tablets, raising dust should be avoided during the cleanup operation.

The product may be harmful by inhalation, ingestion, or skin absorption. Gloves and protective clothing should be worn during preparation and the cleanup operation. The area should be ventilated and the spill site washed after material pick-up is complete. The spilled material should be disposed of as hazardous medical waste in compliance with federal, state, and local regulations.

In case of contact with the powder (eg, from a broken tablet), skin should be washed immediately with soap and copious amounts of water for at least 15 minutes.

In case of contact with the eyes, copious amounts of water should be used to flush the eyes for at least 15 minutes. Medical personnel should be notified.

Patients are to be instructed on proper storage, accountability, and administration of alisertib, including that alisertib is to be taken as intact tablets.

4. ADVERSE EVENTS

4.1.1 Adverse Event Definition

Adverse event (AE) means any untoward medical occurrence in a patient or subject administered a medicinal product; the untoward medical occurrence does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product whether or not it is related to the medicinal product. This includes any newly occurring event, or a previous condition that has increased in severity or frequency since the administration of study drug.

An abnormal laboratory value will not be assessed as an AE unless that value leads to discontinuation or delay in treatment, dose modification, therapeutic intervention, or is considered by the investigator to be a clinically significant change from baseline.

4.2 Moclia database

Clinical data capture called Moclia and CORe will be the electronic database used for this study's electronic case report forms.

4.2.1 Adverse Event Attribution and Severity Definition

Attribution - the determination of whether an adverse event is related to a medical treatment or procedure.

- Definite - the adverse event is clearly related to the investigational agent(s).
- Probable - the adverse event is likely related to the investigational agent(s).
- Possible - the adverse event may be related to the investigational agent(s).
- Unlikely - The adverse event is doubtfully related to the investigational agent(s).
- Unrelated - The adverse event is clearly NOT related to the investigational agent(s).

Severity of the adverse events (AEs)

The severity of the adverse events (AEs) will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) V.5.0. Events not included in the NCI CTCAE will be scored as follows:

General grading:

- Grade 1: Mild: discomfort present with no disruption of daily activity, no treatment required beyond prophylaxis.

- Grade 2: Moderate: discomfort present with some disruption of daily activity, require treatment.

- Grade 3: Severe: discomfort that interrupts normal daily activity, not responding to first line treatment.

- Grade 4: Life Threatening: discomfort that represents immediate risk of death

The investigator (or physician designee) is responsible for verifying and providing source documentation for all adverse events and assigning the attribution for all adverse events for subjects enrolled.

Clarification should be made between a serious AE (SAE) and an AE that is considered severe in intensity (Grade 3 or 4), because the terms serious and severe are NOT synonymous. The general term *severe* is often used to describe the intensity (severity) of a specific event; the event itself, however, may be of relatively minor medical significance (such as a Grade 3 headache). This is NOT the same as *serious*, which is based on patient/event outcome or action criteria described above, and is usually associated with events that pose a threat to a patient's life or ability to function. A severe AE (Grade 3 or 4) does not necessarily need to be considered serious. For example, a white blood cell count of 1000/mm^3 to less than 2000 is considered Grade 3 (severe) but may not be considered serious. Seriousness (not intensity) serves as a guide for defining regulatory reporting obligations.

4.3 Investigator Communications with Puma Biotechnology

AEs may be spontaneously reported by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic

procedures. Any clinically relevant deterioration in laboratory assessments or other clinical finding is considered an AE. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event.

AEs which are serious must be reported to Puma Biotechnology Pharmacovigilance (or designee) from the time of patient consent up to and including *60* days after administration of the last dose of study drug. Any SAE that occurs at any time after completion of study treatment or after the designated follow-up period that the sponsor or investigator considers to be related to any study drug must be reported to Puma Biotechnology Pharmacovigilance (or designee). In addition, new primary malignancies that occur during the follow-up periods must be reported, regardless of causality to study regimen, for a minimum of three years after the last dose of the investigational product, starting from the first dose of study drug. All new cases of primary malignancy must be reported to Puma Biotechnology Pharmacovigilance (or designee).

Planned hospital admissions or surgical procedures for an illness or disease that existed before the patient was enrolled in the trial are not to be considered AEs unless the condition deteriorated in an unexpected manner during the trial (e.g., surgery was performed earlier or later than planned). All SAEs should be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

The principal investigator Dr. Faye Johnson is responsible for reporting serious adverse events (SAEs) to the sponsor's IRB.

Regardless of expectedness or causality, all SAEs must also be reported to Puma Biotechnology Pharmacovigilance or designee:

> **Fatal and Life Threatening SAEs** within 24 hours of the investigator's observation or awareness of the event
>
> **All other serious (non-fatal/non life threatening)** events within 4 calendar days of the investigator's observation or awareness of the event

The SAE report must include at minimum:

- **Event term(s)**
- **Serious criteria**

- **Intensity of the event(s):** Investigator's or sub-investigator's determination. Intensity for each SAE, including any lab abnormalities, will be determined by using the NCI CTCAE version specified in the protocol, as a guideline, whenever possible. The criteria are available online at http://ctep.cancer.gov/reporting/ctc.html.

- **Causality of the event(s):** Investigator's or sub-investigator's determination of the relationship of the event(s) to study drug administration.

Follow-up information on the SAE may be requested by Puma Biotechnology Pharmacovigilance (or designee).

Relationship to all study drugs for each SAE will be determined by the investigator or sub-investigator by responding yes or no to the question: Is there a reasonable possibility that the AE is associated with the study drug(s)?

Investigator must also provide Puma Biotechnology Pharmacovigilance with a copy of all communications with applicable regulatory authorities related to the study product(s) as soon as possible but no later than 4 calendar days of such communication.

Recommended Adverse Event Recording Guidelines

Attribution	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Unrelated	Phase I	Phase I	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III
Unlikely	Phase I	Phase I	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III
Possible	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II Phase III
Probable	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II Phase III
Definitive	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II Phase III

Puma Biotechnology Pharmacovigilance or Designee

SAE and Pregnancy Reporting Contact Information
Email: PumaSAE@parexel.com

Suggested Reporting Form:

- SAE Report Form (a sample will be provided)
- US FDA MedWatch 3500A:

http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm

- Any other form deemed appropriate by the investigator

4.4 Serious Adverse Events Reporting

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or the sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32).

- Important medical events as defined above, may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Principal Investigator or the IND Sponsor, IND Office.
- All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in "The University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Serious Unanticipated Adverse Events for Drugs and Devices".

All SAEs, expected or unexpected, must be reported to the IND Office, regardless of attribution (within 5 working days of knowledge of the event).

- **All life-threatening or fatal events,** that are unexpected, and related to the study drug, must have a written report submitted within **24 hours** (next working day) of knowledge of the event to the Safety Project Manager in the IND Office.
- Unless otherwise noted, the electronic SAE application (eSAE) will be utilized for safety reporting to the IND Office and MDACC IRB.
- Serious adverse events will be captured from the time of the first protocol-specific intervention, until 60 days after the last dose of drug or protocol specific timeline, unless the participant withdraws consent. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.
- Additionally, any serious adverse events that occur after the 60 day time period that are related to the study treatment must be reported to the IND Office. This may include the development of a secondary malignancy.

4.5 Procedures for Reporting To the FDA

- Serious adverse events will be forwarded to FDA by the IND Sponsor (Safety Project Manager IND Office) according to 21 CFR 312.32.

It is the responsibility of the PI and the research team to ensure serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor's guidelines, and Institutional Review Board policy.

4.6 Procedures for Reporting Drug Exposure During Pregnancy and Birth Events

If a woman becomes pregnant or suspects that she is pregnant while participating in this study, she must inform the investigator immediately and permanently discontinue study drug. The investigator must fax a completed Pregnancy Form to the Puma Biotechnology Pharmacovigilance or designee immediately (see Section 4.2). The pregnancy must be followed for the final pregnancy outcome (i.e., delivery, still birth, miscarriage) and Puma Biotechnology Pharmacovigilance or designee will request this information from the investigator.

If a female partner of a male patient becomes pregnant during the male patient's participation in this study, the investigator must also immediately fax a completed Pregnancy Form to the Puma Biotechnology Pharmacovigilance or designee (see Section 4.2). Every effort should be made to follow the pregnancy for the final pregnancy outcome.

4.7 Product Complaints and Medication Errors

A product complaint is a verbal, written, or electronic expression that implies dissatisfaction regarding the identity, strength, purity, quality, or stability of a drug product. Individuals who identify a potential product complaint situation should immediately contact Puma Biotechnology (see below) and report the event. Whenever possible, the associated product should be maintained in accordance with the label instructions pending further guidance from a Puma Biotechnology Quality representative.

A medication error is a preventable event that involves an identifiable patient and that leads to inappropriate medication use, which may result in patient harm. While overdoses and underdoses constitute medication errors, doses missed inadvertently by a patient do not. Individuals who identify a potential medication error situation should immediately contact Puma Biotechnology (see below) and report the event.

For Product Complaints or Medication Errors, call
1-844-MED-PUMA (1-844-633-7862)
E-mail: *Medinfo@pumabiotechnology.com*
Fax: 1-424-248-6501
Hours: Mon-Fri, 9 a.m. – 7 p.m. ET

Product complaints in and of themselves are not AEs. If a product complaint results in an SAE, an SAE form should be completed and sent to Puma Biotechnology Pharmacovigilance (refer to Section 4.2).

5. STATISTICAL PROCEDURES

5.1 Study Design and Overview of Primary and Secondary Endpoints

Bayesian optimal interval design will be applied for the phase I dose escalation part of the study.[7-9] The target toxicity rate for the MTD is 0.3. The maximum sample size is 12. We will enroll and treat patients in cohorts of size 2 starting from Dose Level 1. To guide dose-escalation decisions, if the observed dose-limiting toxicity (DLT) rate at the current dose is $\leqslant 0.236$, the next cohort of patients will be treated at the next higher dose level; if it is $\geqslant 0.359$, the next cohort of patients will be treated at the next lower dose level; otherwise the next cohort of patients will be treated at the same dose. In addition, For the purpose of overdose control, doses j and higher levels will be eliminated from further examination if $\Pr(p_j > 0.3 \mid \text{data}) > 0.95$, where p_j is the true DLT rate of dose level $j, j = 1,\cdots, 3$. This posterior probability is evaluated based on the beta-binomial model $y_j \mid p_j \sim \text{binomial}(p_j)$ with $p_j \sim \text{uniform}(0,1)$, where y_j is the number of patients experienced DLT at dose level j. When the lowest dose is eliminated, stop the trial for safety. The probability cutoff 0.95 is chosen to be consistent with the common practice that when the target DLT rate <=1/6, a dose with 2/3 patients experienced DLT is eliminated.

In a cohort of 2, if no subject has a DLT, then we can proceed to the next higher dose. If $\geqslant$ 1 subjects in a cohort of 2 has a DLT then the rate is $\geqslant 0.359$ and we will enroll at the next lower dose. In a cohort of 4, if no subject has a DLT, then we can proceed to the next higher dose. One subject with a DLT would lead to the next cohort being treated at the same dose and $\geqslant 2$ subjects with a DLT would lead to the next cohort being treated at the lower dose level. The detailed dose escalation / de-escalation rule is given in Table 2.

Table 2. Dose escalation/deescalation rule for the BOIN design

	1	2	3	4	5	6	7	8	9	10	11	12
Number of patients treated at current dose	1	2	3	4	5	6	7	8	9	10	11	12
Escalate if # of DLT <=	0	0	0	0	1	1	1	1	2	2	2	2
Deescalate if # of DLT >=	1	1	2	2	2	3	3	3	4	4	4	5
Eliminate if # of DLT >=	NA	NA	3	3	4	4	5	5	5	6	6	7

Note: # of DLT is the number of patients with at least 1 DLT. When none of the actions (i.e., escalate, de-escalate or eliminate) is triggered, stay at the current dose for treating the next cohort of patients.

We have performed simulation studies and the design will have at least 60% probability selecting the correct dose under various scenarios ranging from low to high toxicity profiles. The operating characteristics in three scenarios are given in Table 3.

Table 3. Operating characteristics of the BOIN design

	Dose Level				
	-1	1	2	Number of Patients	% Early Stopping
Scenario 1					
True DLT Rate	0.05	0.15	**0.3**		
Selection %	2.4	35.6	**62**		0
% Pts Treated	7.3	42.5	**50.2**	12	
Scenario 2					
True DLT Rate	0.1	**0.3**	0.5		
Selection %	22.2	**60.5**	16.9		0.4
% Pts Treated	24.5	**52.3**	23.2	12	
Scenario 3					
True DLT Rate	**0.3**	0.5	0.7		
Selection %	**64**	23.9	1		11.1
% Pts Treated	**52.4**	40.7	6.9	11.5	

For the phase I, any DLT (defined in section 3.3.3) that occurs during the first two cycles (first six weeks) of therapy in the phase I portion of this study will be used to make decisions about proceeding to the subsequent cohort."

In the phase II cohort of immunotherapy-refractory patients, any responses will be of interest. We will use the Bayesian Optimal Phase 2 (BOP2) design.[10] The assumptions are that the null response rate is 5% and the target response rate is 20%. Fourteen patients will be enrolled initially. If there are no responses, then the trial will be terminated due to lack of efficacy. If any responses are observed, an additional 14 patients will be enrolled. The treatment is considered efficacious if there are at least 4 responders in 28 patients and inefficacious otherwise. The design will have 82% power if the response rate is at least 0.2 with a one-sided 5% type I error. The operating characteristics are shown below in Table 4 using the BOP2 web application (BOP2 V1.4.11.0), which is available at http://www.trialdesign.org.

Table 4: Operating characteristics

Scenario	Response Rate	Early Stopping (%)	Claim Promising (%)	Average Sample Size
1	0.05	48.77	4.70	21.2

2	0.20	4.40	82.65	27.4

If patients withdraw consent or drop out for other reasons unrelated to safety or disease progression, they may be replaced. To account for patients who may not be evaluable for the response rate, we will enroll 30 patients in the Phase II part to reach 28 evaluable patients.

To ensure that patients treated at the Phase II part of the study do not experienced excessive toxicity, we will implement the Bayesian toxicity monitoring rule. We will suspend the accrual if excessive toxicity defined as Probability (DLT > 0.3) > 0.65. Assuming the prior of Prob(DLT) follows a prior distribution of Beta(0.3, 0.7), we will suspend accrual if we observe the followings (# of DLT / # of patients): 3/(5-7). 4/(8-10), 5/(11-13), 6/(14-17), 7/(18-20), 8/(21-23), 9/(24-26), 10/(27-29), 11/(30-32).

The operating characteristics are given in Table 5.

Table 5: Operating Characteristics of the Bayesian Toxicity Monitoring Rule.

Scenario	Prob.Of.DLT	Prob.Early.Stop	Prob.Declare.Tox	Avg.N.Patients
1	0.1	0.0326	0.0326	34.1004
2	0.2	0.2433	0.2444	28.8290
3	0.3	0.6337	0.6402	19.5662
4	0.4	0.9137	0.9196	11.6466

5.2 Randomization and Stratification Factors

None.

5.3 Evaluation of Efficacy

See sections 5.1 and 3.10.1

5.4 Evaluation of Safety

See Section 3.10.3.

5.5 Correlative Studies

In HNSCC patients treated with single agent αPD1 therapy, PD-L1 protein expression measured by IHC and scored using the percentage of tumor cells with membranous PD-L1 expression or CPS correlated with response to therapy.[22, 24-27, 42, 43] In multiple pre-clinical cancer models, *RB1* loss or Myc overexpression[51] correlated with sensitivity to Aurora kinase inhibition, although *RB1* was clearly a stronger predictor.[34, 35, 45, 46] To examine potential predictors of therapeutic efficacy, PD-L1, N-Myc C-Myc, L-Myc, and Rb protein levels will be determined by IHC using automated immunostaining. We will calculate the number of tumor cells with Rb, Myc and membranous PD-L1 expression as well as the CPS.

To measure target inhibition, pharmacodynamics studies will be done in pre- and post-treatment tumor. Specifically, we will use IHC to quantify the number of cancer cells that stain for phosphorylation of Histone H3 (Ser10) [5] and MPM 2.[6]

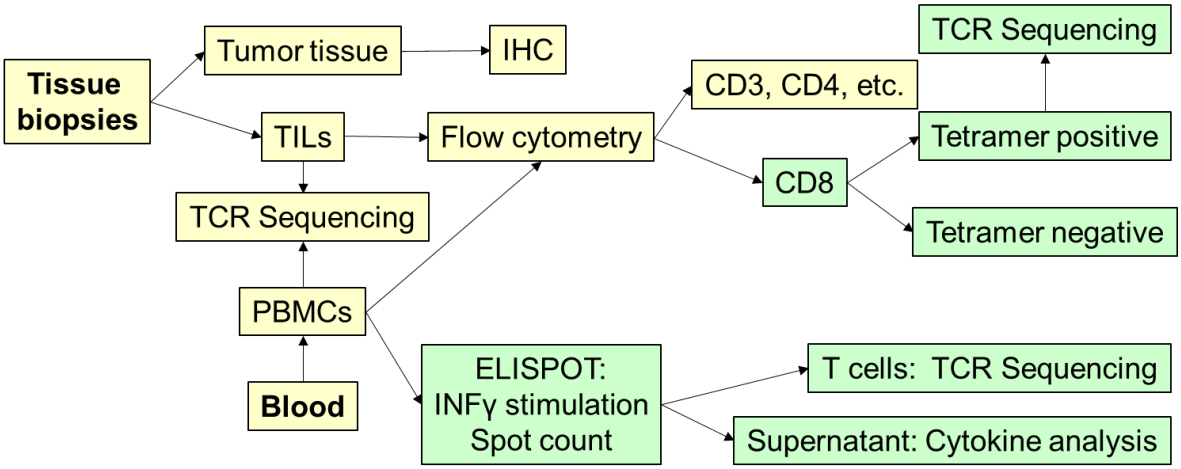
Both peripheral blood mononuclear cells (PBMCs) and TILs will be assessed by flow cytometry for different T cell subsets to evaluate the peripheral and anti-tumor immune response in these patients.[44] Using techniques we described previously,[52-54] we will measure the immune response through two panels targeted at the immune microenvironment and T-cell phenotype. The TME panel will assess CD45 (immune cells), CD3 (T cells), CD19 (B cells), F4/80 (macrophages), CD11c (dendritic cells), CD49b (natural killer cells), Ly6G+Gr-1+ (neutrophils), and live/dead (viability) cells. The T-cell panel will consist of CD45 (immune cells), CD3 (T cells), CD4 (Th cells), CD8 (cytotoxic T cells), FoxP3 and CD25 (Tregs), CD44 and CD62L (memory, effector, and naive T cells), checkpoint molecules PD-1, TIM-3, and LAG-3, proliferation (Ki67), cytokine production (IFNγ, TNFα, IL-2), and live/dead cells.

T-cell recognition is essential to generate a tumor antigen-specific immune response. To quantify HPV-reactive T-cells in tissue and in PBMCs, we will analyze the fraction of E6- and E7-specific CD8+ T cells in tumor tissue using established tetramer reagents.[4] Tetramers are composed of major histocompatibility complex (MHC) and peptide multimers that are highly effective at detecting antigen specific T-cells and can be used in flow cytometry to isolate cell populations as described.[52, 55] These tetramers will be used in combination with markers of activation and exhaustion to determine the features of HPV-

reactive T-cells. To asses HPV-specific T-cell reactivity, we will perform the IFN-γ ELISPOT assay in PBMCs with positive and negative controls as we described.[56] Spots will be developed by 5-bromo-4-chloro-3-indolyl-phosphate in conjunction with nitro blue tetrazolium and counted on an ImmunoSpot ELISPOT reader. We will define the strength of the HPV-specific immune response as the median-specific spot count. At the completion of the IFN-γ ELISPOT assay, the supernatant will be collected to assess production of additional cytokines/chemokines using the luminex multiplex protocol.

The T cell receptor determines the capacity of T cells to react with tumor antigens. Diversification in TIL repertoire is associated with response to therapy.[57, 58] We will use T-cell receptor (TCR) sequencing to characterize the T cell repertoire of TILs before and after treatment using techniques as previously described.[59] Briefly, DNA will be extracted from PBMCs and tissues and undergo two rounds of amplification of the CDR3β region of the T cell receptor which accounts for the greatest amount of contact with antigens (Adaptive Biotechnologies),[60] followed by sequencing of the PCR product by MiSeq and deconvolution of the data by Adaptive Biotechnologies. Analysis will be performed using the Analyzer platform. Metrics such as T cell density, T cell richness (a measure of diversity), and T cell clonality (a measure of reactivity of the T cell repertoire) will be evaluated. Furthermore, we will study changes in the T cell repertoire over time in the periphery and tumor, using the Jaccard and Morisita Overlap Indices which evaluate shared TCR rearrangements across samples/timepoints/compartments to study changes or stability in the T cell repertoire.[59]

Additionally, we will perform TCR sequencing of TILs that react with tetramers (captured by flow cytometry) to identify TCRs that are specific to E6 and E7. TCR sequences from HPV reactive T-cells will be mapped back to TCR sequences in the blood and tissues to track the expansion and contraction of HPV reactive T-cells and we will correlate these findings with response.



We consider that the sample size is also reasonable to evaluate the correlative science endpoints; yet, no explicit sample size justification is made for these exploratory endpoints. Summary statistics (e.g., the mean and standard deviation, median, and minimum/maximum levels of continuous, and frequencies and percentages of categorical variables) will be determined at baseline and post-treatment biomarkers as appropriate. For each pair of specimens, the percent change from baseline of these parameters will also be calculated. Data from biomarker assays may be analyzed using graphical methods and descriptive statistics with additional analyses including linear regression, t-test, and analysis of variance (ANOVA) and the corresponding nonparametric tests as appropriate. Correlations of biomarkers with measures of anti-tumor efficacy will be analyzed using the Spearman's correlation coefficient and the chi-square or Fisher's exact test for the discretized variables.

5.6 Interim Analysis

See section 5.1 for stopping rules in each cohort that require several interim analyses for efficacy and toxicity. In each case, accrual will be suspended temporarily to ensure adequate analysis of the stated endpoint.

The Investigator is responsible for completing Safety and Efficacy Summary Reports and submitting them to the IND office Medical Monitor for review. These should be submitted as follows:

- Escalation Phase:

After the first 2 evaluable subjects complete the first two cycles (first six weeks) of study treatment, and every 2 evaluable subjects thereafter, prior to advancing/changing dose levels.

The study enrollment must be halted until IND Office approval to continue, is obtained.

- Expansion Phase:

After the first 15 evaluable subjects complete 6 months of study treatment, and every 10 evaluable patients thereafter, until enrollment is complete.

5.7 Consent Process and Documentation

This protocol will follow the SOP 04_Informed Consent Process. SOP 04 has been read by the research staff and investigators.

Please indicate what type of consent process will be used (check all that apply):

☒ Remote consent

☒ In-person consent

☐ Waiver of consent

☐ Waiver of written documentation of consent

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7. APPENDICES

7.1 Declaration of Helsinki

World Medical Association Declaration of Helsinki:

Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly Helsinki, Finland, June 1964 and amended by the 29th WMA General Assembly, Tokyo, Japan, October 1975 35th WMA General Assembly, Venice, Italy, October 1983 41st WMA General Assembly, Hong Kong, September 1989 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996 and the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000

A. INTRODUCTION

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.
2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.
3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."
4. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.
5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.
6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.
7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.
8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.
9. Research Investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

1. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.
2. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.
3. Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.
4. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.
5. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.
6. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.
7. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.
8. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.
9. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.
10. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.
11. The subjects must be volunteers and informed participants in the research project.
12. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.
13. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that

the subject has understood the information, the physician should then obtain the subject's freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.

14. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.
15. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.
16. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.
17. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.
18. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

1. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.
2. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists.
3. At the conclusion of the study, every patient entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study.
4. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.
5. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to

evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

7.2 Common Terminology Criteria for Adverse Events Version 5.0

https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf

7.3 New York Heart Association Classification of Cardiac Disease

The following table presents the NYHA classification of cardiac disease.

Class	Functional Capacity	Objective Assessment
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.	No objective evidence of cardiovascular disease.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of minimal cardiovascular disease.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of moderately severe cardiovascular disease.
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.	Objective evidence of severe cardiovascular disease.

Source: The Criteria Committee of New York Heart Association. Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels. 9th Ed. Boston, MA: Little, Brown & Co; 1994:253-256.

7.4 Eastern Cooperative Oncology Group Scale for Performance Status

Grade	Description
0	Normal activity. Fully active, able to carry on all predisease performance without restriction
1	Symptoms but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (eg, light housework, office work)
2	In bed < 50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed > 50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

Source: Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982; 5 (6):649-55.

7.5 Body Surface Area and Creatinine Clearance Calculations

Body surface area (BSA) should be calculated using a standard nomogram that yields the following results in meters squared (m^2):

$$\text{BSA} = \sqrt{\frac{Ht(inches) \times Wt(lbs)}{3131}}$$

or

$$\text{BSA} = \sqrt{\frac{Ht(cm) \times Wt(kg)}{3600}}$$

Creatinine clearance (CrCl) can be calculated using the Cockroft-Gault equation as follows:

CrCl (ml/min) = [(140-age) (body weight in kg) / (72 × serum creatinine in mg/dL)]

OR

[(140-age)(body weight in kg) / (0.81 x serum creatinine in μmol/L)]

For females, use 85% of calculated CrCl value.

Source: Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16(1):31-41.

Note: In markedly obese patients, the Cockroft-Gault formula will tend to overestimate the creatinine clearance. (Adipose tissue tends to contribute little creatinine requiring renal clearance.)

Appendix

7.6 Management of pembrolizumab toxicity

Immune-related AEs	Toxicity grade or conditions (CTCAEv4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
				feasible, fluid and electrolytes should be substituted via IV infusion.
AST / ALT elevation or Increased bilirubin	Grade 2	Withhold	• Administer corticosteroids (initial dose of 0.5 to 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable)
	Grade 3 or 4	Permanently discontinue[1]	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold	• Initiate insulin replacement therapy for participants with T1DM • Administer anti-hyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hyperthyroidism	Grade 2	Continue	• Treat with non-selective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders
	Grade 3 or 4	Withhold or permanently discontinue[2]		
Hypothyroidism	Grade 2-4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyronine) per SOC	• Monitor for signs and symptoms of thyroid disorders
Nephritis and Renal dysfunction	Grade 2	Withhold	• Administer corticosteroids (prednisone 1 to 2 mg/kg or equivalent) followed by taper.	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1 or 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently		

Immune-related AEs	Toxicity grade or conditions (CTCAEv4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
		discontinue		
All other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	• Based on type and severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Guillain-Barre Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		

Abbreviations: AE = adverse event; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CTCAE = Common Terminology Criteria for Adverse Events; GI = gastrointestinal; IV = intravenous; T1DM = type 1 diabetes mellitus

1. A participant with liver metastasis has Grade 2 AST or ALT at the start of study treatment, and the AST or ALT value increases by ≥50% relative to baseline and lasts for ≥1 week, then the participant should permanently discontinue study intervention.
2. Withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician.

NOTE:
For participants with Grade 3 or 4 immune-related endocrinopathy where withhold of pembrolizumab is required, pembrolizumab may be resumed when AE resolves to ≤Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM).

Table 8 Pembrolizumab Dose Modification and Toxicity Management Guidelines for Immune-related AEs

General instructions:

1. Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.
2. For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has been reduced to Grade 1 or 0 and corticosteroid has been tapered. Pembrolizumab must be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤10 mg prednisone or equivalent per day within 12 weeks.
3. For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids.

Immune-related AEs	Toxicity grade or conditions (CTCAEv4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment • Add prophylactic antibiotics for opportunistic infections
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus). • Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not
	Grade 4 or recurrent Grade 3	Permanently discontinue		

Table 9 Pembrolizumab Infusion Reaction Dose Modification and Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at Subsequent Dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires therapy or infusion interruption but responds promptly to symptomatic treatment (eg, antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDs Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. If symptoms resolve within 1 hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (eg, from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the participant should be premedicated for the next scheduled dose. **Participants who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further study drug treatment**	Participant may be premedicated 1.5 h (± 30 minutes) prior to infusion of pembrolizumab with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500 to 1000 mg po (or equivalent dose of analgesic).