Official Protocol Title:	An Open-Label, Phase II Study to Determine the Immunologic Correlates of Pembrolizumab-Mediated Tumor Regression in Subjects with Advanced Melanoma (KEYNOTE-161).
NCT number:	NCT03407170
Document Date:	14-Jun-2019

Title Page

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This protocol amendment is applicable only to US.

Protocol Title: An Open-Label, Phase II Study to Determine the Immunologic Correlates of Pembrolizumab-Mediated Tumor Regression in Subjects with Advanced Melanoma (KEYNOTE-161).

Protocol Number: 161-04

Compound Number: MK-3475

Sponsor Name and Legal Registered Address:

Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc.
(hereafter referred to as the Sponsor or MSD)

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Regulatory Agency Identifying Number(s):

IND NUMBER: 110080

EudraCT NUMBER: Not applicable

Approval Date: 14 June 2019

Sponsor Signatory

Typed Name: Date
Title:

Protocol-specific Sponsor contact information can be found in the Investigator Trial File Binder (or equivalent).

Investigator Signatory

I agree to conduct this clinical study in accordance with the design outlined in this protocol and to abide by all provisions of this protocol.

Typed Name: Date
Title:

DOCUMENT HISTORY

Document	Date of Issue	Overall Rationale
Original Protocol	15-JUL-2015	Not applicable
MK3475-161-01	30-JUN-2016	To align the protocol with the current approved pembrolizumab risk language.
MK3475-161-02	13-NOV-2017	To align the protocol with the current approved pembrolizumab risk language and product label.
MK3475-161-03	23-FEB-2018	To correct the specific blood samples needed and total volume of blood collected during the study.
MK3475-161-04	14-JUN-2019	To clarify the timing of transfer of subjects to the planned pembrolizumab extension study (MK3475-587) because MK3475-161 is closing.

PROTOCOL AMENDMENT SUMMARY OF CHANGES

Amendment 04

Overall Rationale for the Amendment:

To clarify the timing of transfer of subjects to the planned pembrolizumab extension study (MK3475-587) because MK3475-161 is closing.

Summary of Changes Table:

Section # and Name	Description of Change	Brief Rationale
1.1 Synopsis 4.4 Beginning and End of Study Definition 8.1.7 Assignment of Treatment/Randomization Number	Added language to permit rollover into extension once the participant has achieved the study objective or the study has ended.	To clarify the timing of transfer of subjects to the planned pembrolizumb extension study (MK-3475-587) because MK-3475-161 is closing.
5.1 Inclusion Criteria 10.3 Appendix 3: Contraceptive Guidance and Pregnancy Testing	Included contraceptive requirements for male participants.	To remain current with standard contraceptive language.
8.1.1.1 General Informed Consent	Added reconsent language for patients at initial radiographic disease progression.	To establish a mechanism for the patient to acknowledge their continued desire to be in the study when disease progression is determined by the study investigator.
10.7 Appendix 7: Abbreviations	Defined APaT	To update terminology

Table of Contents

LIST OF TABLES

LIST OF FIGURES

1. Protocol Summary

1.1 Synopsis

Protocol Title:

An Open-Label, Phase II Study to Determine the Immunologic Correlates of Pembrolizumab-Mediated Tumor Regression in Subjects with Advanced Melanoma (KEYNOTE-161).

Short Title:

Immunologic determinants of response to pembrolizumab in advanced melanoma

Objectives/Hypotheses and Endpoints:

In all allocated participants receiving treatment for locally advanced or metastatic melanoma:

Objective/Hypothesis	Endpoint
Primary	
• Objective: To compare the baseline CD8+ tumor-infiltrating lymphocytes expressing a predefined single-cell RNA gene signature in participants with locally advanced or metastatic melanoma with primary response to pembrolizumab monotherapy (complete response [CR]/partial response [PR]) vs. those who do not respond (stable disease [SD]/progressive disease [PD]/Other) to pembrolizumab monotherapy as determined by Response Evaluation Criteria in Solid Tumors (RECIST) 1.1. • Hypothesis: Participants who respond to pembrolizumab have a higher FCT at baseline compared to participants who do not respond to pembrolizumab.	• The fraction of cytotoxic tumor-infiltrating T-lymphocytes (FCT), defined as the fraction of CD8+ T-cells expressing a predefined single-cell RNA gene signature to the total tumor infiltrating CD8+T-cells isolated from tumor biopsies.
• Objective: To compare the changes from baseline in CD8+ tumor infiltrating lymphocytes expressing a predefined single-cell RNA gene signature in participants with locally advanced or metastatic melanoma with primary response (CR/PR) to pembrolizumab	• The fold-change from baseline in FCT.

monotherapy vs. those who do not respond (SD/PD/Other) to pembrolizumab monotherapy as determined by RECIST 1.1. • Hypothesis: Participants who respond to pembrolizumab have a higher fold-increase in FCT compared to baseline than participants who do not respond to pembrolizumab.	
• Objective: To compare the expansion of cytotoxic tumor-infiltrating T-cell clones between responders (CR/PR) and non-responders (SD/PD/other) in participants with locally advanced or metastatic melanoma as determined by RECIST 1.1. • Hypothesis: Participants who respond to pembrolizumab have a higher Average Specific Cytotoxic T-lymphocyte Frequency Ratio (ASCTFR) compared to participants who do not respond to pembrolizumab.	• ASCTFR, defined as the arithmetic mean of log10 ratio of the frequency of individual, specific cytotoxic T-cell receptor (TCR) clones on-treatment to pre-treatment (baseline).
Secondary	
• Objective: To evaluate the objective response rate (ORR), progression-free survival (PFS), and overall survival (OS) of participants with locally advanced or metastatic melanoma treated with pembrolizumab.	• ORR, defined as the proportion of participants who have a best response as CR or PR per RECIST 1.1 based on local site review • PFS, defined as the time from start of treatment until the first documented progressive disease (PD), based on local site review using RECIST 1.1, or death due to any cause, whichever occurs first. • OS, defined as the time from the start of treatment to date of death due to any cause
• Objective: To evaluate the safety and tolerability of pembrolizumab.	• Number of participants with adverse events (AEs) and serious AEs

	• Number of participants discontinuing study treatment due to AEs.
• Objective: To compare the tumor reactivity and antigenic determinants of CD8+ TCR clones in tumor tissue from participants who respond (CR/PR) to pembrolizumab compared to participants who do not respond (SD/PD/other) to pembrolizumab.	• TCRs from CD8+ T-cell clones will be identified by single cell RNA sequencing (scRNAseq) and their killing function will be confirmed by TCR-transduced T-cells recognizing autologous tumor-derived cell lines.
• Objective: To evaluate the relationship between neoepitope burden and clinical/immunological response to pembrolizumab.	• Neoepitope sequencing will be generated based on scRNAseq, whole exome sequencing, and an epitope prediction algorithm to obtain neoepitope burden.

Overall Design:

Study Phase	Phase II
Clinical Indication	Advanced melanoma
Population	Patients with advanced melanoma
Study Type	Interventional
Type of Design	Multi-site, single arm
Type of Control	No treatment control
Study Blinding	Unblinded open-label
Estimated Duration of Study	The Sponsor estimates that the study will require approximately 30 months (or until the last survival data are available) from the time the first participant signs the informed consent until the last participant's last study-related telephone call or visit.

Number of Participants:

Approximately 60 participants will be allocated.

Treatment Groups and Duration:

Treatment Groups	Pembrolizumab 200 mg every 3 weeks (Q3W)
Duration of Participation	Each participant will participate in the study from the time the participant signs the Informed Consent Form (ICF) through the final protocol-specified contact. After a screening phase of 28 days, each participant will be assigned to receive study treatment until disease progression is radiographically documented and, when clinically appropriate, confirmed by the sites per immune-modified RECIST 1.1 for immune-based therapeutics (iRECIST), unacceptable adverse event(s) (AEs), intercurrent illness that prevents further administration of treatment, investigator's decision to withdraw the participant, noncompliance with trial treatment or procedure requirements or administrative reasons requiring cessation of treatment, or until the participant has received 35 administrations of pembrolizumab (approximately 2 years). After the end of treatment, each participant will be followed for the occurrence of AEs and spontaneously reported pregnancy as described under Section 8.4.1. Participants who discontinue for reasons other than radiographic disease progression will have post-treatment follow-up imaging for disease status until disease progression is documented radiographically per RECIST 1.1, confirmed by the site per iRECIST, initiating a non-trial cancer treatment, withdrawing consent, or becoming lost to follow-up. All participants will be followed by telephone for OS until death, withdrawal of consent, or the end of the study. Once the participant has achieved the study objective or the study has ended, the participant is discontinued from this study and will be enrolled in a pembrolizumab extension study (MK3475-587) to continue protocol-defined assessments and treatment.

Study Governance:

Study Governance Committees	There are no governance committees in this study.

A list of abbreviations used in this document can be found in Appendix 7.

1.2 Schema

The study design is depicted in Figure 1

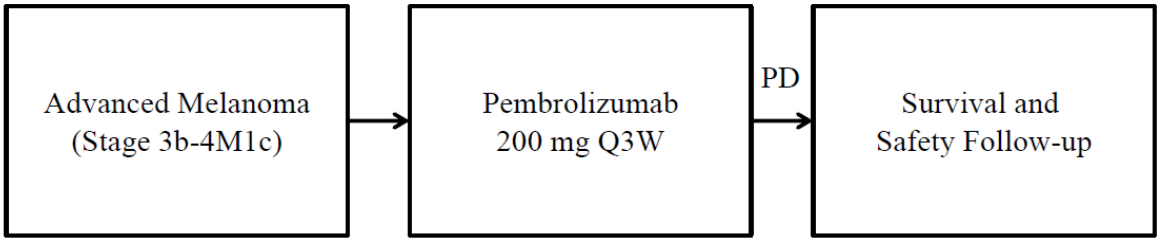


Figure 1 Study Design

1.3 Schedule of Activities (SoA)

Study Period:	Screening Phase	Treatment Cycles (3-week cycles)						EOT	Post-Treatment			Notes
		Through Cycle 35										• Assessments and procedures are to be performed on Day 1 of each treatment cycle unless otherwise specified. • Results of a test performed prior to signing the ICF as part of routine clinical management are acceptable in lieu of a screening test if performed within the specified time frame (e.g., within 42 days prior to the first dose of study treatment). • If the DC visit occurs ≥30 days from last dose of study treatment, a Safety Follow-up Visit is not required. • For participants who DC for reasons other than confirmed PD, imaging continues until confirmed PD or initiation of a new anti-neoplastic therapy. • Follow-Up visits may be scheduled to coincide with Follow-Up imaging
Treatment Cycle/Title:	Screening (Visit 1)	1	2	3	4	5	6+	DC	Safety Follow-up	Follow Up Visits	Survival Follow-Up	
Scheduling Window (Days):	-28 to -1	+3	±3	±3	±3	±3	±3	At time of DC	30 days after last dose (±3 days)	Every 12 weeks post DC (±7 days)	Every 12 weeks (±7 days)	
Administrative Procedures												
ICF	X											Written ICF must be obtained prior to performing any protocol-specified procedure. Screening number assigned at the time the ICF is signed.
FBR ICF	X											Participants can still participate in the study if they decline to sign the FBR ICF.
Inclusion/exclusion criteria	X											
Participant identification card	X											
Demographics and medical history	X											
Prior and concomitant medication review	X	X	X	X	X	X	X	X	X			Record all medications taken within 30 days of the first dose of study treatment and all new medications started during the study, including medications for AEs.
Subsequent anti-neoplastic therapy								X	X	X	X	

Study Period:	**Screening Phase**	**Treatment Cycles (3-week cycles)**						**EOT**	**Post-Treatment**			**Notes**
		Through Cycle 35										
Treatment Cycle/Title:	Screening (Visit 1)	1	2	3	4	5	6+	DC	Safety Follow-up	Follow Up Visits	Survival Follow-Up	• Assessments and procedures are to be performed on Day 1 of each treatment cycle unless otherwise specified. • Results of a test performed prior to signing the ICF as part of routine clinical management are acceptable in lieu of a screening test if performed within the specified time frame (e.g., within 42 days prior to the first dose of study treatment). • If the DC visit occurs ≥30 days from last dose of study treatment, a Safety Follow-up Visit is not required. • For participants who DC for reasons other than confirmed PD, imaging continues until confirmed PD or initiation of a new anti-neoplastic therapy. • Follow-Up visits may be scheduled to coincide with Follow-Up imaging
Scheduling Window (Days):	-28 to -1	+3	±3	±3	±3	±3	±3	At time of DC	30 days after last dose (±3 days)	Every 12 weeks post DC (±7 days)	Every 12 weeks (±7 days)	
Survival status		←----------					----------→				X	After Investigator determined PD or start of new anticancer treatment. In addition, upon Sponsor request, participants may be contacted for survival status at any time during the course of the study.
Administration of Study Treatment												
Pembrolizumab		X	X	X	X	X	X					200 mg Q3W Study personnel will administer treatment in Cycle 1 within 3 days of treatment allocation.

Study Period:	**Screening Phase**	**Treatment Cycles (3-week cycles)**						**EOT**	**Post-Treatment**			**Notes**
		Through Cycle 35										
Treatment Cycle/Title:	Screening (Visit 1)	1	2	3	4	5	6+	DC	Safety Follow-up	Follow Up Visits	Survival Follow-Up	• Assessments and procedures are to be performed on Day 1 of each treatment cycle unless otherwise specified. • Results of a test performed prior to signing the ICF as part of routine clinical management are acceptable in lieu of a screening test if performed within the specified time frame (e.g., within 42 days prior to the first dose of study treatment). • If the DC visit occurs ≥30 days from last dose of study treatment, a Safety Follow-up Visit is not required. • For participants who DC for reasons other than confirmed PD, imaging continues until confirmed PD or initiation of a new anti-neoplastic therapy. • Follow-Up visits may be scheduled to coincide with Follow-Up imaging
Scheduling Window (Days):	-28 to -1	+3	±3	±3	±3	±3	±3	At time of DC	30 days after last dose (±3 days)	Every 12 weeks post DC (±7 days)	Every 12 weeks (±7 days)	
Efficacy Measurements												**Scans performed as part of routine clinical management are acceptable for use as the screening scan if they are of diagnostic quality and performed within 28 days prior to the first dose of study treatment.** **The same imaging technique should be used in a participant throughout the study.**
Tumor imaging	X				X		X	X		X		The first on-study imaging will be performed at 9 weeks (±7 days) after the date of allocation and then every 9 weeks (± 7 days) thereafter for the first year, or more frequently if clinically indicated. After 52 weeks (12 months), imaging will be performed every 12 weeks (±7 days). Imaging should be performed at the time of DC (±4 weeks). If imaging was obtained within 4 weeks prior to DC, then imaging at DC is not mandatory. Imaging timing should follow **calendar days** and should not be adjusted for delays in cycle starts of pembrolizumab.

Study Period:	Screening Phase	Treatment Cycles (3-week cycles)						EOT	Post-Treatment			Notes
		Through Cycle 35										
Treatment Cycle/Title:	Screening (Visit 1)	1	2	3	4	5	6+	DC	Safety Follow-up	Follow Up Visits	Survival Follow-Up	• Assessments and procedures are to be performed on Day 1 of each treatment cycle unless otherwise specified. • Results of a test performed prior to signing the ICF as part of routine clinical management are acceptable in lieu of a screening test if performed within the specified time frame (e.g., within 42 days prior to the first dose of study treatment). • If the DC visit occurs ≥30 days from last dose of study treatment, a Safety Follow-up Visit is not required. • For participants who DC for reasons other than confirmed PD, imaging continues until confirmed PD or initiation of a new anti-neoplastic therapy. • Follow-Up visits may be scheduled to coincide with Follow-Up imaging
Scheduling Window (Days):	-28 to -1	+3	±3	±3	±3	±3	±3	At time of DC	30 days after last dose (±3 days)	Every 12 weeks post DC (±7 days)	Every 12 weeks (±7 days)	
Digital photography: cutaneous lesions	X				X		X	X		X		Digital photography is recommended, but option for this study.
Safety Assessments												**All on-treatment assessments should be performed prior to treatment administration.**
Review AEs	X	X	X	X	X	X	X	X	X	X		All AEs and ECIs from the time of treatment allocation through 30 days following cessation of study treatment. All SAEs from the time of treatment allocation through 90 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anticancer therapy, whichever is earlier.
12-lead ECG	X											12-lead ECG performed using local standard procedures. Additional ECGs performed as clinically indicated.
Complete physical exam	X											
Directed physical exam		X	X	X	X	X	X	X				Directed physical exam performed as clinically indicated.
Vital signs	X	X	X	X	X	X	X	X	X	X		Includes temperature, pulse, RR, DBP, SBP, and weight

Study Period:	Screening Phase	Treatment Cycles (3-week cycles)						EOT	Post-Treatment			Notes
		Through Cycle 35										• Assessments and procedures are to be performed on Day 1 of each treatment cycle unless otherwise specified.
Treatment Cycle/Title:	Screening (Visit 1)	1	2	3	4	5	6+	DC	Safety Follow-up	Follow Up Visits	Survival Follow-Up	• Results of a test performed prior to signing the ICF as part of routine clinical management are acceptable in lieu of a screening test if performed within the specified time frame (e.g., within 42 days prior to the first dose of study treatment).
Scheduling Window (Days):	-28 to -1	+3	±3	±3	±3	±3	±3	At time of DC	30 days after last dose (±3 days)	Every 12 weeks post DC (±7 days)	Every 12 weeks (±7 days)	• If the DC visit occurs ≥30 days from last dose of study treatment, a Safety Follow-up Visit is not required. • For participants who DC for reasons other than confirmed PD, imaging continues until confirmed PD or initiation of a new anti-neoplastic therapy. • Follow-Up visits may be scheduled to coincide with Follow-Up imaging
Height	X											
ECOG performance status	X	X	X	X	X	X	X	X	X	X		Screening assessment to be performed within 10 days prior to treatment initiation.
Local Laboratory Procedures/ Assessments												**All on-treatment samples should be collected on Day 1 of each cycle, prior to treatment administration but may be collected up to 72 hours prior to the scheduled time point.**
Urine or serum β-hCG pregnancy test – WOCBP only	X											WOCBP require a negative urine test within 72 hours prior to the first dose of study treatment. Serum test is only required if urine test is positive or is not evaluable. Additional urine pregnancy testing can be conducted if required by local regulations or clinically indicated.
Serum FSH – WOCBP only	X											Only for women < 45 years old with no menses for ≥1 year (12 months) and not currently on HRT or hormonal contraception.

Study Period:	**Screening Phase**	**Treatment Cycles (3-week cycles)**						**EOT**	**Post-Treatment**			**Notes**
		Through Cycle 35										
Treatment Cycle/Title:	Screening (Visit 1)	1	2	3	4	5	6+	DC	Safety Follow-up	Follow Up Visits	Survival Follow-Up	• Assessments and procedures are to be performed on Day 1 of each treatment cycle unless otherwise specified. • Results of a test performed prior to signing the ICF as part of routine clinical management are acceptable in lieu of a screening test if performed within the specified time frame (e.g., within 42 days prior to the first dose of study treatment). • If the DC visit occurs ≥30 days from last dose of study treatment, a Safety Follow-up Visit is not required. • For participants who DC for reasons other than confirmed PD, imaging continues until confirmed PD or initiation of a new anti-neoplastic therapy. • Follow-Up visits may be scheduled to coincide with Follow-Up imaging
Scheduling Window (Days):	-28 to -1	+3	±3	±3	±3	±3	±3	At time of DC	30 days after last dose (±3 days)	Every 12 weeks post DC (±7 days)	Every 12 weeks (±7 days)	
PT, INR, aPTT	X											Screening samples are to be collected within 10 days of treatment initiation. Participants receiving anticoagulant therapy should have coagulation factors monitored closely throughout the study
CBC with differential	X		X	X	X	X	X	X	X			Screening samples are to be collected within 10 days of treatment initiation. For participants with a baseline estimated creatinine clearance below the normal institutional laboratory range, a baseline measured creatinine clearance should be performed.
Chemistry panel	X		X	X	X	X	X	X	X			
LDH	X											
Urinalysis	X		X		X		X		X			
T3, FT4, TSH	X		X		X		X		X			Screening samples are to be collected within 10 days of treatment initiation. Thyroid function tests will be performed on Day 1 of every 6 weeks (e.g., Cycles 2, 4, 6, etc.). Free T3 is acceptable if T3 is not available.
BRAF testing	X											To be performed in participants without documented BRAF status

Study Period:	Screening Phase	Treatment Cycles (3-week cycles)						EOT	Post-Treatment			Notes
		Through Cycle 35										• Assessments and procedures are to be performed on Day 1 of each treatment cycle unless otherwise specified. • Results of a test performed prior to signing the ICF as part of routine clinical management are acceptable in lieu of a screening test if performed within the specified time frame (e.g., within 42 days prior to the first dose of study treatment). • If the DC visit occurs ≥30 days from last dose of study treatment, a Safety Follow-up Visit is not required. • For participants who DC for reasons other than confirmed PD, imaging continues until confirmed PD or initiation of a new anti-neoplastic therapy. • Follow-Up visits may be scheduled to coincide with Follow-Up imaging
Treatment Cycle/Title:	Screening (Visit 1)	1	2	3	4	5	6+	DC	Safety Follow-up	Follow Up Visits	Survival Follow-Up	
Scheduling Window (Days):	-28 to -1	+3	±3	±3	±3	±3	±3	At time of DC	30 days after last dose (±3 days)	Every 12 weeks post DC (±7 days)	Every 12 weeks (±7 days)	
Central Laboratory Procedures/ Assessments												
Blood for plasma biomarker analysis (EDTA tube)		X	X	X				X				Samples to be collected predose. Additional samples should be collected at the time of initial documentation of radiographic response or progression and whenever a biopsy is performed.
Blood for serum biomarker analysis (serum tube)		X	X	X				X				
Blood for RNA analysis (RNA PaxGene)		X	X	X				X				
Blood for genetic analysis (DNA PaxGene)		X										
Protocol-Specific Laboratory Assessments												
PBMC collection (sodium heparin tube)		X	X	X			X	X				Samples to be collected predose. Additional samples should be collected at the time of initial documentation of radiographic response or progression and whenever a biopsy is performed.
Blood for correlative DNA studies (EDTA tube)		X										

Study Period:	Screening Phase	Treatment Cycles (3-week cycles)						EOT	Post-Treatment			Notes
		Through Cycle 35										• Assessments and procedures are to be performed on Day 1 of each treatment cycle unless otherwise specified.
Treatment Cycle/Title:	Screening (Visit 1)	1	2	3	4	5	6+	DC	Safety Follow-up	Follow Up Visits	Survival Follow-Up	• Results of a test performed prior to signing the ICF as part of routine clinical management are acceptable in lieu of a screening test if performed within the specified time frame (e.g., within 42 days prior to the first dose of study treatment). • If the DC visit occurs ≥30 days from last dose of study treatment, a Safety Follow-up Visit is not required. • For participants who DC for reasons other than confirmed PD, imaging continues until confirmed PD or initiation of a new anti-neoplastic therapy. • Follow-Up visits may be scheduled to coincide with Follow-Up imaging
Scheduling Window (Days):	-28 to -1	+3	±3	±3	±3	±3	±3	At time of DC	30 days after last dose (±3 days)	Every 12 weeks post DC (±7 days)	Every 12 weeks (±7 days)	
Tumor Tissue Collection												
Fresh tumor tissue collection	X		X									If a biopsy is obtained at Cycle 2 (Week 6; 42 days ±3 days), then a second on-treatment biopsy obtained at an imaging visit that shows initial evidence of response or progression is optional.

Abbreviations: AE = adverse event; aPTT = activated partial thromboplastin time; CBC = complete blood count; DBP = diastolic blood pressure; DC = discontinuation; ECG = electrocardiogram; ECIs = events of clinical interest; ECOG = Easter Cooperative Oncology Group; EOT = end of treatment; FBR = future biomedical research; FSH = follicle stimulating hormone; FT4 = free thyroxine; hCG = human chorionic gonadotropin; HRT = hormone replacement therapy; ICF = informed consent form; INR = international normalized ratio; LDH = lactate dehydrogenase; PBMC = peripheral blood mononuclear cells; PD = progressive disease; PT = prothrombin time; Q3W = every 3 weeks; RNAseq = RNA sequencing; RR = respiratory rate; SAEs = serious adverse events; SBP = systolic blood pressure; T3 = triiodothyronine; TCR = T-cell receptor; TSH = thyroid stimulating hormone; WOCBP = women of childbearing potential.

2. Introduction

2.1 Study Rationale

Pembrolizumab-mediated tumor regression is thought to be affected by tumor antigen-specific T-cells that are quiescent in the setting of programmed cell death 1 (PD-1) receptor-mediated inhibitory signaling, but are re-activated by PD-1 blockade to acquire cytolytic antitumor function [Disis, M. L. 2010]. However, these antitumor T-cells have not previously been directly studied in detail in participants with documented clinical response to PD-1 checkpoint blockade. In participants with advanced melanoma, pembrolizumab has exhibited a high degree of clinical efficacy, with many participants achieving durable clinical response [Deschoolmeester, V. 2010] [Sasaki, A., et al 2008] [Shen, Z., et al 2010]. However, a significant proportion of participants develop progressive disease despite pembrolizumab therapy. Therefore, melanoma represents an ideal setting in which differences in clinical response patterns (e.g. response vs. progression) can be directly correlated with differences in antigen-specific T cell phenotype and function to gain deeper insights into the mechanistic determinants of tumor regression following checkpoint blockade.

In addition to the immunologic state of anti-tumor T-cells, the antigenic burden of the tumor may also be a critical determinant of pembrolizumab-mediated antitumor immunity. Emerging data suggest that neoepitopes created by tumor-specific mutations may serve as important targets of antitumor T-cell immunity in melanoma and other malignancies [Talmadge, J. E., et al 2007] [Thompson, R. H., et al 2007]. However, the relative contribution of neoepitopes vs. epitopes from common germline antigens, such as cancer-testes antigens and tissue-specific differentiation antigens, has not previously been empirically determined. Insights into this issue can be gained by evaluating the antigen specificity of dominant T-cell clones exhibiting significant cytotoxicity in the tumors of participants experiencing a clinical and radiographic response to pembrolizumab. Melanoma represents an ideal setting because of both the high mutational burden of this tumor type from UV-related mutagenesis [Andersen, R. S., et al 2012] and the extensive characterization of dominant epitopes from common tumor-associated antigens [Deschoolmeester, V. 2010] [Sasaki, A., et al 2008] [Shen, Z., et al 2010] [Dudley, M. E., et al 2005].

In order to interrogate the biology of individual tumor-specific T-cells within the tumor microenvironment, a technical approach requiring dissociation of freshly-obtained tumor tissue is necessary to obtain and characterize viable tumor-infiltrating lymphocytes. In addition, close observation is necessary to time on-treatment biopsies with clinical and/or radiographic evidence of response or progression. A dedicated clinical trial will allow optimization of both clinical observation and tissue collection in order to obtain these data.

2.2 Background

Pembrolizumab is a potent humanized immunoglobulin G4 (IgG4) monoclonal antibody (mAb) with high specificity of binding to the PD-1 receptor, thus inhibiting its interaction with PD-1 ligand 1 (PD-L1) and PD-1 ligand 2 (PD-L2). Based on preclinical in vitro data, pembrolizumab has high affinity and potent receptor blocking activity for PD-1. Pembrolizumab has an acceptable preclinical safety profile and is in clinical development as

an intravenous (IV) immunotherapy for advanced malignancies. Keytruda® (pembrolizumab) is indicated for the treatment of patients across a number of indications.

Refer to the Investigator's Brochure (IB)/approved labeling for detailed background information on pembrolizumab.

2.2.1 Pharmaceutical and Therapeutic Background

2.2.1.1 Inhibition of PD-1 as a Target for Cancer

The importance of intact immune surveillance function in controlling outgrowth of neoplastic transformations has been known for decades [Disis, M. L. 2010]. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes in cancer tissue and favorable prognosis in various malignancies. In particular, the presence of cluster of differentiation 8 positive (CD8+) T-cells and the ratio of CD8+ effector T-cells (Teff)/FoxP3+ regulatory T-cells (Tregs) correlates with improved prognosis and long-term survival in solid malignancies, such as ovarian, colorectal, and pancreatic cancer; hepatocellular carcinoma; malignant melanoma; and renal cell carcinoma. Tumor-infiltrating lymphocytes can be expanded *ex vivo* and reinfused, inducing durable objective tumor responses in cancers such as melanoma [Dudley, M. E., et al 2005] [Hunder, N. N., et al 2008].

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) that has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD L1 and/or PD-L2) [Greenwald, R. J., et al 2005] [Okazaki, T., et al 2001].

The structure of murine PD-1 has been resolved [Zhang, X., et al 2004]. PD-1 and its family members are type I transmembrane glycoproteins containing an Ig-variable–type (IgV type) domain responsible for ligand binding and a cytoplasmic tail responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif, and an immunoreceptor tyrosine-based switch motif. Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases, SHP-1 and SHP-2, to the immunoreceptor tyrosine-based switch motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3 zeta (CD3ζ), protein kinase C-theta (PKCθ), and zeta-chain-associated protein kinase (ZAP70), which are involved in the CD3 T-cell signaling cascade [Chemnitz, J. M., et al 2004] [Sheppard, K-A, et al 2004] [Riley, J. L. 2009] [Okazaki, T., et al 2001]. The mechanism by which PD-1 down-modulates T cell responses is similar to, but distinct from, that of CTLA-4, because both molecules regulate an overlapping set of signaling proteins [Parry, R. V., et al 2005] [Francisco, L. M., et al 2010]. As a consequence, the PD 1/PD-L1 pathway is an attractive target for therapeutic intervention in locally advanced or metastatic melanoma.

2.2.2 Preclinical and Clinical Studies

Refer to the pembrolizumab IB for preclinical and clinical study data.

2.2.3 Ongoing Clinical Studies

Refer to the pembrolizumab IB for ongoing clinical study data.

2.3 Benefit/Risk Assessment

It cannot be guaranteed that participants in clinical studies will directly benefit from treatment during participation, as clinical studies are designed to provide information about the safety and effectiveness of an investigational medicine.

Additional details regarding specific benefits and risks for participants participating in this clinical study may be found in the accompanying IB and Informed Consent documents.

3. Objectives/Hypotheses and Endpoints

In all allocated participants receiving treatment for locally advanced or metastatic melanoma:

Objective/Hypothesis	Endpoint
Primary	
• Objective: To compare the baseline CD8+ tumor-infiltrating lymphocytes expressing a predefined single-cell RNA gene signature in participants with locally advanced or metastatic melanoma with primary response to pembrolizumab monotherapy (complete response [CR]/partial response [PR]) vs. those who do not respond (stable disease [SD]/progressive disease [PD]/other) to pembrolizumab monotherapy as determined by Response Evaluation Criteria in Solid Tumors (RECIST) 1.1. • Hypothesis: Participants who respond to pembrolizumab have a higher FCT at baseline compared to participants who do not respond to pembrolizumab.	• The fraction of cytotoxic tumor-infiltrating T-lymphocytes (FCT), defined as the fraction of CD8+ T-cells expressing a predefined single-cell RNA gene signature to the total tumor infiltrating CD8+ T-cells isolated from tumor biopsies.
• Objective: To compare the changes from baseline in CD8+ tumor infiltrating lymphocytes expressing a predefined single-cell RNA gene signature in participants with locally advanced or metastatic melanoma with primary response (CR/PR) to pembrolizumab monotherapy vs. those who do not respond (SD/PD/other) to pembrolizumab monotherapy as determined by RECIST 1.1.	• The fold-change from baseline in FCT.

Objective/Hypothesis	Endpoint
• Hypothesis: Participants who respond to pembrolizumab have a higher fold-increase in FCT compared to baseline than participants who do not respond to pembrolizumab.	
• Objective: To compare the expansion of cytotoxic tumor-infiltrating T-cell clones between responders (CR/PR) and non-responders (SD/PD/other) in participants with locally advanced or metastatic melanoma. • Hypothesis: Participants who respond to pembrolizumab have a higher Average Specific Cytotoxic T-lymphocyte Frequency Ratio (ASCTFR) compared to participants who do not respond to pembrolizumab.	• ASCTFR, defined as the arithmetic mean of log10 ratio of the frequency of individual, specific cytotoxic T-cell receptor (TCR) clones on-treatment to pre-treatment (baseline).
Secondary	
• Objective: To evaluate the objective response rate (ORR), progression-free survival (PFS), and overall survival (OS) of participants with locally advanced or metastatic melanoma treated with pembrolizumab.	• ORR, defined as the proportion of participants who have a best response as CR or PR per RECIST 1.1 based on local site review. • PFS, defined as the time from start of treatment until the first documented progressive disease (PD), as determined by local site review using RECIST 1.1, or death due to any cause, whichever occurs first. • OS, defined as the time from the start of treatment to date of death due to any cause.
• Objective: To evaluate the safety and tolerability of pembrolizumab.	• Number of participants with adverse events (AEs) and serious AEs. • Number of participants discontinuing study treatment due to AEs.
• Objective: To compare the tumor reactivity and antigenic determinants of CD8+ TCR clones in tumor tissue from participants who respond (CR/PR) to pembrolizumab compared to participants who do not respond (SD/PD/other) to pembrolizumab.	• TCRs from CD8+ T-cell clones will be identified by single cell RNA sequencing (scRNAseq) and their killing function will be confirmed by TCR-transduced T-cells recognizing autologous tumor-derived cell lines.

Objective/Hypothesis	Endpoint
• Objective: To evaluate the relationship between neoepitope burden and clinical/immunological response to pembrolizumab.	• Neoepitope sequencing will be generated based on scRNAseq, whole exome sequencing, and an epitope prediction algorithm to obtain neoepitope burden.
Exploratory	
• Objective: To evaluate ORR and PFS based on modified RECIST 1.1 for immune-based therapeutics (iRECIST).	• ORR and PFS as determined by local site per iRECIST.
• Objective: To characterize the gene expression profile of T-cells in participants who experience clinical benefit or nonclinical benefit.	• scRNAseq of freshly sorted tumor-infiltrating T-cells and the RNAseq profile of the whole tumor will be used to obtain a gene expression profile of T-cells.
• Objective: To correlate the change in gene expression profile of CD8+ T-cell clones with proven tumor reactivity from participants with locally advanced or metastatic melanoma who do or do not experience tumor regression following treatment with pembrolizumab.	• scRNAseq of freshly sorted tumor infiltrating T-lymphocytes and the RNAseq profile of the whole tumor from on-treatment tissue will be compared to baseline tissue. CD8+ T-cell clone killing function will be confirmed by TCR-transduced T-cells recognizing autologous tumor-derived cell lines.
• Objective: To evaluate the change in relative frequency of CD8+ T-cell clones with proven tumor reactivity in tumor tissue from participants with locally advanced or metastatic melanoma who do or do not experience tumor regression following treatment with pembrolizumab	• TCRs from CD8+ T-cell clones with proven tumor reactivity will be evaluated for changes from pembrolizumab-treated tissue samples.
• Objective: To compare the relative ratio of CD8+ effector T lymphocytes (Teff) to CD4+FOXP3+ regulatory T-cells (Tregs) in participants with locally advanced or metastatic melanoma who exhibit primary response vs. non-response.	• The Teff/Treg ratio will be estimated by coupling scRNAseq with immunohistochemistry (IHC) staining data for markers (not funded by this proposal) as well as performing RNAseq of bulk samples.

Objective/Hypothesis	Endpoint
• Objective: To profile the intra-tumoral heterogeneity of malignant, immune, and stromal cells before and after treatment with pembrolizumab.	• scRNAseq in combination with computational analysis will be used to reconstruct tumor circuits, immune circuits, and tumor-immune interactions from paired baseline and on-treatment samples.
• Objective: To evaluate the correlation between intra-tumoral and peripheral blood dynamics of specific TCR T-cell clones in responders (PR/CR) and non-responders (SD/PD/Other) treated with pembrolizumab.	• TCR data from blood and tumor will be generated from scRNAseq or adaptive TCR sequencing.
• Objective: To explore the relationship between genetic variation and response to the treatment(s) administered	• Variation across the human genome will be analyzed for association with clinical data collected in this study.

4. Study Design

4.1 Overall Design

This is a single-arm, open label, trial of pembrolizumab in participants with locally advanced/ or metastatic melanoma to be conducted in conformance with Good Clinical Practice (GCP). Approximately 60 participants with advanced/metastatic melanoma, RECIST 1.1-measureable disease, and lesions amenable to surgical resection or core biopsy will be enrolled. Participants will undergo a baseline excisional, core, or punch biopsy. Blood samples for biomarker analysis will also be collected at the time of each biopsy. All participants will receive pembrolizumab at a dose of 200 mg every 3 weeks (Q3W). Participants will undergo one or more on-treatment excisional, core, or punch biopsies. Additional blood samples for biomarker analyses will be collected from all participants during treatment and follow-up.

Participants will be evaluated with radiographic imaging to assess response to treatment at regular intervals. On study imaging will be assessed approximately every 9 weeks (63 days ± 7 days) until verified progressive disease (PD) or initiation of a new anticancer regimen. All imaging obtained on study will be reviewed by the Investigator using RECIST 1.1 for the determination of objective response rate (ORR) and progression-free survival (PFS). Treatment-based decisions may utilize site-assessed modified RECIST 1.1 for immune-based therapeutics (iRECIST) criteria (Section 8.2.1.5), which allows for participants with initial site-assessed PD to continue treatment until PD is confirmed by the site at least 4 weeks later. Skin lesions and other superficial lesions that are only assessed by physical examination will be classified as non-target lesions and their assessment should follow the imaging assessment schedule. Skin lesions will be documented at baseline with qualitative digital photography, and followed for response assessment in the same way at each scheduled tumor assessment.

Adverse Event (AE) monitoring will be ongoing throughout the trial and graded in severity according to the guidelines outlined in the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Treatment with pembrolizumab will continue until 35 administrations of pembrolizumab (approximately 2 years) of therapy have been completed, documented disease progression, unacceptable AE(s), intercurrent illness that prevents further administration of treatment, investigator's decision to withdraw the participant, participant withdraws consent, pregnancy, noncompliance with trial treatment or procedure requirements, or administrative reasons.

After the end of treatment, each participant will be followed for a minimum of 30 days to monitor for AEs and Events of Clinical Interest (ECI). Serious AEs (SAEs) will be collected for up to 90 days after the end of treatment, or 30 days following cessation of treatment if the participant initiates new anticancer therapy, whichever is earlier. Participants will have post-treatment follow-up for disease status, including initiating a non-study cancer treatment and, experiencing disease progression, death, withdrawal of consent, or lost to follow-up.

The primary endpoints of the trial are based on the assessments of the frequency and clonality of cytotoxic tumor-infiltrating T-lymphocytes in participants with evidence of radiographic response versus progression, as assessed by RECIST 1.1. Details of these correlative endpoints are described in Section 4.2.1.3.

Specific procedures to be performed during the study, as well as their prescribed times and associated visit windows, are outlined in the Schedule of Activities (SoA), Section 1.3. Details of each procedure are provided in Section 8.

4.2 Scientific Rationale for Study Design

4.2.1 Rationale for Endpoints

4.2.1.1 Efficacy Endpoints

Clinical efficacy endpoints will include ORR, PFS and overall survival (OS). Response and progressive disease will be assessed by RECIST 1.1.

Overall survival has been recognized as the gold standard for the demonstration of superiority of a new antineoplastic therapy in randomized clinical studies.

This trial will also use ORR and PFS based on RECIST 1.1 criteria as assessed by the investigator as secondary endpoints. ORR and PFS are acceptable measures of clinical benefit for a late stage trial that demonstrates superiority of a new antineoplastic therapy, especially if the magnitude of the effect is large and the therapy has an acceptable risk/benefit profile.

Biomarker data will be assessed comparatively in responders and non-responders.

4.2.1.1.1 RECIST 1.1

RECIST 1.1 will be used by the local site when assessing images for efficacy measures and when determining eligibility (Section 8.2.1.4). Although traditional RECIST 1.1 references a maximum of 5 target lesions in total and 2 per organ, this protocol has implemented a modification to RECIST 1.1 to allow a maximum of 10 target lesions in total and 5 per organ.

4.2.1.1.2 Modified RECIST 1.1 for Immune-based Therapeutics (iRECIST)

RECIST 1.1 will be adapted to account for the unique tumor response characteristics seen following treatment with pembrolizumab (Section 8.2.1.5). Immunotherapeutic agents such as pembrolizumab may produce antitumor effects by potentiating endogenous cancer-specific immune responses. The response patterns seen with such an approach may extend beyond the typical time course of responses seen with cytotoxic agents, and patients treated with pembrolizumab may manifest a clinical response after an initial increase in tumor burden or even the appearance of new lesions. Thus, standard RECIST 1.1 may, not provide an accurate response assessment of immunotherapeutic agents such as pembrolizumab. Based on an analysis of participants with melanoma enrolled in KEYNOTE-001 (KN001), 7% of evaluable participants experienced delayed or early tumor pseudo-progression. Of note, participants who had progressive disease (PD) by RECIST 1.1 but not by the immune-related response criteria [Wolchok, J. D., et al 2009] had longer overall survival than participants with PD by both criteria [Hodi, F. S., et al 2014]. Additionally, the data suggest that RECIST 1.1 may underestimate the benefit of pembrolizumab in approximately 15% of participants. These findings support the need to apply a modification to RECIST 1.1 that takes into account the unique patterns of atypical responses in immunotherapy and enables treatment beyond initial radiographic progression, if the participant is clinically stable.

iRECIST assessment has been developed and published by the RECIST Working Group, with input from leading experts from industry and academia, along with participation from the US Food and Drug Administration and the European Medicines Agency [Seymour, L., et al 2017]. The unidimensional measurement of target lesions, qualitative assessment of non-target lesions, and response categories are identical to RECIST 1.1, until progression is seen by RECIST 1.1. However, if a participant is clinically stable, additional imaging may be performed to confirm radiographic progression. iRECIST will be used by Investigators to assess tumor response and progression and make treatment decisions as well as for exploratory efficacy analyses where specified.

4.2.1.2 Safety Endpoints

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 4.0.

4.2.1.3 Planned Exploratory Biomarker Research

Cancer immunotherapies represent an important and novel class of antitumor agents. However, the mechanism of action of these exciting new therapies is not completely understood and much remains to be learned regarding how best to leverage these new drugs in treating patients. Thus, to aid future patients, it is important to investigate the determinants of response or resistance to cancer immunotherapy and other treatments administered, as well as determinants of AEs in the course of our clinical studies. These efforts may identify novel predictive/PD biomarkers and generate information that may better guide single-agent and combination therapy with immuno-oncology drugs. To identify novel biomarkers, biospecimens (ie, blood components, tumor material) will be collected to support analyses of

cellular components (eg, protein, DNA, RNA, metabolites) and other circulating molecules. Investigations may include but are not limited to:

Germline (blood) genetic analyses (eg, SNP analyses, whole exome sequencing, whole genome sequencing)

This research may evaluate whether genetic variation within a clinical study population correlates with response to the treatment(s) under evaluation. If genetic variation is found to predict efficacy or adverse events, the data might inform optimal use of therapies in the patient population. Furthermore, it is important to evaluate germline DNA variation across the genome in order to interpret tumor-specific DNA mutations. Finally, microsatellite instability (MSI) may be evaluated as this is an important biomarker for some cancers (ie, colorectal cancer).

Genetic (DNA) analyses from tumor

The application of new technologies, such as next generation sequencing, has provided scientists the opportunity to identify tumor-specific DNA changes (ie, mutations, methylation status, microsatellite instability). Key molecular changes of interest to immuno-oncology drug development include the mutational burden of tumors and the clonality of T-cells in the tumor microenvironment. Increased mutational burden (sometimes referred to as a 'hyper-mutated' state) may generate neo-antigen presentation in the tumor microenvironment. To conduct this type of research, it is important to identify tumor-specific mutations that occur across all genes in the tumor genome. Thus, genome-wide approaches may be used for this effort. Note that in order to understand tumor-specific mutations, it is necessary to compare the tumor genome with the germline genome. Microsatellite instability may also be evaluated as this is an important biomarker for some cancers (ie, colorectal cancer). Circulating tumor DNA and/or RNA may also be evaluated from blood samples.

Tumor and blood RNA analyses

Both genome-wide and targeted messenger RNA (mRNA) expression profiling and sequencing in tumor tissue and in blood may be performed to define gene signatures that correlate to clinical response to treatment with immunotherapies and/or other treatments administered. Immunotherapies induce a response in tumors that likely reflects an inflamed/immune phenotype. Specific immune-related gene sets (ie, those capturing interferon-gamma transcriptional pathways) may be evaluated and new signatures may be identified. Individual genes related to the immune system may also be evaluated (eg, IL-10). MicroRNA profiling may also be pursued as well as exosomal profiling.

Proteomics and immunohistochemistry (IHC) using blood or tumor

Tumor and blood samples from this study may undergo proteomic analyses (eg, PD-L1 IHC). PD L1 protein level in tumor sections, assessed by IHC, has been shown to correlate with response to immunotherapy in patients with NSCLC, and an in vitro diagnostic (IVD) device has been developed for use with immunotherapy in NSCLC. Preliminary data indicates that this association may also be true in additional cancer types (ie, triple negative breast cancer, head and neck, and gastric). Additional tumor or blood-derived proteins may also correlate with response to immunotherapy. Therefore, tumor tissue may be subjected to proteomic analyses using a variety of platforms that could include but are not limited to immunoassays

and liquid chromatography/mass spectrometry. This approach could identify novel protein biomarkers that could aid in patient selection for immunotherapy and/or treatments.

Other biomarkers

In addition to expression on the tumor tissue, PD-L1 and other tumor derived proteins can be shed from tumor and released into the blood. Assays such as enzyme-linked immunoassay (ELISA) that measure proteins may also be evaluated from blood samples. Correlation of these biomarkers with response to immunotherapy and/or treatments may identify new approaches for predictive biomarkers in blood, representing a major advance from today's reliance on assessing tumor biomarkers. This research would serve to develop such assays for future clinical use.

Other molecular changes of interest include the subtype of T-cells in the tumor microenvironment. The T-cell repertoire from tumor tissue and blood components may be evaluated.

4.2.1.4 Future Biomedical Research

The Sponsor will conduct future biomedical research on specimens for which consent was provided during this study. This research may include genetic analyses (DNA), gene expression profiling (ribonucleic acid [RNA]), proteomics, metabolomics (serum, plasma) and/or the measurement of other analytes, depending on which specimens are consented for future biomedical research.

Such research is for biomarker testing to address emergent questions not described elsewhere in the protocol (as part of the main study) and will only be conducted on specimens from appropriately consented participants. The objective of collecting/retaining specimens for future biomedical research is to explore and identify biomarkers that inform the scientific understanding of diseases and/or their therapeutic treatments. The overarching goal is to use such information to develop safer, more effective drugs/vaccines, and/or to ensure that participants receive the correct dose of the correct drug/vaccine at the correct time. The details of this future biomedical research substudy are presented in Appendix 2.

4.2.1.5 Planned Genetic Analysis

Understanding genetic determinants of drug response is an important endeavor during medical research. This research will evaluate whether genetic variation within a clinical trial population correlates with response to the treatment(s) under evaluation. If genetic variation is found to predict efficacy or adverse events, the data might inform optimal use of therapies in the patient population. This research contributes to understanding genetic determinants of efficacy and safety associated with the treatments in this study.

4.3 Justification for Dose

The planned dose of pembrolizumab for this study is 200 mg Q3W. Based on the totality of data generated in the Keytruda development program, 200 mg Q3W 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 Q3W to 10 mg/kg every 2 weeks (Q2W),

- Clinical data showing meaningful improvement in benefit-risk including overall survival at 200 mg Q3W 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 Q3W

Among the 8 randomized dose-comparison studies, a total of 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 Q3W versus 10 mg/kg Q2W (KEYNOTE-001 [KN001] Cohort B2, KN001 Cohort D, KN002, KN010, and KN021), and 3 studies compared 10 mg/kg Q3W versus 10 mg/kg Q2W (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) Q3W 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 Q3W 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 Q3W. First, PK data in KN001 evaluating target-mediated drug disposition (TMDD) conclusively demonstrated saturation of PD-1 in systemic circulation at doses much lower than 200 mg Q3W. 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 Q3W 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 Q3W fixed dose and 2 mg/kg Q3W dose. Supported by these PK characteristics, and given that fixed-dose has advantages of reduced dosing complexity and reduced potential of dosing errors, the 200 mg Q3W fixed-dose was selected for evaluation across all pembrolizumab protocols.

4.4 Beginning and End of Study Definition

The overall study begins when the first participant signs the Informed Consent Form (ICF). The overall study ends when the last participant completes the last study-related telephone-call or visit, withdraws from the study or is lost to follow-up (ie, the participant is unable to be contacted by the investigator). Upon study completion, participants are discontinued and enrolled in a pembrolizumab extension study (ML3475-587).

4.4.1 Clinical Criteria for Early Study Termination

There are no prespecified criteria for terminating the study early.

5. Study Population

Male/Female participants with a histologically-confirmed diagnosis of locally advanced or metastatic melanoma and who are at least 18 years of age on the day of signing the informed consent will be enrolled in this study.

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

5.1 Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

Type of Participant and Disease Characteristics

1. Male/female participants who are 18 years of age on the day of signing the informed consent with histologically confirmed diagnosis of unresectable stage III or metastatic melanoma not amenable to local therapy.
    - Participant may not have a diagnosis of uveal or ocular melanoma.
    - Participant who have not received prior systemic treatment (excluding adjuvant or neoadjuvant therapy) for melanoma or who have received one or more prior systemic treatments for melanoma are both eligible.
    - Participant must have testing for a BRAF mutation prior to study entry. Participants with BRAF V600E mutant melanoma may have received prior BRAF inhibitor therapy as first-line systemic therapy and are eligible for this study as second-line treatment. Participants with BRAF V600E mutant melanoma who have NOT received a BRAF inhibitor are also eligible for this study as first-line treatment.
2. Have measurable disease per RECIST 1.1 as assessed by the local site investigator/radiology. Cutaneous lesions and other superficial lesions that are detectable only by physical examination are not considered measurable lesions for the purposes of this protocol, but may be considered as non-target lesions. Tumor lesions situated in a previously irradiated area are considered measurable if progression has been demonstrated in such lesions.
3. Be willing and able to undergo pre-treatment and on-treatment baseline excisional and/or image-guided core or punch biopsies, and have lesions that are amenable to excisional biopsy, high pass core needle, and/or punch biopsy. Participants must have enough accessible lesions that, in the investigator's assessment, such biopsies can be performed both at baseline and at least 1 subsequent time point (i.e. at 6 weeks of treatment).

4. Have resolution of toxic effect(s) of the most recent prior chemotherapy to Grade 1 or less (except alopecia). If the participant received major surgery or radiation therapy of >30 Gy, they must have recovered from the toxicity and/or complications from the intervention.

   Note: Participants with ≤Grade 2 neuropathy or ≤Grade 2 alopecia are an exception to this criterion and may qualify for the study.

   Note: If the participant received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

Demographics

5. Have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, as assessed within 10 days prior to treatment initiation

Male Participants

Contraceptive use by men should be consistent with local regulations regarding the methods of contraception for those participating in clinical studies.

6. Male participants are eligible to participate if they agree to the following during the intervention period and for at least 120 days after the last dose of study intervention:

   Refrain from donating sperm

   PLUS either:

   Be abstinent from heterosexual intercourse as their preferred and usual lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent

   OR

   Must agree to use contraception, unless confirmed to be azoospermic (vasectomized or secondary to medical cause [Appendix 3]), as detailed below:

   Agree to use a male condom plus partner use of an additional contraceptive method when having penile-vaginal intercourse with a woman of child-bearing potential (WOCBP) who is not currently pregnant. Note: Men with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile-vaginal penetration.

7. Male participants must agree to use male condom when engaging in any activity that allows for passage of ejaculate to another person of any sex.

Female Participants

Contraceptive use by women should be consistent with local regulations regarding the methods of contraception for those participating in clinical studies.

8. A female participant is eligible to participate if she is not pregnant (see Appendix 3), not breastfeeding, and at least one of the following conditions applies:
   a.) Not a woman of childbearing potential (WOCBP) as defined in Appendix 3
   OR
   b.) A WOCBP who agrees to follow the contraceptive guidance in Appendix 3 during the treatment period and for at least 120 days after the last dose of study treatment.

Informed Consent

9. The participant (or legally acceptable representative if applicable) provides written informed consent/assent for the study. The participant may also provide consent/assent for future biomedical research. However the participant may participate in the main study without participating in future biomedical research.

Laboratory Values

10. Participant has adequate organ function as defined in Table 1; all screening laboratory tests should be performed within 10 days of treatment initiation.

Table 1 Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1500/µL
Platelets	≥100 000/µL
Hemoglobin	≥9.0 g/dL or ≥5.6 mmol/L[1]
Renal	
Creatinine OR Measured or calculated[2] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 × ULN OR ≥30 mL/min for participant with creatinine levels >1.5 × institutional ULN
Hepatic	
Total bilirubin	≤1.5 ×ULN OR direct bilirubin ≤ULN for participants with total bilirubin levels >1.5 × ULN
AST (SGOT) and ALT (SGPT)	≤2.5 × ULN (≤5 × ULN for participants with liver metastases)
Coagulation	
International normalized ratio (INR) OR prothrombin time (PT) Activated partial thromboplastin time (aPTT)	≤1.5 × ULN unless participant is receiving anticoagulant therapy as long as PT or aPTT is within therapeutic range of intended use of anticoagulants

Abbreviations: ALT (SGPT) = alanine aminotransferase (serum glutamic pyruvic transaminase); AST (SGOT) = aspartate aminotransferase (serum glutamic oxaloacetic transaminase); CrCl = creatinine clearance; GFR = glomerular filtration rate; ULN = upper limit of normal.

1. Criteria must be met without erythropoietin dependency and without packed red blood cell (pRBC) transfusion within last 2 weeks.
2. Creatinine clearance (CrCl) should be calculated per institutional standard.

Note: This table includes eligibility-defining laboratory value requirements for treatment; laboratory value requirements should be adapted according to local regulations and guidelines for the administration of specific chemotherapies.

5.2 Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

Medical Conditions

1. Has disease that is suitable for local therapy administered with curative intent.
2. Has a history of interstitial lung disease.
3. A WOCBP who has a positive urine pregnancy test within 72 hours before the first dose of study treatment (see Appendix 3). If the urine test cannot be confirmed as negative, a serum pregnancy test is required. In such cases, the participant must be excluded from participation if the serum pregnancy result is positive.

Prior/Concomitant Therapy

4. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent or with an agent directed to another stimulatory or co-inhibitory T-cell receptor (eg, CTLA-4, OX-40, CD137).
5. Has received prior systemic anti-cancer therapy including investigational agents within 4 weeks prior to allocation.

   Note: Participants must have recovered from all AEs due to previous therapies to ≤Grade 1 or baseline. Participants with ≤Grade 2 neuropathy may be eligible.

   Note: If participant received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting study treatment.
6. Has received prior radiotherapy within 2 weeks of start of study treatment. Participants must have recovered from all radiation-related toxicities, not require corticosteroids, and not have had radiation pneumonitis. A 1-week washout is permitted for palliative radiation (≤2 weeks of radiotherapy) to non-CNS disease.
7. Has received a live vaccine within 30 days prior to the first dose of study drug. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster (chicken pox), yellow fever, rabies, Bacillus Calmette–Guérin (BCG), and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (eg, FluMist®) are live attenuated vaccines and are not allowed.
8. Has received transfusion of blood products (including platelets or red blood cells) or administration of colony stimulating factors (including granulocyte colony stimulating factor [G-CSF], granulocyte macrophage colony stimulating factor [GM-CSF] or recombinant erythropoietin) within 4 weeks prior to study Day 1.

Prior/Concurrent Clinical Study Experience

9. Is currently participating in or has participated in a study of an investigational agent or has used an investigational device within 4 weeks prior to the first dose of study treatment.

Note: Participants who have entered the follow-up phase of an investigational study may participate as long as it has been 4 weeks after the last dose of the previous investigational agent.

Diagnostic Assessments

10. Has a diagnosis of immunodeficiency or is receiving chronic systemic steroid therapy (in dosing exceeding 10 mg daily of prednisone equivalent) or any other form of immunosuppressive therapy within 7 days prior the first dose of study drug.
11. Has a known additional malignancy that is progressing or has required active treatment within the past 3 years.

    Note: Participants with basal cell carcinoma of the skin, squamous cell carcinoma of the skin, transitional cell carcinoma of urothelial cancer, or carcinoma in situ (eg, breast carcinoma, cervical cancer in situ) that have undergone potentially curative therapy are not excluded.
12. Has known active CNS metastases and/or carcinomatous meningitis. Participants with previously treated brain metastases may participate provided they are radiologically stable, ie, without evidence of progression for at least 4 weeks by repeat imaging (note that the repeat imaging should be performed during study screening), clinically stable and without requirement of steroid treatment for at least 14 days prior to first dose of study treatment.
13. Has severe hypersensitivity (≥Grade 3) to pembrolizumab and/or any of its excipients.
14. Has an active autoimmune disease that has required systemic treatment in past 2 years (ie, with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg, thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency) is not considered a form of systemic treatment and is allowed.
15. Has a history of (non-infectious) pneumonitis that required steroids or has current pneumonitis.
16. Has an active infection requiring systemic therapy.
17. Has a known history of human immunodeficiency virus (HIV) infection. No HIV testing is required unless mandated by local health authority.
18. Has a known history of Hepatitis B (defined as Hepatitis B surface antigen [HBsAg] reactive) or known active Hepatitis C virus (defined as HCV RNA [qualitative] is detected) infection.
    Note: No testing for Hepatitis B and Hepatitis C is required unless mandated by local health authority.
19. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the study, interfere with the participant's participation for the full duration of the study, or is not in the best interest of the participant to participate, in the opinion of the treating investigator.

20. Has known psychiatric or substance abuse disorders that would interfere with cooperating with the requirements of the study.

Other Exclusions

21. Is pregnant or breastfeeding or expecting to conceive or father children within the projected duration of the study, starting with the screening visit through 120 days after the last dose of study treatment.
22. Is or has an immediate family member (eg, spouse, parent/legal guardian, sibling or child) who is investigational site or sponsor staff directly involved with this trial.

5.3 Lifestyle Considerations

5.3.1 Meals and Dietary Restrictions

Participants should maintain a normal diet unless modifications are required to manage an AE such as diarrhea, nausea, or vomiting.

5.3.2 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Refer to Appendix 3 for approved methods of contraception.

Participants should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study, participants of childbearing potential must adhere to the contraception requirement (Appendix 3) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 120 days after the last dose of study medication. If there is any question that a participant of childbearing potential will not reliably comply with the requirements for contraception, that participant should not be entered into the study.

5.3.3 Pregnancy

If a participant inadvertently becomes pregnant while on treatment with pembrolizumab, the participant will be immediately discontinued from study treatment. The site will contact the participant at least monthly and document the participant's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor without delay and within 24 hours if the outcome is an SAE (eg, death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn). The study Investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor. If a male participant impregnates his female partner, the study personnel at the site must be informed immediately and the pregnancy must be reported to the Sponsor and followed as described in Section 8.4.1.

5.3.4 Use in Nursing Women

It is unknown whether pembrolizumab is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, participants who are breastfeeding are not eligible for enrollment.

5.4 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any adverse events (AEs) or serious adverse events (SAEs) meeting reporting requirements as outlined in the data entry guidelines.

5.5 Participant Replacement Strategy

A participant who discontinues from study treatment will not be replaced.

6. Treatments

Study treatment is defined as any investigational treatment(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol.

Clinical supplies [study treatment(s) provided by the Sponsor] will be packaged to support enrollment. Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

6.1 Treatments Administered

The study treatment(s) to be used in this study is outlined below in Table 2.

Table 2 Study Treatment(s)

Study Treatment Name	Dose Formulation	Unit Dose Strength(s)	Dosage Level(s)	Route of Administration	Use	Sourcing
Pembrolizumab	Solution for infusion	100 mg/vial	200 mg Q3W	IV infusion	Experimental	Provided centrally by the sponsor
Abbreviations: IV = intravenous; Q3W = every 3 weeks						

All supplies indicated in Table 2 will be provided per the 'Sourcing' row depending upon local country operational requirements. Every attempt should be made to source these supplies from a single lot/batch number where possible (eg, not applicable in the case where multiple lots or batches may be required due to the length of the study etc.).

Refer to Section 8.1.8 for details regarding administration of the study treatment.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Dose Preparation

Details on preparation and administration of pembrolizumab are provided in the Pharmacy manual.

6.2.2 Handling, Storage and Accountability

The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatment received and any discrepancies are reported and resolved before use of the study treatment.

Only participants enrolled in the study may receive study treatment and only authorized site staff may supply or administer study treatment. All study treatments 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 treatment accountability, reconciliation, and record maintenance (ie, 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 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 treatments in accordance with the protocol and any applicable laws and regulations.

6.3 Measures to Minimize Bias: Randomization and Blinding

6.3.1 Method of Treatment Assignment

Participants participating in this study will be allocated by nonrandom assignment.

6.3.1.1 Stratification

No stratification based on age, sex or other characteristics will be used in this study.

6.3.2 Blinding

This study is an open-label study; therefore, the Sponsor, investigator and participant will know the treatment administered.
See Section 8.1.10 for a description of the method of unblinding a participant during the study, should such action be warranted.

6.4 Treatment Compliance

Interruptions from the protocol specified treatment plan for greater than 12 weeks between pembrolizumab doses require consultation between the investigator and the Sponsor and written documentation of the collaborative decision on participant management.

6.5 Concomitant Therapy

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing study. If there is a clinical indication for any medication or vaccination specifically prohibited, discontinuation from study treatment may be required. The investigator should discuss any questions regarding this with the Sponsor Clinical Director. The final decision on any supportive therapy or vaccination rests with the investigator and/or the participant's primary physician. However, the decision to continue the participant on study treatment requires the mutual agreement of the investigator, the Sponsor and the participant.

6.5.1 Acceptable Concomitant Therapy

All treatments that the investigator considers necessary for a participant's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF.

All concomitant medications received within 30 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs as defined in Section 8.4.

6.5.2 Prohibited Concomitant Therapy

Participants are prohibited from receiving the following therapies during the Screening and Treatment Phase (including retreatment for post-complete response relapse) of this trial:

- Antineoplastic systemic chemotherapy or biological therapy not specified in this protocol
- Immunotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy

  Note: Radiation therapy to a symptomatic lesion or to the brain may be considered on an exceptional case by case basis after consultation with Sponsor. The participant must have clear measurable disease outside the radiated field. Administration of palliative radiation therapy will be considered clinical progression for the purposes of determining PFS.

- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chickenpox, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed. However, intranasal influenza vaccines (e.g. FluMist®) are live attenuated vaccines, and are not allowed.

- Systemic glucocorticoids for any purpose other than to modulate symptoms from events of suspected immunologic etiology. The use of glucocorticoids for AEs not attributed to pembrolizumab is allowed with approval from the Sponsor. Physiologic doses of corticosteroids (not exceeding 10 mg of prednisone equivalent daily) are allowed.

Note: Inhaled steroids are allowed for management of asthma.

Participants who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial. Participants may receive other medications that the investigator deems to be medically necessary.

The Exclusion Criteria describe other medications that are prohibited in this trial.

There are no prohibited therapies during the Post-Treatment Follow-up Phase.

6.5.3 Rescue Medications and Supportive Care

Participants should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of AEs with potential immunologic etiology are outlined along with the dose modification guidelines in Section 6.6.1, Table 3. Where appropriate, these guidelines include the use of oral or IV treatment with corticosteroids, as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the Investigator determines the events to be related to pembrolizumab.

Note: If after the evaluation of the event, it is determined not to be related to pembrolizumab, the Investigator does not need to follow the treatment guidance. Refer to Table 3 in Section 6.6.1 for guidelines regarding dose modification and supportive care.

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event.

6.6 Dose Modification

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

AEs associated with pembrolizumab exposure may represent an immunologic etiology. These immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than one body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. 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. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab are provided in Table 3.

Table 3 Dose Modification and Toxicity Management Guidelines for Immune-related AEs Associated with Pembrolizumab

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 should 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.

irAEs	Toxicity grade or conditions (NCI CTCAE v4.0)	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 1-2 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 1-2 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 feasible, fluid and electrolytes should be substituted via IV infusion.
	Grade 4	Permanently discontinue		

irAEs	Toxicity grade or conditions (NCI CTCAE v4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
AST / ALT elevation or Increased bilirubin	Grade 2	Withhold	• Administer corticosteroids (initial dose of 0.5-1 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	• Administer corticosteroids (initial dose of 1-2 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[1]		
Hypothyroidism	Grade 2-4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyroinine) per standard of care	• Monitor for signs and symptoms of thyroid disorders.

irAEs	Toxicity grade or conditions (NCI CTCAE v4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
Nephritis and Renal dysfunction	Grade 2	Withhold	• Administer corticosteroids (prednisone 1-2 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 discontinue		
All other irAEs	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-Barré 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; irAE = immune-related adverse event; IV = intravenous; NCI = National Cancer Institute; T1DM = type 1 diabetes mellitus.

1. 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).

6.6.2 Dose Modification and Toxicity Management of Infusion-reactions Related to Pembrolizumab

Pembrolizumab may cause severe or life threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines on pembrolizumab associated infusion reaction are provided in Table 4.

Table 4 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 (e.g., 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 (e.g. 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 500-1000 mg PO (or equivalent dose of analgesic).

NCI CTCAE Grade	Treatment	Premedication at Subsequent Dosing
Grades 3 or 4 **Grade 3**: Prolonged (ie, not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) **Grade 4**: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: • Epinephrine** • IV fluids • Antihistamines • NSAIDs • Acetaminophen • Narcotics • Oxygen • Pressors • Corticosteroids Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately. **Participant is permanently discontinued from further study drug treatment.**	No subsequent dosing

Abbreviations: CTCAE = Common Terminology Criteria for Adverse Events; IV – intravenous; NCI – National Cancer Institute; NSAIDS = nonsteroidal anti-inflammatory drugs; PO = by mouth.

Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration.

For further information, please refer to the CTCAE v4.0 at http://ctep.cancer.gov

6.6.3 Other Allowed Dose Interruption for Pembrolizumab

Pembrolizumab may be interrupted for situations other than treatment-related AEs such as medical / surgical events or logistical reasons not related to study therapy. Participants should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the participant's study record.

6.7 Treatment After the End of the Study

There is no study-specified treatment following the end of the study.

6.8 Clinical Supplies Disclosure

This study is open-label; therefore, the participant, the study site personnel, the Sponsor and/or designee are not blinded. Study treatment (name, strength or potency) is included in the label text; random code/disclosure envelopes or lists are not provided.

7. Discontinuation of Study Treatment and Participant Withdrawal

7.1 Discontinuation of Study Treatment

Discontinuation of study treatment does not represent withdrawal from the study.

As certain data on clinical events beyond study treatment discontinuation may be important to the study, they must be collected through the participant’s last scheduled follow-up, even if the participant has discontinued study treatment. Therefore, all participants who discontinue study treatment prior to completion of the protocol-specified treatment period will still continue to participate in the study as specified in Section 1.3 and Section 8.10.3.

Participants may discontinue study treatment at any time for any reason or be discontinued from the study treatment at the discretion of the investigator should any untoward effect occur. In addition, a participant may be discontinued from study treatment by the investigator or the Sponsor if study treatment is inappropriate, the study plan is violated, or for administrative and/or other safety reasons. Specific details regarding procedures to be performed at study treatment discontinuation are provided in Section 8.1.9.

A participant must be discontinued from study treatment but continue to be monitored in the study for any of the following reasons:

- The participant or participant’s legally acceptable representative requests to discontinue study treatment.
- Radiographic disease progression as determined by local/site assessment

  Note: For unconfirmed radiographic disease progression, or confirmed progression with reduction in disease burden from baseline please see Section 8.2.1.4
- Any progression or recurrence of any malignancy, or any occurrence of another malignancy that requires active treatment
- Noncompliance with study treatment or procedure requirements
- Recurrent Grade 2 pneumonitis

- Unacceptable AEs as described in Section 6.6
- Intercurrent illness that prevents further administration of study treatment
- The participant has a confirmed positive serum pregnancy test
- Investigator's decision to withdraw the participant
- The participant is lost to follow-up (Section 7.3)
- Completed 35 cycles (~2 years) of treatment with pembrolizumab
- Administrative reasons

For participants who are discontinued from study treatment but continue to be monitored in the study, see Section 1.3 and Section 8.10.3 for those procedures to be completed at each specified visit.

Discontinuation from study treatment is "permanent." Once a participant is discontinued, he/she shall not be allowed to restart study treatment.

7.2 Participant Withdrawal From the Study

A participant must be withdrawn from the study if the participant or participant's legally acceptable representative withdraws consent from the study.

If a participant withdraws from the study, they will no longer receive study treatment or be followed at scheduled protocol visits.

Specific details regarding procedures to be performed at the time of withdrawal from the study, as well as specific details regarding withdrawal from future biomedical research are outlined in Section 8.1.9. The procedures to be performed should a participant repeatedly fail to return for scheduled visits and/or if the study site is unable to contact the participant are outlined in Section 7.3.

7.3 Lost to Follow-up

If a participant fails to return to the clinic for a required study visit and/or if the site is unable to contact the participant, the following procedures are to be performed:

- The site must attempt to contact the participant and reschedule the missed visit. If the participant is contacted, the participant should be counseled on the importance of maintaining the protocol-specified visit schedule.
- The investigator or designee must make every effort to regain contact with the participant at each missed visit (eg, telephone calls and/or a certified letter to the participant's last known mailing address or locally equivalent methods). These contact attempts should be documented in the participant's medical record.
- Note: A participant is not considered lost to follow-up until the last scheduled visit for the individual participant. The missing data for the participant will be managed via the prespecified statistical data handling and analysis guidelines.

8. Study Assessments and Procedures

- Study procedures and their timing are summarized in the SoA.
- Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- The investigator is responsible for assuring that procedures are conducted by appropriately qualified or trained staff. Delegation of study site personnel responsibilities will be documented in the Investigator Trial File Binder (or equivalent).
- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the participant's routine clinical management (eg, blood count) and obtained before signing of ICF may be utilized for screening or baseline purposes provided the procedure met the protocol-specified criteria and were performed within the time frame defined in the SoA.
- Additional evaluations/testing may be deemed necessary by the investigator and or the Sponsor for reasons related to participant safety. In some cases, such evaluation/testing may be potentially sensitive in nature (eg, HIV, Hepatitis C, etc.), and thus local regulations may require that additional informed consent be obtained from the participant. In these cases, such evaluations/testing will be performed in accordance with those regulations.

Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

8.1 Administrative and General Procedures

8.1.1 Informed Consent

The investigator or qualified designee must obtain documented consent from each potential participant or each participant's legally acceptable representative prior to participating in a clinical study or future biomedical research. If there are changes to the participant's status during the study (eg, health or age of majority requirements), the investigator or qualified designee must ensure the appropriate consent is in place.

8.1.1.1 General Informed Consent

Consent must be documented by the participant's dated signature or by the participant's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion.

A copy of the signed and dated consent form should be given to the participant before participation in the study.

The initial ICF, any subsequent revised written ICF and any written information provided to the participant must receive the Institutional Review Board/Independent Ethics Committee's (IRB/IEC's) approval/favorable opinion in advance of use. The participant or his/her legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the participant's willingness to continue participation in the study. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the participant's dated signature or by the participant's legally acceptable representative's dated signature.

The participant or his/her legally acceptable representative will be asked to sign consent at the point of initial radiographic disease progression.

Specifics about a study and the study population will be added to the consent form template at the protocol level.

The informed consent will adhere to IRB/IEC requirements, applicable laws and regulations and Sponsor requirements.

8.1.1.2 Consent and Collection of Specimens for Future Biomedical Research

The investigator or qualified designee will explain the future biomedical research consent to the participant, answer all of his/her questions, and obtain written informed consent before performing any procedure related to the future biomedical research substudy. A copy of the informed consent will be given to the participant.

8.1.2 Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the participant qualifies for the study.

8.1.3 Participant Identification Card

All participants will be given a Participant Identification Card identifying them as participants in a research study. The card will contain study site contact information (including direct telephone numbers) to be utilized in the event of an emergency. The investigator or qualified designee will provide the participant with a Participant Identification Card immediately after the participant provides written informed consent. At the time of treatment allocation/randomization, site personnel will add the treatment/randomization number to the Participant Identification Card.

The participant identification card also contains contact information for the emergency unblinding call center so that a health care provider can obtain information about study treatment in emergency situations where the investigator is not available.

8.1.4 Medical History

A medical history will be obtained by the investigator or qualified designee.

8.1.5 Prior and Concomitant Medications Review

8.1.5.1 Prior Medications

The investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the participant within 30 days before the first dose of study medication.

8.1.5.2 Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the participant during the study.

8.1.6 Assignment of Screening Number

All consented participants will be given a unique screening number that will be used to identify the participant for all procedures that occur prior to treatment allocation. Each participant will be assigned only one screening number. Screening numbers must not be re-used for different participants.

Any participant who is screened multiple times will retain the original screening number assigned at the initial screening visit.

Specific details on the screening visit requirements (screening/rescreening) are provided in Section 8.10.1.

8.1.7 Assignment of Treatment/Randomization Number

All eligible participants will be allocated, by nonrandom assignment, and will receive a treatment/randomization number. The treatment/randomization number identifies the participant for all procedures occurring after treatment allocation/randomization. Once a treatment/randomization number is assigned to a participant, it can never be re-assigned to another participant.

A single participant cannot be assigned more than 1 treatment/randomization number.

In a situation where treatment reallocation of the participants is planned (eg, study extension periods), it will be based on a new allocation schedule; however, each participant will retain his/her original treatment/randomization number. Only the study intervention regimen associated with the treatment reallocation period or phase may change.

8.1.8 Treatment Administration

Administration of study medication will be witnessed by the investigator and/or study staff.

Study Treatment should begin within 3 days of treatment allocation.

8.1.8.1 Timing of Dose Administration

Pembrolizumab will be administered on Day 1 of each 3-week treatment cycle after all procedures and assessments have been completed as detailed in the SoA.

Pembrolizumab can be administered ±3 days of the targeted Day 1 for each cycle, except Cycle 1 where treatment can only be administered +3 days of the targeted Day 1.

Pembrolizumab will be administered at a dose of 200mg using a 30-minute IV infusion. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window between -5 minutes and +10 minutes is permitted (i.e., the infusion time is 30 minutes: -5 min/+10 min).

8.1.9 Discontinuation and Withdrawal

Participants who discontinue study treatment prior to completion of the treatment period should be encouraged to continue to be followed for all remaining study visits.

When a participant withdraws from participation in the study, all applicable activities scheduled for the final study visit should be performed (at the time of withdrawal). Any AEs which are present at the time of withdrawal should be followed in accordance with the safety requirements outlined in Section 8.4 .

8.1.9.1 Withdrawal From Future Biomedical Research

Participants may withdraw their consent for future biomedical research. Participants may withdraw consent at any time by contacting the principal investigator for the main study. If medical records for the main study are still available, the investigator will contact the Sponsor using the designated mailbox (clinical.specimen.management@merck.com). Subsequently, the participant's consent for future biomedical research will be withdrawn. A letter will be sent from the Sponsor to the investigator confirming the withdrawal. It is the responsibility of the investigator to inform the participant of completion of withdrawal. Any analyses in progress at the time of request for withdrawal or already performed prior to the request being received by the Sponsor will continue to be used as part of the overall research study data and results. No new analyses would be generated after the request is received.

In the event that the medical records for the main study are no longer available (eg, if the investigator is no longer required by regulatory authorities to retain the main study records) or the specimens have been completely anonymized, there will no longer be a link between the participant’s personal information and their specimens. In this situation, the request for specimen withdrawal cannot be processed.

8.1.10 Participant Blinding/Unblinding

This is an open label study; there is no blinding for this study.

8.1.11 Calibration of Equipment

The investigator or qualified designee has the responsibility to ensure that any device or instrument used for a clinical evaluation/test during a clinical study that provides information about inclusion/exclusion criteria and/or safety or efficacy parameters shall be suitably calibrated and/or maintained to ensure that the data obtained is reliable and/or reproducible. Documentation of equipment calibration must be retained as source documentation at the study site.

8.2 Efficacy Assessments

8.2.1 Tumor Imaging and Assessment of Disease

. Tumor imaging is strongly preferred to be acquired by computed tomography (CT). For the abdomen and pelvis, contrast-enhanced magnetic resonance imaging (MRI) may be used when CT with iodinated contrast is contraindicated, or when mandated by local practice. MRI is the strongly preferred modality for imaging the brain. The same imaging technique regarding modality, ideally the same scanner, and the use of contrast should be used in a participant throughout the study to optimize the reproducibility of the assessment of existing and new tumor burden and improve the accuracy of the assessment of response or progression based on imaging. Note: for the purposes of assessing tumor imaging, the term "Investigator" refers to the local investigator at the site and/or the radiological reviewer located at the site or at an offsite facility.

Participant eligibility will be determined using local assessment (Investigator assessment) based on RECIST 1.1. In addition, images (including via other modalities) that are obtained at an unscheduled time point to determine disease progression, as well as imaging obtained for other reasons, but which demonstrate radiologic progression, should also be reviewed by the investigator.

If the Investigator considers the participant has progressed, but elects to implement iRECIST, the Investigator will assess for confirmation of progression by iRECIST at subsequent time points.

8.2.1.1 Initial Tumor Imaging

Initial tumor imaging must be performed within 28 days prior to the first dose of trial treatment. The site study team must review screening images to confirm the participant has measurable disease per RECIST 1.1.

Tumor imaging performed as part of routine clinical management is acceptable for use as screening tumor imaging if it is of diagnostic quality and performed within 28 days prior to the date of allocation.

If brain imaging is performed to document the stability of existing metastases, MRI should be used if possible. If MRI is medically contraindicated, CT with contrast is an acceptable alternative.

8.2.1.2 Tumor Imaging During the Study

The first on-study imaging assessment should be performed at 9 weeks (63 days ±7 days) from the date of allocation. Subsequent imaging should be performed every 9 weeks (63 days ±7 days) or more frequently if clinically indicated. After 12 months (52 weeks ±7 days), participants who remain on treatment will have imaging performed every 12 weeks (84 days ±7 days). Imaging timing should follow calendar days and should not be adjusted for delayed for delays in cycle starts. Imaging should continue to be performed until disease progression is identified by the Investigator (unless the Investigator elects to continue treatment and follow iRECIST), the start of new anticancer treatment, withdrawal of consent, death, or the end of the study, whichever occurs first.

Imaging should continue to be performed until disease recurrence is assessed identified by the investigator (unless the Investigator elects to continue treatment and follow iRECIST), the start of new anti-cancer treatment, withdrawal of consent, death, or the end of the study, whichever occurs first.

Objective response should be confirmed by a repeat imaging assessment. Tumor imaging to confirm PR or CR should be performed at least 4 weeks after the first indication of a response is observed. Participants will then return to regular scheduled imaging, starting with the next scheduled imaging time point. Participants who receive additional imaging for confirmation do not need to undergo the next scheduled tumor imaging if it is less than 4 weeks later; tumor imaging may resume at the subsequent scheduled imaging time point.

Per iRECIST (Section 8.2.1.5), disease progression should be confirmed by the site 4 to 8 weeks after site-assessed first radiologic evidence of PD in clinically stable participants. Participants who have unconfirmed disease progression may continue on treatment at the discretion of the Investigator until progression is confirmed by the site, provided they have met the conditions detailed in Section 8.2.1.5. Participants who receive confirmatory imaging do not need to undergo the next scheduled tumor imaging if it is less than 4 weeks later; tumor imaging may resume at the subsequent scheduled imaging time point, if clinically stable. Participants who have confirmed disease progression by iRECIST, as assessed by the site, will discontinue study treatment. Exceptions are detailed in Section 8.2.1.5.

8.2.1.3 End of Treatment and Follow-up Imaging

For participants who discontinue study treatment, tumor imaging should be performed at the time of treatment discontinuation (±4 week window). If previous imaging was obtained within 4 weeks prior to the date of discontinuation, then imaging at treatment discontinuation is not mandatory. For participants who discontinue study treatment due to documented disease progression, this is the final required tumor imaging if the Investigator elects not to implement iRECIST.

For participants who discontinue study treatment without documented disease progression, every effort should be made to continue monitoring disease status by tumor imaging using the same imaging schedule used while on treatment (approximately every 9 weeks in Year 1 or approximately every 12 weeks after Year 1) until the start of a new anticancer treatment, disease progression, pregnancy, death, withdrawal of consent, or the end of the study, whichever occurs first.

8.2.1.4 RECIST 1.1 Assessment of Disease

RECIST 1.1 will be used by the investigator as the primary measure for assessment of tumor response, date of disease progression, and as a basis for all protocol guidelines related to disease status (eg, discontinuation of study treatment). Although RECIST 1.1 references a maximum of 5 target lesions in total and 2 per organ, this protocol allows a maximum of 10 target lesions in total and 5 per organ, if clinically relevant to enable a broader sampling of tumor burden. Figure 2 illustrates the imaging flow involving verification of PD for clinically stable participants.

8.2.1.5 iRECIST Assessment of Disease

iRECIST is based on RECIST 1.1, but adapted to account for the unique tumor response seen with immunotherapeutic drugs. iRECIST will be used by the Investigator to assess tumor response and progression, and make treatment decisions. When clinically stable, participants should not be discontinued until progression is confirmed by the Investigator, working with local radiology, according to the rules outlined in Appendix 6. This allowance to continue treatment despite initial radiologic PD takes into account the observation that some participants can have a transient tumor flare in the first few months after the start of immunotherapy, and then experience subsequent disease response. This data will be captured in the clinical database.

Clinical stability is defined as the following:

- Absence of symptoms and signs indicating clinically significant progression of disease
- No decline in ECOG performance status
- No requirements for intensified management, including increased analgesia, radiation, or other palliative care

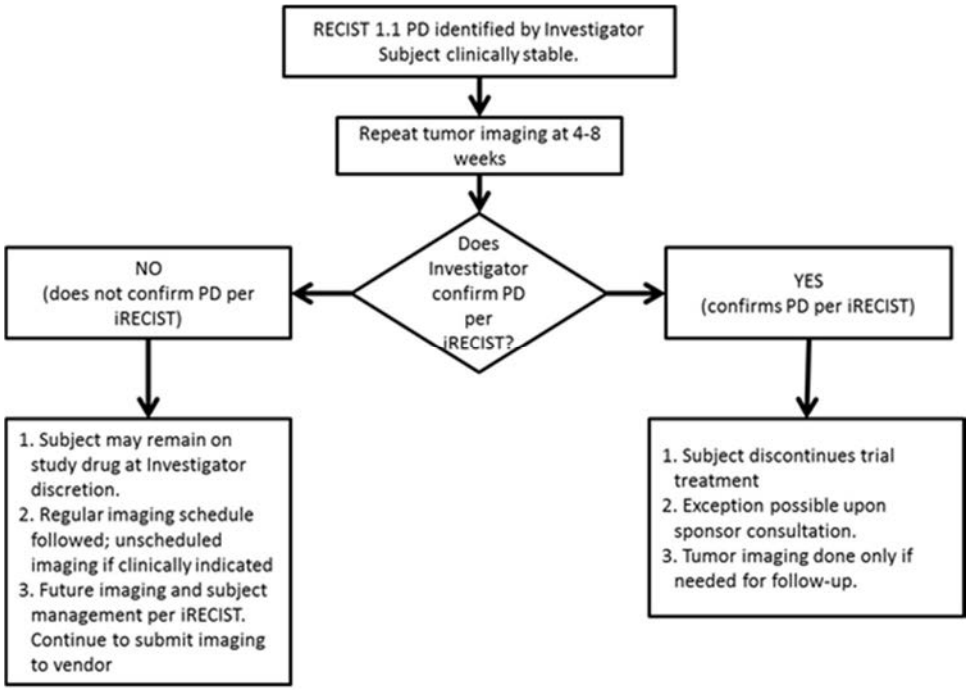
Any participant deemed **clinically unstable** should be discontinued from study treatment at site-assessed first radiologic evidence of PD, and is not required to have repeat tumor imaging for confirmation of PD by iRECIST.

If the Investigator decides to continue treatment, the participant may continue to receive study treatment and the tumor assessment should be repeated 4 to 8 weeks later to confirm PD by iRECIST, per Investigator assessment.

If repeat imaging does not confirm PD per iRECIST, as assessed by the Investigator, and the participant continues to be clinically stable, study treatment may continue and follow the regular imaging schedule. If PD is confirmed, participants will be discontinued from study treatment.

If a participant has confirmed radiographic progression (iCPD) as defined in Appendix 6, study treatment should be discontinued; however, if the participant is achieving a clinically meaningful benefit, an exception to continue study treatment may be considered following consultation with the Sponsor. In this case, if study treatment is continued, tumor imaging should continue to be performed following the intervals as outlined in Section 1.3.

A description of the adaptations and iRECIST process is provided in Appendix 6, with additional details in the iRECIST publication [Seymour, L., et al 2017]. A summary of imaging and treatment requirements after first radiologic evidence of progression is provided in Table 5 and illustrated as a flowchart in Figure 2.



Abbreviations: iRECIST=modified Response Evaluation Criteria in Solid Tumors 1.1 for immune-based therapeutics; PD=progressive disease; RECIST 1.1=Response Evaluation Criteria in Solid Tumors 1.1.

Figure 2 Imaging and Treatment for Clinically Stable Participants Treated with Pembrolizumab After First Radiologic Evidence of PD by the Investigator

Table 5 Imaging and Treatment After First Radiologic Evidence of Progressive Disease

	Clinically Stable		Clinically Unstable	
	Imaging	**Treatment**	**Imaging**	**Treatment**
First radiologic evidence of PD by RECIST 1.1	Repeat imaging at 4 to 8 weeks to confirm PD.	May continue study treatment at the Investigator's discretion while awaiting confirmatory tumor imaging by site by iRECIST.	Repeat imaging at 4 to 8 weeks to confirm PD per Investigator's discretion only.	Discontinue treatment
Repeat tumor imaging confirms PD (iCPD) by iRECIST per Investigator assessment	No additional imaging required.	Discontinue treatment (exception is possible upon consultation with Sponsor).	No additional imaging required.	Not applicable
Repeat tumor imaging shows iUPD by iRECIST per Investigator assessment	Repeat imaging at 4 to 8 weeks to confirm PD. May occur at next regularly scheduled imaging visit.	Continue study treatment at the Investigator's discretion.	Repeat imaging at 4 to 8 weeks to confirm PD per Investigator's discretion only.	Discontinue treatment
Repeat tumor imaging shows iSD, iPR, or iCR by iRECIST per Investigator assessment.	Continue regularly scheduled imaging assessments.	Continue study treatment at the Investigator's discretion.	Continue regularly scheduled imaging assessments.	May restart study treatment if condition has improved and/or clinically stable per Investigator's discretion. Next tumor imaging should occur according to the regular imaging schedule.

Abbreviations: iCPD=iRECIST confirmed progressive disease; iCR=iRECIST complete response; iRECIST=modified Response Evaluation Criteria in Solid Tumors 1.1 for immune-based therapeutics; iSD=iRECIST stable disease; iUPD=iRECIST unconfirmed progressive disease; PD=progressive disease; RECIST 1.1=Response Evaluation Criteria in Solid Tumors 1.1.

Note: If progression has been verified, further management is by the site, based on iRECIST. Any further imaging should still be reviewed by the investigator. If RECIST 1.1 disease progression has not been verified, ideally the site should continue treatment. Whether or not treatment continues, imaging should be collected and by the Investigator until PD is verified

8.2.2 Photography for Cutaneous Lesions

Skin lesions will be classified as non-target lesions and their assessment should follow the imaging assessment schedule. Skin lesions will be documented at baseline with qualitative digital photography, and followed for response assessment in the same way at each scheduled tumor assessment. The timing for capturing cutaneous lesion photographs should follow the same schedule as the imaging scans.

8.3 Safety Assessments

Details regarding specific safety procedures/assessments to be performed in this study are provided below. The total amount of blood/tissue to be drawn/collected over the course of the study (from prestudy to poststudy visits), including approximate blood/tissue volumes drawn/collected by visit and by sample type per participant can be found in Section 8.

Planned time points for all safety assessments are provided in the SoA.

8.3.1 Physical Examinations

8.3.1.1 Complete Physical Examination

The investigator or qualified designee will perform a complete physical exam during screening, at Day 1 of each cycle, and during follow up, as specified in Section 1.3. After the consent form is signed, new clinically significant abnormal physical examination findings should be recorded as AEs.

8.3.1.2 Directed Physical Examination

The investigator or qualified designee will perform a directed physical exam as per Section 1.3 and as clinically indicated. New clinically significant abnormal findings should be recorded as AEs.

8.3.2 Vital Signs

The investigator or qualified designee will take vital signs as specified in the SoA (Section 1.3). Vital signs should include temperature, pulse, respiratory rate, and blood pressure. Weight will be recorded as specified in the SoA. Height will be measured at screening only.

8.3.3 Electrocardiograms

A standard 12-lead ECG will be performed using local standard procedures at Screening. Clinically significant abnormal findings should be recorded as medical history. Additional time points for standard 12-lead ECGs will be performed as per Section 1.3. Clinically significant abnormal findings seen on the follow-up ECGs should be recorded as AEs.

8.3.4 Eastern Cooperative Oncology Group Performance Scale

The investigator or qualified designee will assess ECOG status at every visit.

8.3.5 Clinical Safety Laboratory Assessments

Refer to Appendix 5 for the list of clinical laboratory tests to be performed and to the SoA for the timing and frequency.

- The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the case report form (CRF). The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.
- All protocol-required laboratory assessments, as defined in Appendix 5, must be conducted in accordance with the laboratory manual and the SoA.
- If laboratory values from nonprotocol specified laboratory assessments performed at the institution's local laboratory require a change in study participant management or are considered clinically significant by the investigator (eg, SAE or AE or dose modification), then the results must be recorded in the appropriate CRF (eg, SLAB).
- For any laboratory tests with values considered clinically significantly abnormal during participation in the study or within 30 days after the last dose of study treatment, every attempt should be made to perform repeat assessments until the values return to normal or baseline or if a new baseline is established as determined by the investigator.

8.3.5.1 Pregnancy Testing

All women who are being considered for participation in the trial, and who are not surgically sterilized or postmenopausal, must be tested for pregnancy within 72 hours of each cycle of trial treatment and 30 days post-treatment. If a urine test is positive or not evaluable, a serum test will be required. Participants must be excluded/discontinued from the trial in the event of a positive or borderline-positive test result.

8.4 Adverse Events (AEs), Serious Adverse Events (SAEs), and Other Reportable Safety Events

The definitions of an AE or SAE, as well as the method of recording, evaluating, and assessing causality of AE and SAE and the procedures for completing and transmitting AE, SAE, and other reportable safety event reports can be found in Appendix 4.

Progression of the cancer under study is not considered an AE as described in Section 8.4.5 and Appendix 4.

AE, SAEs, and other reportable safety events will be reported by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative).

The investigator, who is a qualified physician, and any designees are responsible for detecting, assessing, documenting, and reporting events that meet the definition of an AE or SAE as well as other reportable safety events. Investigators remain responsible for following up AE, SAEs, and other reportable safety events for outcome according to Section 8.4.3.

8.4.1 Time Period and Frequency for Collecting AE, SAE, and Other Reportable Safety Event Information

All AEs, SAEs, and other reportable safety events that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if the participant is receiving placebo run-in or other run-in treatment, if the event causes the participant to be excluded from the study, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, or a procedure.

- All AEs from the time of treatment allocation/randomization through 30 days following cessation of study treatment must be reported by the investigator.
- All AEs meeting serious criteria, from the time of treatment allocation/randomization through 90 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anticancer therapy, whichever is earlier must be reported by the investigator.
- All pregnancies and exposure during breastfeeding, from the time of treatment allocation/randomization through 120 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anticancer therapy must be reported by the investigator.
- Additionally, any SAE brought to the attention of an investigator at any time outside of the time period specified above must be reported immediately to the Sponsor if the event is considered to be drug-related.

Investigators are not obligated to actively seek AE or SAE or other reportable safety events in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study treatment or study participation, the investigator must promptly notify the Sponsor.

All initial and follow-up AEs, SAEs and other reportable safety events will be recorded and reported to the Sponsor or designee within the time frames as indicated in Table 6.

Table 6 Reporting Time Periods and Time Frames for Adverse Events and Other Reportable Safety Events

Type of Event	Time Period			Time Frame to Report Event and Follow-up Information to SPONSOR:
	Consent to Randomization / Allocation	Randomization / Allocation through Protocol-Specified Follow-up Period	After the Protocol Specified Follow-up Period	
Non-Serious Adverse Event (NSAE)	Report if: - due to protocol-specified intervention - causes exclusion - participant is receiving placebo run-in or other run-in treatment	Report all	Not required	Per data entry guidelines
Serious Adverse Event (SAE) including Cancer and Overdose	Report if: - due to protocol-specified intervention - causes exclusion - participant is receiving placebo run-in or other run-in treatment	Report all	Report if: - drug/vaccine related. (Follow ongoing to outcome)	Within 24 hours of learning of event
Pregnancy/Lactation Exposure	Report if: - due to intervention - causes exclusion	Report all	Previously reported – Follow to completion/termination ; report outcome	Within 24 hours of learning of event
Event of Clinical Interest (require regulatory reporting)	Report if: - due to intervention - causes exclusion	Report - Potential DILI - Require regulatory reporting	Not required	Within 24 hours of learning of event
Event of Clinical Interest (Do not require regulatory reporting)	Report if: - due to intervention - causes exclusion	Report - non-DILI ECIs and those not requiring regulatory reporting	Not required	Within 5 calendar days of learning of event

8.4.2 Method of Detecting AEs, SAEs, and Other Reportable Safety Events

Care will be taken not to introduce bias when detecting AE and/or SAE and other reportable safety events. Open-ended and nonleading verbal questioning of the participant is the preferred method to inquire about AE occurrence.

8.4.3 Follow-up of AE, SAE and Other Reportable Safety Event Information

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All AE, SAE, and other reportable safety events including pregnancy and exposure during breastfeeding, events of clinical interest (ECIs), Cancer and Overdose will be followed until resolution, stabilization, until the event is otherwise explained, or the participant is lost to follow-up (as defined in Section 7.3). In addition, the investigator will make every attempt to follow all nonserious AEs that occur in randomized participants for outcome. Further information on follow-up procedures is given in Appendix 4.

8.4.4 Regulatory Reporting Requirements for SAE

- Prompt notification (within 24 hours) by the investigator to the Sponsor of SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study treatment under clinical investigation are met.
- The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study treatment under clinical investigation. All AEs will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations, ie, per ICH Topic E6 (R2) Guidelines for GCP.
- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSARs) according to local regulatory requirements and Sponsor policy and forwarded to investigators as necessary.
- An investigator who receives an investigator safety report describing an SAE or other specific safety information (eg, summary or listing of SAE) from the Sponsor will file it along with the IB and will notify the IRB/IEC, if appropriate according to local requirements.

8.4.5 Disease-related Events and/or Disease-related Outcomes Not Qualifying as AEs or SAEs

Efficacy endpoints as outlined in this section will not be reported to the Sponsor as described in Section 8.4.1.

Specifically, the suspected/actual events covered in this exception include any event that is disease progression of the cancer under study.

The Sponsor will monitor unblinded aggregated efficacy endpoint events and safety data to ensure the safety of the participants in the study. Any suspected endpoint which upon review is not progression of the cancer under study will be forwarded to Global Pharmacovigilance as an SAE within 24 hours of determination that the event is not progression of the cancer under study.

8.4.6 Pregnancy and Exposure During Breastfeeding

Although pregnancy and infant exposure during breastfeeding are not considered AEs, any pregnancy or infant exposure during breastfeeding in a participant (spontaneously reported to the investigator or their designee) that occurs during the study are reportable to the Sponsor.

All reported pregnancies must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

8.4.7 Events of Clinical Interest (ECIs)

Selected nonserious and serious adverse events are also known as ECIs and must be reported to the Sponsor.

Events of clinical interest for this study include:

1. an overdose of Sponsor's product, as defined in Section 8.5, that is not associated with clinical symptoms or abnormal laboratory results.
2. an elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

*Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology. The study site guidance for assessment and follow-up of these criteria can be found in the Investigator Trial File Binder (or equivalent).

8.5 Treatment of Overdose

For this study, an overdose of pembrolizumab will be defined as any dose of 1000 mg or greater (≥5 times the indicated dose).

No specific information is available on the treatment of overdose of pembrolizumab. In the event of overdose, the participant should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

8.6 Pharmacokinetics

PK parameters will not be evaluated in this study.

8.6.1 Blood Collection for Biomarker Analyses

Sample collection, storage and shipment instructions for blood and plasma samples will be provided in the procedures manual.

8.7 Pharmacodynamics

8.7.1 Tumor Tissue Collection

Fresh tumor tissue will be obtained at baseline (during the screening period) and on-treatment. On-treatment biopsies prior to radiographic determination of response or progression should be obtained at Week 6 (42 days ± 3 days) to evaluate pharmacodynamics in participants who subsequently respond, progress, or have SD. If a biopsy is obtained at this time point, then a second on-treatment biopsy timed with response/progression is optional. Excisional biopsy, punch biopsy and high-pass core needle biopsy will be permitted. Additional information on biopsy requirements and tissue handling can be found in the Procedures Manual.

8.8 Biomarkers

The following correlative blood samples will be collected:

- PBMCs
- Whole blood (for RNA analysis)
- Whole blood (for DNA analysis)
- Serum for biomarker analysis
- Plasma for biomarker analysis

Please refer to the Procedures Manual for approximate blood volumes to be collected.

8.8.1 Single Cell RNA Sequencing

To ensure that high quality data is generated from scRNAseq, rigorous quality control (QC) efforts will be performed for this study for both the experimental procedures and computational analysis.

Experimental QC: Tissue dissociation will be performed using an optimized protocol to ensure a standardized procedure is followed for every participant-derived sample. Tissue will be processed in a rapid manner so that the profile of cellular RNA will remain as authentic as possible. By the end of the process, every sample will be assessed for cellular viability, the presence of ambient debris, and cell number. This QC test will determine if the sample is ready for further processing for sequencing or if additional steps are needed to improve the quality of the sample. If the quality of the sample needs to be improved, it will be treated with a magnetic bead-based method to enrich for viable cells, deplete debris, and reduce ambient RNA. If a second QC test confirms improvement in the quality of the sample, the cells will then be further processed. Quality control measures will be included for library construction. Bulk RNAseq will be performed on samples and the RNA integrity number (RIN) scores will be checked for the population.

Computational QC: scRNAseq data is noisy, and includes false negatives ("drop-outs"): genes expressed in the cell but not detected by the assay. The Klarman Cell Observatory (KCO) at the Broad Institute has developed and leveraged state of the art tools, many

developed within the laboratory as well as from other groups, for pre-processing and QC of scRNAseq data.

The KCO pipeline uses RNAseq analysis tools such as Trinity, Tuxedo, and RSEM to align reads, transcript reconstruction/annotation, abundance estimation, read quality trimming, and sample quality analysis. Library quality is scored based on alignment rates to the genome and transcriptome, the proportion of ribosomal (rRNA) reads, the uniformity of coverage, 3′ and 5′ biases, biases associated with length or sequence composition, library complexity, reproducibility of expression estimates, and the relationship between single cell and bulk population profiles. The pipeline is optimized for plate, single nucleus sequencing (sNuc-Seq), Droplet-sequencing (Drop-Seq), DroNc-Seq, and 10x Genomics data, addressing each data type's unique characteristics (e.g., unique molecular identifiers [UMIs], 3′-directed libraries, etc.). Tools will be incorporated that increase efficiency by pseudo-alignment, and address unique quality issues that arise at polymerase chain reaction, such as cell barcode chimeras.

To calculate expression levels, alternatives to standard approaches that typically rely on assumptions that are violated in single cell profiles of heterogeneous cell populations will be considered. For example, in a population of T-cells, some may be active and others "quiescent" with the former having radically larger and more complex transcriptomes. This is addressed by introducing a scaling factor reflecting the expected number of transcripts (or UMIs) in each condition and by revising differential expression tests, to account for the varying number of transcripts and genes in each condition.

8.8.2 Planned Genetic Analysis Sample Collection

The sample for genetic analysis should be drawn for planned exploratory biomarker research. This sample will not be collected at the site if there is either a local law or regulation prohibiting collection, or if the IRB/IEC does not approve the collection of the sample for these purposes. If the sample is collected, leftover extracted DNA will be stored for future biomedical research if the participant signs the future biomedical research consent. If the genetic sample collection is not approved, but future biomedical research is approved and consent is given, this sample will be collected for the purpose of future biomedical research.

8.9 Future Biomedical Research Sample Collection

If the participant signs the future biomedical research consent, the following specimens will be obtained as part of future biomedical research:

- DNA for future research
- Leftover plasma and serum from biomarker analyses
- Leftover DNA and RNA
- Leftover tumor tissue, including tumor cell lines
- Leftover sorted single cells and PBMCs

8.10 Visit Requirements

Visit requirements are outlined in Section 1.3. Specific procedure-related details are provided above in Section 8.

8.10.1 Screening

Written consent must be obtained prior to performing any protocol-specific procedure. Results of a test performed prior to the participant signing consent as part of routine clinical management are acceptable in lieu of a screening test if performed within the specified time frame. Screening procedures are to be completed within 28 days prior to the first dose of study treatment except for the following:

- Consent Form signed within 42 days. The consent form must be signed prior to completing any protocol protocol-specified procedure.
- Laboratory tests and evaluation of ECOG status are to be performed within 10 days prior to the first dose of study treatment
- For women of reproductive potential, a urine pregnancy test will be performed within 72 hours prior to the first dose of study treatment. If urine pregnancy results cannot be confirmed as negative, a serum pregnancy test will be required (performed by the local study site laboratory)

Participants may be rescreened after initially failing to meet the inclusion/exclusion criteria. Results from assessments during the initial screening period are acceptable in lieu of a repeat screening test if performed within the specified time frame and the corresponding inclusion/exclusion criteria is met. Participants who are rescreened will retain their original screening number.

8.10.2 Treatment Period

Visit requirements are outlined in Section 1.3. Specific procedure-related details are provided above in Section 8.1.

8.10.3 Poststudy

8.10.3.1 Safety Follow-up Visit

The mandatory Safety Follow-up Visit should be conducted approximately 30 days after the last dose of study treatment or before the initiation of a new anticancer treatment, whichever comes first.

All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Participants with an AE of Grade >1 will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anticancer therapy, whichever occurs first. Serious AEs that occur within 90 days of the end of treatment or before initiation of a new anticancer treatment should also be followed and recorded.

8.10.3.2 Follow-up Visits

Participants who discontinue trial treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed every 9 weeks (63 days ±7 days) during the first year (52 weeks) and every 12 weeks (84 days ±7 days) thereafter, by radiologic imaging to monitor disease status. Every effort should be made to collect information regarding disease status until the start of new anti-cancer therapy, disease progression, death, or end of study. Information regarding post-study anticancer treatment will be collected if new treatment is initiated.

8.10.3.3 Survival Follow-up

Participants who experience confirmed disease progression or start a new anticancer therapy, will move into the Survival Follow-Up Phase and should be contacted by telephone approximately every 12 weeks to assess for survival status until death, withdrawal of consent, or the end of the trial, whichever occurs first.

8.10.3.4 Survival Status

To ensure current and complete survival data is available at the time of database locks, updated survival status may be requested during the course of the study by the Sponsor. For example, updated survival status may be requested prior to but not limited to an external Data Monitoring Committee (eDMC) review, interim and/or final analysis. Upon Sponsor notification, all participants who do not/will not have a scheduled study visit or study contact during the Sponsor defined time period will be contacted for their survival status (excluding participants that have a previously recorded death event in the collection tool).

9. Statistical Analysis Plan

This section outlines the statistical analysis strategy and procedures for the study. Changes to analyses made after the protocol has been finalized, but prior to final database lock, will be documented in a supplemental statistical analysis plan (sSAP) and referenced in the Clinical Study Report (CSR) for the study. Post hoc exploratory analyses will be clearly identified in the CSR. Details around the analysis approach for biomarker endpoints related to the secondary and exploratory objectives are deemed out of scope for this SAP, but may be the subject of sSAPs.

9.1 Statistical Analysis Plan Summary

Key elements of the statistical analysis plan are summarized below; the comprehensive plan is provided in Sections 9.2 through 9.12.

Study Design Overview	This is a single-arm, open label, trial of pembrolizumab in participants with advanced melanoma.
Treatment Assignment	All participants will receive pembrolizumab at a dose of 200 mg Q3W by non-random assignment in an unblinded fashion.
Analysis Populations	Primary objectives: The All Participants as Treated (APaT) population will be used as the analyses population for primary objectives. Efficacy objective (secondary objective): APaT population. Safety: APaT population.
Primary Endpoints	1. Fraction of cytotoxic tumor-infiltrating T-lymphocytes (FCT) at baseline. 2. FCT fold change from baseline. 3. Average Specific Cytotoxic T-lymphocyte Frequency Ratio (ASCTFR)
Key Secondary Endpoints / Variables	1. Objective response rate (ORR) by RECIST 1.1 (investigator-assessed). 2. Progression-free-survival (PFS) by RECIST 1.1 (investigator-assessed). 3. Overall survival (OS). 4. Safety measures
Statistical Methods for Key Biomarker Analyses	The primary objectives will be evaluated by estimating the posterior distribution of the difference in means of the primary endpoints for responders vs. non-responders via the Bayesian framework.
Statistical Methods for Key Efficacy Analyses	The secondary efficacy objective will be evaluated by reporting summary statistics and 95% confidence intervals for ORR (with Clopper-Pearson CI), PFS, and OS (via Kaplan-Meier method).
Statistical Methods for Key Safety Analyses	Descriptive summary statistics will be provided as appropriate.
Interim Analyses	Interim analyses that will not change the conduct of the study may be performed during the course of the study. Final analyses: the final analyses will be carried out after all participants have had a 90-day follow-up after the end of treatment (unless lost to follow-up). If by that time the death rate is <70%, for OS and OS-related analyses, the final analyses will occur after death has occurred in approximately 70% of the subjects (~42 deaths). If the estimated interval between the 2 analyses is less than 3 months, then the first analysis may be delayed and both analyses will be conducted at the later of the two.
Multiplicity	No multiplicity adjustment will be applied.
Sample Size and Power	The study will enroll approximately 60 participants. If there are at least 10 responders, reasonable power supporting the primary objectives can be expected (~90% power for baseline FCT with a mean difference of 0.10; ~80% power for FCT fold-change from baseline with an effect size of 0.50 on natural log scale; and ~80% power for ASCTFR with an effect size of 0.50).

9.2 Responsibility for Analyses/In-house Blinding

The statistical analysis of the data obtained from this study will be conducted by, or under the direct auspices of, the Early Clinical Development Statistics Department in collaboration with Genetics and Pharmacogenomics Department of the Sponsor.

This trial is being conducted as an open-label study, i.e., participants, investigators, and Sponsor personnel will be aware of participant treatment assignments after each participant is enrolled and treatment is assigned.

Biomarker assays used to assess the primary, secondary and exploratory objectives in this study will be performed by personnel blinded to participants' clinical outcome data, as appropriate, in order to preserve an objective evaluation of the study objectives. Separation between the generation of the biomarker data and knowledge of clinical outcome will permit a flexible assessment strategy for the complex biomarker data without impinging on the validity of the eventual comparison between responders and non-responders. If deemed appropriate, clinical outcome data of a subset of participants may be unblinded to certain biomarker personnel(s) and these data will be used as a testing dataset to generate specific hypotheses surrounding the biomarker data. These additional hypotheses will be tested in the corresponding validation dataset (i.e., participants not part of the testing dataset). If this testing and validation strategy is utilized, additional details will be provided in the sSAP prior to implementation.

Interim analyses and timing of the final analyses are described in Section 9.7.

9.3 Hypotheses/Estimation

Objectives of the study are stated in Section 3.

9.4 Analysis Endpoints

Biomarker, efficacy, and safety endpoints that will be evaluated are outlined in the following sections.

9.4.1 Biomarker Endpoints

9.4.1.1 Primary Biomarker Endpoints

The primary intra-tumoral biomarker measures that will be compared between responders and non-responders in evaluation of the primary objectives are

1) FCT, defined as the fraction of cytotoxic CD8+ T-cells expressing a predefined single-cell RNA gene signature to the total tumor infiltrating CD8+ T-cells isolated from tumor biopsies.

To quantify FCT based on scRNAseq data we will use a previously defined signature of cytotoxic T-cells [Tirosh, I., et al 2016]. RNAseq reads will be used to assemble TCR and B-cell receptor sequences and then identify T-cell or B-cell clones. Dimensionality reduction and graph clustering will then be used to partition cell types by their expression profiles and associate them with cell-specific signatures. Statistical scoring will be adapted for single cell data to identify key cell states and their respective signatures, within and across cell types (e.g., phases of the cell cycle, inflammation, activation states, "activation-independent

exhaustion, drug resistance signatures). The cell type and state signatures will be compared to a large and diverse set of immune cells from various cancer cohorts [Tirosh, I., et al 2016] [Venteicher, A. S., et al 2017] [Zheng, C., et al 2017]. T-cells with an overall high expression of the cytotoxic signature will be defined as cytotoxic tumor-infiltrating T-lymphocytes. A cytotoxic score will be calculated for each cell to quantify the expression level of the cytotoxic signature (details in next paragraph). If the cells' cytotoxic score has a bimodal distribution, a natural cutoff will define highly cytotoxic T-cells. If it is not bimodal, we will define an "artificial" cutoff using the continuous scores (e.g., 90th percentile). The FCT will be calculated as the relative abundance of these cells compared to the overall number of sequenced CD8+ T-cells, i.e., the number of cytotoxic CD8+ T-cells (with high cytotoxic scores) divided by the number of total CD8+ T-cells that were sequenced in this tumor.

To calculate the cytotoxic score, genes will be binned into 50 *expression bins* according to their average expression across CD8+ T-cells. Let $\boldsymbol{T_{ij}}$ be the normalized expression of gene $\boldsymbol{i}$ in cell $\boldsymbol{j}$. Let $\boldsymbol{S}$ be the gene signature of the cytotoxic T-cells, and $\tilde{\boldsymbol{S}}$ a random signature with the same distribution of genes across the *expression bins* as $\boldsymbol{S}$. The cytotoxic score (***CytS***) of a cell is defined as:

$$CytS_j = \frac{\sum_{i \in S} T_{ij}}{\mathrm{E}[\sum_{i \in \tilde{S}} T_{ij}]}$$

2) FCT fold-change from baseline, and

3) Average Specific Cytotoxic T-lymphocyte Frequency Ratio (ASCTFR): defined as the arithmetic mean of log10 ratio of the frequency of individual specific cytotoxic TCR clones on-treatment to pre-treatment (baseline). In this definition, pre-treatment frequencies of unique (as determined by TCR Vβ chain sequencing) specific cytotoxic clones will be used to normalize the on-treatment frequencies. Each participant's ASCTFR will then be calculated as the arithmetic mean of the log10 ratios of the on-treatment to pre-treatment frequencies across the unique specific cytotoxic clones identified within that participant. For example, consider one participant who has two specific cytotoxic TCR clones, Clone A and Clone B, and for each of these two clones the participant has pre-treatment frequency measurements A0/B0 and on-treatment frequency measurements A1/B1, then the ASCTFR for this participant will be calculated as:

$$ASCTFR = \frac{\log_{10}(\frac{A1}{A0}) + \log_{10}\left(\frac{B1}{B0}\right)}{2}$$

The specific TCR clones will be selected based on their frequency as well as the interferon-γ signature. Here the frequency is in reference to the specific tumor-infiltrating lymphocyte TCR Vβ frequency distribution (obtained by TCR deep sequencing) in the pre-treatment and on-treatment biopsies. The cytolytic function of these TCR clones will be determined based on *CytS* obtained from the scRNAseq (as used in the definition of FCT).

9.4.1.2 Secondary and Exploratory Biomarker Endpoints

Details around other biomarker related endpoints associated with secondary and exploratory objectives, including tumor-associated epitopes, the pipeline surrounding the identification of neoepitopes, what will define the response of T-cell clones to such epitopes, as well as details for the processing and normalization of the complex data sources to be generated by the set of methods described in Section 4.2.1.3 are deemed out of scope for this SAP, but will be described in the CSR and may be the subject of sSAPs.

9.4.2 Efficacy Endpoints

Secondary Efficacy Endpoints

1) ORR is defined as the proportion of the participants in the analysis population who have a best response as CR or PR. Responses are based on local site review using RECIST 1.1.
2) PFS is defined as the time from start of treatment to the first documented PD (based on local site review using RECIST 1.1) or death due to any cause, whichever occurs first.
3) OS is defined as the time from start of treatment to death due to any cause. Participants without documented death at the time of the analysis will be censored at the date of last follow-up. See Section 9.6.2 for the definition of censoring.

9.4.3 Safety Endpoints

Refer to Section 4.2.1.2 for the description of the safety measures.

9.5 Analysis Populations

9.5.1 Biomarker Analysis Population

The All Participants as Treated (APaT) population will be used for the analysis of biomarker endpoints. The APaT population consists of all allocated participants who received at least one dose of study treatment. For analysis related to post-baseline biomarker values, at least one post-baseline measurement is required for inclusion in the analysis of each specific biomarker endpoint.

9.5.2 Efficacy Analysis Population

The APaT population will be used for the analysis of ORR, PFS and OS. Details on the approach to handle missing data are provided in Section 9.6 Statistical Methods.

9.5.3 Safety Analysis Population

The APaT population will be used for the analysis of safety data in this study. At least one laboratory or vital sign measurement obtained subsequent to at least one dose of study treatment is required for inclusion in the analysis of each specific parameter. To assess change from baseline, a baseline measurement is also required.

9.6 Statistical Methods

For the primary study objectives enumerated in Section 3, the overall analysis strategy is to formulate reasonable parametric models for the distributions of the measures describing the nature and frequency of cytotoxic tumor-infiltrating T-lymphocytes. These models, formulated in a Bayesian framework, will be used to understand the differences for responders vs. non-responders by evaluating and comparing their posterior distributions of the model parameters. Details are described in Section 9.6.1.

Analyses methods for efficacy and safety endpoints are described in Section 9.6.2 and Section 9.6.3, respectively.

9.6.1 Statistical Methods for Biomarker Analysis

This section describes the statistical methods that address the primary objectives. Data will be examined for departures from the assumptions of the distribution model(s) as appropriate. Suitable data transformation may be applied if a serious departure from the assumptions of the model(s) is observed, or other suitable distributions may be used in the Bayesian models.

Methods related to secondary and/or exploratory biomarker related objectives may be described in sSAPs.

Baseline FCT

To address the first primary objective, the difference in mean baseline FCT between responders and non-responders will be quantified by calculating the posterior probability that the mean baseline FCT for responders exceeds that of non-responders, under a Bayesian model assuming the participant FCTs arise from a Beta distribution. Non-informative (vague) priors will be used: e.g., a Uniform (0, 1) prior for both the means of responders and non-responders. The first primary hypothesis will be supported if the posterior probability that the mean baseline FCT for responders exceeds that of non-responders is greater than 70%. The 95% posterior credible interval will also be provided for the difference in the mean baseline FCT between responders and non-responders.

Supportive analyses may be performed by comparing the average baseline *CytS* scores of the two populations by using a hierarchical linear model (HLM) that captures the hierarchical structure of the data.

FCT Fold Change from Baseline

Individual FCT fold change from baseline values will be natural log transformed prior to statistical analysis. It is expected that the mean FCT log fold-change from baseline in responders is higher than that in non-responders. The difference in mean FCT log fold-change from baseline between responders and non-responders will be quantified by calculating the posterior probability that the difference exceeds zero under the Bayesian framework. The planned approach will be to assume that these participant-level FCT log fold-change from baseline values arise from normal distributions with means and variances that potentially differ for responders and non-responders. Non-informative (vague) priors will be used: e.g., a diffuse normal distribution centered at 0 with precision 1e-06 as prior for the mean, and a Gamma (1e-04, 1e-04) distribution as prior for the precision. The same priors will be used for both responders and non-responders. The second primary hypothesis

will be supported if the posterior probability that the mean FCT log fold-change from baseline of responders exceeds that of non-responders is greater than 70%. The 95% posterior credible interval will also be provided for the difference in mean FCT log fold-change from baseline between responders and non-responders.

Supportive analysis may be performed by adding to the hierarchical linear model a covariate time point to indicate whether the sample was taken before or after treatment, and an interaction term between the response and time point.

ASCTFR

Similarly, the third primary objective to compare the ASCTFR between responders and non-responders will also be addressed using a Bayesian framework to compare means between the 2 groups of participants. It is expected that on-treatment specific tumor-infiltrating cytotoxic T-lymphocytes represent a set of expanded, higher frequency clones among responders compared to non-responders. The analysis method for ASCTFR will be similar to that for the FCT fold-change from baseline: we assume that these participant-level average log frequency ratios arise from normal distributions with means and variances that potentially differ for responders and non-responders. The key quantity being estimated will be the posterior probability that the mean ASCTFR is larger in responders vs. non-responders. Non-informative (vague) priors will be used: e.g., a diffuse normal distribution centered at 0 with precision 1e-06 as prior for the mean, and a Gamma (1e-04, 1e-04) distribution as prior for the precision. The same priors will be used for both responders and non-responders. The third primary hypothesis will be supported if the posterior probability that the mean ASCTFR for responders exceeds that of non-responders is greater than 70%. The 95% posterior credible interval will also be provided for the difference in mean ASCTFR between responders and non-responders.

Other Biomarker Endpoints

Details around the analysis approach for biomarker endpoints related to the secondary and exploratory objectives are deemed out of scope for this SAP, but may be the subject of sSAPs

9.6.2 Statistical Methods for Efficacy Analyses

The chief purpose of determining efficacy for this study is to define the appropriate subsets to be compared in the evaluation of the biology of intra-tumoral changes. Participants will be separated as responder vs. non-responder, as assessed by RECIST 1.1 per investigator review.

This section describes the statistical methods that address the secondary efficacy objective (ORR, PFS and OS).

Objective response rate (ORR)

The ORR point estimate and its 95% Clopper-Pearson CI will be reported. Participants without disease assessment after the start of treatment will be considered as non-responders in ORR estimation.

Progression-free-survival (PFS)

The Kaplan-Meier method will be used to estimate the PFS curve. The median PFS and its associated two-sided 95% confidence interval (CI) will be estimated and reported. The Kaplan-Meier estimates of the PFS rate at 6 months and other time points of interest will also be reported. Since disease progression is assessed periodically, PD can occur any time in the time interval between the last assessment where PD was not documented and the assessment when PD is documented. For this analysis, for participants who have PD, the true date of disease progression will be approximated by the date of first assessment at which PD is objectively documented using RECIST 1.1, regardless of discontinuation of study drug. Death is always considered as a confirmed PD event. Participants without documented PD/death will be censored at the last disease assessment date. In the event that participants without documented PD/death have new anticancer treatment initiated, the participants will be considered censored at last disease assessment before new anticancer treatment.

Overall Survival (OS)

The Kaplan-Meier method will be used to estimate the OS curve. The median OS and its associated two-sided 95% CI will be estimated and reported. The Kaplan-Meier estimates of the OS rate at 6 months and other time points of interest will also be reported. A sensitivity analysis of OS that censors participants at the time of initiation of new anticancer treatment will also be performed. Table 7 summarizes the efficacy analyses.

Table 7 Analysis Strategy for Efficacy Variables

Endpoint/ Variable (Description, Time Point)	**Primary vs. Supportive Approach**	**Statistical Method**	**Analysis Population**	**Missing Data Approach**
Secondary Objective				
ORR	P	Point estimate with 95% Clopper-Pearson CI for ORR will be reported.	APaT	Participants with missing data are considered non-responders
PFS	P	Kaplan-Meier method for PFS curve estimation, and summary statistics with 95% CIs will be reported.	APaT	Censored at last assessment (before new anticancer treatment)
OS	P	Kaplan-Meier method for OS curve estimation, and summary statistics with 95% CIs will be reported.	APaT	Censored at last date
OS	S	Kaplan-Meier method for OS curve estimation, and summary statistics with 95% CIs will be reported.	APaT	Censored at last date or initiation of new anticancer treatment

Abbreviations: APaT = All Participants as Treated; ORR = objective response rate; OS = overall survival; P = Primary approach; PFS = progression-free survival; S = Supportive approach.

9.6.3 Statistical Methods for Safety Analyses

Safety and tolerability will be assessed by clinical review of all relevant parameters including AEs, laboratory tests, and vital signs.

Descriptive summary statistics will be provided for safety endpoints. See Table 8 for the list of safety endpoints to be summarized. For continuous measures such as changes from baseline in laboratory tests and vital signs, summary statistics for baseline, on-treatment, and change from baseline values will be provided in table format. Additional safety endpoints may also be summarized, as deemed clinically appropriate.

Table 8 Analysis Strategy for Safety Parameters

Safety Endpoint[†]	Descriptive Statistics
Any AE	X
Any Grade 3-5 AE	X
Any Serious AE	X
Onset and Duration of First Grade 3-5 AE	X
Any Drug-Related AE	X
Any Serious and Drug-Related AE	X
Any Grade 3-5 and Drug-Related AE	X
Discontinuation due to AE	X
Death	X
Specific AEs, SOCs	X
Change from Baseline Results (Labs, Vital Signs)	X

Abbreviations: AE = adverse event; SOC = system organ class
[†]Adverse events refer to both Clinical and Laboratory AEs.
Note: X = results will be provided.

9.6.4 Summaries of Baseline Characteristics, Demographics, and Other Analyses

The number and percentage of participants screened, allocated, the primary reasons for screen failure, and the primary reasons for discontinuation will be displayed. Demographic variables (such as age), baseline characteristics, and prior and concomitant therapies will be summarized either by descriptive statistics or categorical tables.

9.7 Interim Analyses

During the course of the study, preliminary analyses of biomarker, efficacy and safety endpoints may be performed as data become available. The primary purpose of these analyses is to provide an early assessment of the study objectives. There is no intention to change the conduct of the current study based on the results of these analyses.

The final analyses will be carried out after all participants have had a 90-day follow-up after the end of treatment (unless lost to follow-up). If, by that time, the death rate is <70%, for OS and OS-related analyses, the final analyses will occur after death has occurred in

approximately 70% of the participants (~42 deaths). If the estimated interval between the 2 analyses is less than 3 months, then the first analysis may be delayed and both analyses will be conducted at the later of the two.

9.8 Multiplicity

As all analyses are exploratory in nature, no multiplicity adjustments will be applied.

9.9 Sample Size and Power Calculations

9.9.1 Sample Size and Power for Biomarker Analyses

This study will enroll approximately 60 participants. Based on response rates observed previously for melanoma patients, it is assumed that the response rate is ~30%.

For the endpoints of the primary objectives, there is no pre-specified difference in the means of prior interest. Hence, the principal summary of results around the study objectives will be via the posterior probability that the mean difference exceeds 0 in the direction of responders showing the higher mean. For the primary objectives, the posterior probability threshold to claim statistical significance is set as 70% (or a ~2-to-1 odds), which is a reasonable level of certainty addressing these objectives.

Table 9 shows the power to detect a difference in mean baseline FCT between responders and non-responders, assuming a few different sample sizes and true differences. It is assumed that baseline FCT in non-responders follows a Beta distribution with alpha and beta parameters equal to 0.022 and 1, respectively, which puts ~95% of baseline FCT values below 0.10. True mean differences shown were obtained by selecting higher values of the alpha parameter in the responders' distribution, relative to non-responders, while keeping the beta parameter fixed at 1. Bayesian models were fit allowing for different parameter values of responders and non-responders. The results suggest that for a true mean difference at or above 0.10 and at least 10 responders, we expect to observe ≥90% power (with the assumed Beta distributions).

Table 9 Power for Baseline FCT

N Responder	N Non-Responder	True Mean Difference: δ [a]	% Power [b]
10	50	0.05	68
		0.10	90
15	45	0.05	80
		0.10	96
20	40	0.05	83
		0.10	98

a. Assuming that baseline FCT in non-responders follows a Beta distribution with alpha and beta parameters equal to 0.022 and 1, respectively, which puts 95% of baseline FCT values below 0.10. Mean differences shown were obtained by selecting higher values of the alpha parameter in the responders' distribution, relative to non-responders, while keeping the beta parameter fixed at 1.
b. Power is defined as Prob ($PP_{(\delta > 0)}$ >70%); computed over 500 trial simulations.

Table 10 below provides the power for detecting a difference in mean FCT log fold-change from baseline and ASCTFR between responders and non-responders. A common standard deviation for the responders and non-responder was assumed for simplicity, although the Bayesian models were fit allowing for unique standard deviations. It is shown that with at least 10 responders, for effect sizes at or above 0.50, we expect to observe ≥80% power.

Table 10 Power for FCT Log Fold-Change From Baseline and ASCTFR

N Responder	N Non-Responder	True Mean Difference: δ [a]	% Power [b]
10	50	0.25	55
		0.50	81
15	45	0.25	61
		0.50	88
20	40	0.25	64
		0.50	90

a. Assuming standard deviation of 1 for each of the responder and non-responder groups, as would result if first dividing each of the non-responder and responder groups by their respective true (albeit unknown) standard deviations, and so this δ is essentially the effect size for this exercise.
b. Power is defined as Prob ($PP_{(\delta > 0)}$ >70%); computed over 500 trial simulations.

As an illustration of precision associated with secondary and exploratory biomarker related objectives that may involve a large set of features being compared between responders and non-responders, Table 11 presents the power for comparing features between these 2 groups testing for significance at a false discovery rate (FDR) of 0.20.

Table 11 Power for Other Biomarker Endpoints

N Responder	N Non-Responder	Effect Size[a]	% Power[b]
10	50	0.50	12.3
		0.75	30.7
		1.00	59.0
		1.50	95.4
15	45	0.50	16.7
		0.75	44.3
		1.00	75.4
		1.50	98.7
20	40	0.50	20.9
		0.75	54.7
		1.00	82.7
		1.50	99.7

a. Standardized difference in the biomarker means between responders and non-responders.
b. Testing for significance at an FDR of 0.20 and assuming 90% of hypotheses are null and 10% have the given effect size.

9.9.2 Sample Size and Power for Efficacy Analyses

The estimation precision of efficacy analyses is illustrated with the expected precision for ORR. Assuming a total of 60 participants included in the analysis with a 30% ORR, the 95% Clopper-Pearson CI for ORR will be (19%, 43%); assuming a total of 60 participants with a 20% ORR, the 95% Clopper-Pearson CI for ORR will be (11%, 32%).

9.9.3 Sample Size and Power for Safety Analyses

For any specific AE, the probability of observing at least 1 AE in this study depends on the number of participants treated and the underlying percentage of participants with an AE in the study population. Assuming a total of 60 participants included in the analysis, if the underlying incidence rate of a given type of AE is 1% or 3%, there is 45.3% or 83.9% chance of observing at least 1 AE among the 60 participants, respectively. If no AE of a given type is observed among the 60 participants, this study will provide 95% confidence that the underlying percentage of participants with the AE is <4.9% in the study population treated with the study treatment.

9.10 Subgroup Analyses

No subgroup analyses are planned for this study.

9.11 Compliance (Medication Adherence)

Drug accountability data for trial treatment will be collected during the study. Compliance with trial treatment administration will be measured by participants: 1) receiving unscheduled study agent infusions/injections; 2) missing an infusion/injection. Numbers and percentages of participants and infusion/injection visits with any deviation in these measures will be reported for the APaT population.

9.12 Extent of Exposure

The Extent of Exposure to study treatment will be evaluated by summary statistics (e.g. N, mean, median, standard deviation etc.) for duration of treatment in cycles.

10. Supporting Documentation and Operational Considerations

10.1 Appendix 1: Regulatory, Ethical and Study Oversight Considerations

Merck Code of Conduct for Clinical Trials

Merck*
Code of Conduct for Clinical Trials

I. Introduction

A. Purpose

Merck, through its subsidiaries, conducts clinical trials worldwide to evaluate the safety and effectiveness of our products. As such, we are committed to designing, implementing, conducting, analyzing and reporting these trials in compliance with the highest ethical and scientific standards. Protection of participant safety is the overriding concern in the design of clinical trials. In all cases, Merck clinical trials will be conducted in compliance with local and/or national regulations and in accordance with the ethical principles that have their origin in the Declaration of Helsinki.

B. Scope

Such standards shall be endorsed for all clinical interventional investigations sponsored by Merck irrespective of the party (parties) employed for their execution (eg, contract research organizations, collaborative research efforts). This Code is not intended to apply to trials which are observational in nature, or which are retrospective. Further, this Code does not apply to investigator-initiated trials which are not under the control of Merck.

II. Scientific Issues

A. Trial Conduct

1. Trial Design

Except for pilot or estimation trials, clinical trial protocols will be hypothesis-driven to assess safety, efficacy and/or pharmacokinetic or pharmacodynamic indices of Merck or comparator products. Alternatively, Merck may conduct outcomes research trials, trials to assess or validate various endpoint measures, or trials to determine participant preferences, etc.

The design (ie, participant population, duration, statistical power) must be adequate to address the specific purpose of the trial. Research participants must meet protocol entry criteria to be enrolled in the trial.

2. Site Selection

Merck selects investigative sites based on medical expertise, access to appropriate participants, adequacy of facilities and staff, previous performance in Merck trials, as well as budgetary considerations. Prior to trial initiation, sites are evaluated by Merck personnel to assess the ability to successfully conduct the trial.

3. Site Monitoring/Scientific Integrity

Trial sites are monitored to assess compliance with the trial protocol and general principles of Good Clinical Practice. Merck reviews clinical data for accuracy, completeness and consistency. Data are verified versus source documentation according to standard operating procedures. Per Merck policies and procedures, if fraud, misconduct or serious GCP-noncompliance are suspected, the issues are promptly investigated. When necessary, the clinical site will be closed, the responsible regulatory authorities and ethics review committees notified and data disclosed accordingly.

B. Publication and Authorship

To the extent scientifically appropriate, Merck seeks to publish the results of trials it conducts. Some early phase or pilot trials are intended to be hypothesis-generating rather than hypothesis testing. In such cases, publication of results may not be appropriate since the trial may be underpowered and the analyses complicated by statistical issues of multiplicity.

Merck's policy on authorship is consistent with the requirements outlined in the ICH-Good Clinical Practice guidelines. In summary, authorship should reflect significant contribution to the design and conduct of the trial, performance or interpretation of the analysis, and/or writing of the manuscript. All named authors must be able to defend the trial results and conclusions. Merck funding of a trial will be acknowledged in publications.

III. Participant Protection

A. IRB/IEC review

All clinical trials will be reviewed and approved by an independent IRB/IEC before being initiated at each site. Significant changes or revisions to the protocol will be approved by the IRB/IEC prior to implementation, except that changes required urgently to protect participant safety and well-being may be enacted in anticipation of IRB/IEC approval. For each site, the IRB/IEC and Merck will approve the participant informed consent form.

B. Safety

The guiding principle in decision-making in clinical trials is that participant welfare is of primary importance. Potential participants will be informed of the risks and benefits of, as well as alternatives to, trial participation. At a minimum, trial designs will take into account the local standard of care. Participants are never denied access to appropriate medical care based on participation in a Merck clinical trial.

All participation in Merck clinical trials is voluntary. Participants are enrolled only after providing informed consent for participation. Participants may withdraw from a Merck trial at any time, without any influence on their access to, or receipt of, medical care that may otherwise be available to them.

C. Confidentiality

Merck is committed to safeguarding participant confidentiality, to the greatest extent possible. Unless required by law, only the investigator, Sponsor (or representative) and/or regulatory authorities will have access to confidential medical records that might identify the research participant by name.

D. Genomic Research

Genomic Research will only be conducted in accordance with informed consent and/or as specifically authorized by an Ethics Committee.

IV. Financial Considerations

A. Payments to Investigators

Clinical trials are time- and labor-intensive. It is Merck's policy to compensate investigators (or the sponsoring institution) in a fair manner for the work performed in support of Merck trials. Merck does not pay incentives to enroll participants in its trials. However, when enrollment is particularly challenging, additional payments may be made to compensate for the time spent in extra recruiting efforts.

Merck does not pay for participant referrals. However, Merck may compensate referring physicians for time spent on chart review to identify potentially eligible participants.

B. Clinical Research Funding

Informed consent forms will disclose that the trial is sponsored by Merck, and that the investigator or sponsoring institution is being paid or provided a grant for performing the trial. However, the local IRB/IEC may wish to alter the wording of the disclosure statement to be consistent with financial practices at that institution. As noted above, publications resulting from Merck trials will indicate Merck as a source of funding.

C. Funding for Travel and Other Requests

Funding of travel by investigators and support staff (eg, to scientific meetings, investigator meetings, etc.) will be consistent with local guidelines and practices including, in the U.S., those established by the American Medical Association (AMA).

V. Investigator Commitment

Investigators will be expected to review Merck's Code of Conduct as an appendix to the trial protocol, and in signing the protocol, agree to support these ethical and scientific standards.

* In this document, "Merck" refers to Merck Sharp & Dohme Corp. and Schering Corporation, each of which is a subsidiary of Merck & Co., Inc. Merck is known as MSD outside of the United States and Canada. As warranted by context, Merck also includes affiliates and subsidiaries of Merck & Co., Inc."

Financial Disclosure

Financial Disclosure requirements are outlined in the US Food and Drug Administration Regulations, Financial Disclosure by Clinical Investigators (21 CFR Part 54). It is the Sponsor's responsibility to determine, based on these regulations, whether a request for Financial Disclosure information is required. It is the investigator's/subinvestigator's responsibility to comply with any such request.

The investigator/subinvestigator(s) agree, if requested by the Sponsor in accordance with 21 CFR Part 54, to provide his/her financial interests in and/or arrangements with the Sponsor to allow for the submission of complete and accurate certification and disclosure statements. The investigator/subinvestigator(s) further agree to provide this information on a Certification/Disclosure Form, commonly known as a financial disclosure form, provided by the Sponsor. The investigator/subinvestigator(s) also consent to the transmission of this information to the Sponsor in the United States for these purposes. This may involve the transmission of information to countries that do not have laws protecting personal data.

Data Protection

Participants will be assigned a unique identifier by the Sponsor. Any participant records or datasets that are transferred to the Sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.

The participant must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant.

The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

Confidentiality of Data

By signing this protocol, the investigator affirms to the Sponsor that information furnished to the investigator by the Sponsor will be maintained in confidence, and such information will be divulged to the IRB, IEC, or similar or expert committee; affiliated institution and employees, only under an appropriate understanding of confidentiality with such board or committee, affiliated institution and employees. Data generated by this study will be considered confidential by the investigator, except to the extent that it is included in a publication as provided in the Publications section of this protocol.

Confidentiality of Participant Records

By signing this protocol, the investigator agrees that the Sponsor (or Sponsor representative), IRB/IEC, or regulatory authority representatives may consult and/or copy study documents in order to verify worksheet/CRF data. By signing the consent form, the participant agrees to this process. If study documents will be photocopied during the process of verifying worksheet/CRF information, the participant will be identified by unique code only; full names/initials will be masked prior to transmission to the Sponsor.

By signing this protocol, the investigator agrees to treat all participant data used and disclosed in connection with this study in accordance with all applicable privacy laws, rules and regulations.

Confidentiality of IRB/IEC Information

The Sponsor is required to record the name and address of each IRB/IEC that reviews and approves this study. The Sponsor is also required to document that each IRB/IEC meets regulatory and ICH GCP requirements by requesting and maintaining records of the names and qualifications of the IRB/IEC members and to make these records available for regulatory agency review upon request by those agencies.

Publication Policy

The results of this study may be published or presented at scientific meetings. The Sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.

If publication activity is not directed by the Sponsor, the investigator agrees to submit all manuscripts or abstracts to the Sponsor before submission. This allows the Sponsor to protect proprietary information and to provide comments.

Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

Compliance with Study Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Amendments Act (FDAAA) of 2007 and the European Medicines Agency (EMA) clinical trial Directive 2001/20/EC, the Sponsor of the study is solely responsible for determining whether the study and its results are subject to the requirements for submission to http://www.clinicaltrials.gov, www.clinicaltrialsregister.eu or other local registries. MSD, as Sponsor of this study, will review this protocol and submit the information necessary to fulfill these requirements. MSD entries are not limited to FDAAA or the EMA clinical trial directive mandated trials. Information posted will allow participants to identify potentially appropriate studies for their disease conditions and pursue participation by calling a central contact number for further information on appropriate study locations and site contact information.

By signing this protocol, the investigator acknowledges that the statutory obligations under FDAAA, the EMA clinical trials directive or other locally mandated registries are that of the Sponsor and agrees not to submit any information about this study or its results to those registries.

Compliance with Law, Audit, and Debarment

By signing this protocol, the investigator agrees to conduct the study in an efficient and diligent manner and in conformance with this protocol; generally accepted standards of GCP (eg, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Good Clinical Practice: Consolidated Guideline and other

generally accepted standards of GCP); and all applicable federal, state and local laws, rules and regulations relating to the conduct of the clinical study.

The Code of Conduct, a collection of goals and considerations that govern the ethical and scientific conduct of clinical investigations sponsored by MSD, is provided in this appendix under the Merck Code of Conduct for Clinical Trials.

The investigator agrees not to seek reimbursement from participants, their insurance providers or from government programs for procedures included as part of the study reimbursed to the investigator by the Sponsor.

The investigator will promptly inform the Sponsor of any regulatory authority inspection conducted for this study.

The investigator agrees to provide the Sponsor with relevant information from inspection observations/findings to allow the Sponsor to assist in responding to any citations resulting from regulatory authority inspection, and will provide the Sponsor with a copy of the proposed response for consultation before submission to the regulatory authority.

Persons debarred from conducting or working on clinical studies by any court or regulatory authority will not be allowed to conduct or work on this Sponsor's studies. The investigator will immediately disclose in writing to the Sponsor if any person who is involved in conducting the study is debarred or if any proceeding for debarment is pending or, to the best of the investigator's knowledge, threatened.

Data Quality Assurance

All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the Sponsor or designee electronically (eg, laboratory data). The investigator or qualified designee is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.

Detailed information regarding Data Management procedures for this protocol will be provided separately.

The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.

The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.

Study documentation will be promptly and fully disclosed to the Sponsor by the investigator upon request and also shall be made available at the study site upon request for inspection, copying, review and audit at reasonable times by representatives of the Sponsor or any regulatory authorities. The investigator agrees to promptly take any reasonable steps that are requested by the Sponsor or any regulatory authorities as a result of an audit or inspection to cure deficiencies in the study documentation and worksheets/CRFs.

The Sponsor or designee is responsible for the data management of this study including quality checking of the data.

Study monitors will perform ongoing source data review and verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from

source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

Records and documents, including signed ICF, pertaining to the conduct of this study must be retained by the investigator for 15 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

Source Documents

Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.

Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

Study and Site Closure

The Sponsor or its designee may stop the study or study site participation in the study for medical, safety, regulatory, administrative, or other reasons consistent with applicable laws, regulations, and GCP.

In the event the Sponsor prematurely terminates a particular study site, the Sponsor will promptly notify that study site's IRB/IEC.

10.2 Appendix 2: Collection and Management of Specimens for Future Biomedical Research

1. Definitions

a. Biomarker: A biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process or of a condition or disease. A biomarker may be used to see how well the body responds to a treatment for a disease or condition.[1]

b. Pharmacogenomics: The investigation of variations of DNA and RNA characteristics as related to drug/vaccine response.[2]

c. Pharmacogenetics: A subset of pharmacogenomics, pharmacogenetics is the influence of variations in DNA sequence on drug/vaccine response.[2]

d. DNA: Deoxyribonucleic acid.

e. RNA: Ribonucleic acid.

2. Scope of Future Biomedical Research

The specimens consented and/or collected in this study as outlined in Section 8.9 will be used in various experiments to understand:

- The biology of how drugs/vaccines work
- Biomarkers responsible for how a drug/vaccine enters and is removed by the body
- Other pathways drugs/vaccines may interact with
- The biology of disease

The specimen(s) may be used for future assay development and/or drug/vaccine development.

It is now well recognized that information obtained from studying and testing clinical specimens offers unique opportunities to enhance our understanding of how individuals respond to drugs/vaccines, enhance our understanding of human disease and ultimately improve public health through development of novel treatments targeted to populations with the greatest need. All specimens will be used by the Sponsor or those working for or with the Sponsor.

3. Summary of Procedures for Future Biomedical Research

a. Participants for Enrollment

All participants enrolled in the clinical study will be considered for enrollment in the future biomedical research substudy.

b. Informed Consent

Informed consent for specimens (ie, DNA, RNA, protein, etc.) will be obtained during screening for protocol enrollment from all participants or legal guardians, at a study visit by the investigator or his or her designate. Informed consent for future biomedical research should be presented to the participants on the visit designated in the SoA. If delayed, present consent at next possible Participant Visit. Consent forms

signed by the participant will be kept at the clinical study site under secure storage for regulatory reasons.

A template of each study site's approved informed consent will be stored in the Sponsor's clinical document repository.

c. eCRF Documentation for Future Biomedical Research Specimens

Documentation of participant consent for future biomedical research will be captured in the eCRFs. Any specimens for which such an informed consent cannot be verified will be destroyed.

d. Future Biomedical Research Specimen(s)

Collection of specimens for future biomedical research will be performed as outlined in the SoA. In general, if additional blood specimens are being collected for future biomedical research, these will usually be obtained at a time when the participant is having blood drawn for other study purposes.

4. Confidential Participant Information for Future Biomedical Research

In order to optimize the research that can be conducted with future biomedical research specimens, it is critical to link participant' clinical information with future test results. In fact little or no research can be conducted without connecting the clinical study data to the specimen. The clinical data allow specific analyses to be conducted. Knowing participant characteristics like gender, age, medical history and treatment outcomes are critical to understanding clinical context of analytical results.

To maintain privacy of information collected from specimens obtained for future biomedical research, the Sponsor has developed secure policies and procedures. All specimens will be single-coded per ICH E15 guidelines as described below.

At the clinical study site, unique codes will be placed on the future biomedical research specimens. This code is a random number which does not contain any personally identifying information embedded within it. The link (or key) between participant identifiers and this unique code will be held at the study site. No personal identifiers will appear on the specimen tube.

5. Biorepository Specimen Usage

Specimens obtained for the Sponsor will be used for analyses using good scientific practices. Analyses utilizing the future biomedical research specimens may be performed by the Sponsor, or an additional third party (eg, a university investigator) designated by the Sponsor. The investigator conducting the analysis will follow the Sponsor's privacy and confidentiality requirements. Any contracted third party analyses will conform to the specific scope of analysis outlined in this substudy. Future biomedical research specimens remaining with the third party after specific analysis is performed will be reported to the Sponsor.

6. Withdrawal From Future Biomedical Research

Participants may withdraw their consent for future biomedical research and ask that their biospecimens not be used for future biomedical research. Participants may withdraw consent at any time by contacting the principal investigator for the main study. If medical

records for the main study are still available, the investigator will contact the Sponsor using the designated mailbox (clinical.specimen.management@merck.com). Subsequently, the participant's specimens will be flagged in the biorepository and restricted to main study use only. If specimens were collected from study participants specifically for future biomedical research, these specimens will be removed from the biorepository and destroyed. Documentation will be sent to the investigator confirming withdrawal and/or destruction, if applicable. It is the responsibility of the investigator to inform the participant of completion of the withdrawal and/or destruction, if applicable. Any analyses in progress at the time of request for withdrawal/destruction or already performed prior to the request being received by the Sponsor will continue to be used as part of the overall research study data and results. No new analyses would be generated after the request is received.

In the event that the medical records for the main study are no longer available (eg, if the investigator is no longer required by regulatory authorities to retain the main study records) or the specimens have been completely anonymized, there will no longer be a link between the participant's personal information and their specimens. In this situation, the request for withdrawal of consent and/or destruction cannot be processed.

7. Retention of Specimens

Future biomedical research specimens will be stored in the biorepository for potential analysis for up to 20 years from the end of the main study. Specimens may be stored for longer if a regulatory or governmental authority has active questions that are being answered. In this special circumstance, specimens will be stored until these questions have been adequately addressed.

Specimens from the study site will be shipped to a central laboratory and then shipped to the Sponsor-designated biorepository. If a central laboratory is not utilized in a particular study, the study site will ship directly to the Sponsor-designated biorepository. The specimens will be stored under strict supervision in a limited access facility which operates to assure the integrity of the specimens. Specimens will be destroyed according to Sponsor policies and procedures and this destruction will be documented in the biorepository database.

8. Data Security

Databases containing specimen information and test results are accessible only to the authorized Sponsor representatives and the designated study administrator research personnel and/or collaborators. Database user authentication is highly secure, and is accomplished using network security policies and practices based on international standards to protect against unauthorized access.

9. Reporting of Future Biomedical Research Data to Participants

No information obtained from exploratory laboratory studies will be reported to the participant, family, or physicians. Principle reasons not to inform or return results to the participant include: Lack of relevance to participant health, limitations of predictive capability, and concerns regarding misinterpretation.

If important research findings are discovered, the Sponsor may publish results, present results in national meetings, and make results accessible on a public website in order to

rapidly report this information to doctors and participants. Participants will not be identified by name in any published reports about this study or in any other scientific publication or presentation.

10. Future Biomedical Research Study Population

Every effort will be made to recruit all participants diagnosed and treated on Sponsor clinical studies for future biomedical research.

11. Risks Versus Benefits of Future Biomedical Research

For future biomedical research, risks to the participant have been minimized and are described in the future biomedical research informed consent.

The Sponsor has developed strict security, policies and procedures to address participant data privacy concerns. Data privacy risks are largely limited to rare situations involving possible breach of confidentiality. In this highly unlikely situation there is risk that the information, like all medical information, may be misused.

12. Questions

Any questions related to the future biomedical research should be e-mailed directly to clinical.specimen.management@merck.com.

13. References

1. National Cancer Institute [Internet]: Available from https://www.cancer.gov/publications/dictionaries/cancer-terms?cdrid=45618
2. International Conference on Harmonization [Internet]: E15: Definitions for Genomic Biomarkers, Pharmacogenomics, Pharmacogenetics, Genomic Data and Sample Coding Categories. Available from http://www.ich.org/products/guidelines/efficacy/efficacy-single/article/definitions-for-genomic-biomarkers-pharmacogenomics-pharmacogenetics-genomic-data-and-sample-cod.html
3. Industry Pharmacogenomics Working Group [Internet]: Understanding the Intent, Scope and Public Health Benefits of Exploratory Biomarker Research: A Guide for IRBs/IECs and Investigational Site Staff. Available at http://i-pwg.org/
4. Industry Pharmacogenomics Working Group [Internet]: Pharmacogenomics Informational Brochure for IRBs/IECs and Investigational Site Staff. Available at http://i-pwg.org/

10.3 Appendix 3: Contraceptive Guidance and Pregnancy Testing

Definitions

Women of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming postmenopausal unless permanently sterile (see below)

Women in the following categories are not considered WOCBP:

- Premenarchal
- Premenopausal female with 1 of the following:
  - Documented hysterectomy
  - Documented bilateral salpingectomy
  - Documented bilateral oophorectomy

  Note: Documentation can come from the site personnel's review of the participant's medical records, medical examination, or medical history interview.
- Postmenopausal female
  - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
    - A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormone replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with two FSH measurements in the postmenopausal range is required.
  - Females on HRT and whose menopausal status is in doubt will be required to use one of the nonhormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Requirements

Male Participants

Male participants with female partners of childbearing potential are eligible to participate if they agree to one of the following during the protocol defined time frame in section 5.1:

- Be abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent.
- Use a male condom plus partner use of an additional contraceptive method when having penile-vaginal intercourse with a WOCBP who is not currently pregnant.
    - The following are not acceptable methods of contraception:
        - Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhoea method (LAM).
        - Male condom with cap, diaphragm or sponge with spermicide.
        - Male and female condom cannot be used together.
    - Note: Men with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration.

Female Participants

Female participants of childbearing potential are eligible to participate if they agree to use a highly effective method of contraception consistently and correctly as described in Table 12 during the protocol-defined time frame in Section 5.1.

Table 12 Highly Effective Contraception Methods

Highly Effective Contraceptive Methods That Are User Dependent [a] *Failure rate of <1% per year when used consistently and correctly.*
● Combined (estrogen- and progestogen- containing) hormonal contraception [b] ○ Oral ○ Intravaginal ○ Transdermal ○ Injectable
● Progestogen-only hormonal contraception [b] ○ Oral ○ Injectable
Highly Effective Methods That Have Low User Dependency *Failure rate of <1% per year when used consistently and correctly.*
● Progestogen-only contraceptive implant [b] ● Intrauterine hormone-releasing system (IUS) ● Intrauterine device (IUD) ● Bilateral tubal occlusion
● Vasectomized partner A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.
● Sexual abstinence Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.
Notes: Use should be consistent with local regulations regarding the use of contraceptive methods for participants of clinical studies. a) Typical use failure rates are higher than perfect-use failure rates (ie, when used consistently and correctly). b) If locally required, in accordance with Clinical Trial Facilitation Group (CTFG) guidelines, acceptable hormonal contraceptives are limited to those which inhibit ovulation.

Pregnancy Testing

WOCBP should only be included after a negative highly sensitive urine or serum pregnancy test.

Following initiation of treatment additional pregnancy testing will be performed whenever an expected menstrual cycle is missed or when pregnancy is otherwise suspected, at the time points specified in the Schedule of Activities, and as required locally.

10.4 Appendix 4: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

Definition of AE

AE definition
• An AE is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of study treatment, whether or not considered related to the study treatment. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a study treatment. • NOTE: for purposes of AE definition, study treatment (also referred to as Sponsor's product) includes any pharmaceutical product, biological product, vaccine, device, diagnostic agent or protocol specified procedure whether investigational (including placebo or active comparator product) or marketed, manufactured by, licensed by, provided by or distributed by the Sponsor for human use in this study.

Events <u>meeting</u> the AE definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital signs measurements), including those that worsen from baseline, or are considered clinically significant in the medical and scientific judgment of the investigator. • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study treatment administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication. • For all reports of overdose (whether accidental or intentional) with an associated AE, the AE term should reflect the clinical symptoms or abnormal test result. An overdose of study treatment without any associated clinical symptoms or abnormal laboratory results is reported using the terminology "accidental or intentional overdose without adverse effect." • Any new cancer (that is not a condition of the study). Note: Progression of the cancer under study is not a reportable event. Refer to Section 8.4.5 for additional details.

Events NOT meeting the AE definition
● Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE. ● Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). ● Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen. ● Surgery planned prior to informed consent to treat a pre-existing condition that has not worsened. ● Refer to Section 8.4.5 for protocol specific exceptions

Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met

An SAE is defined as any untoward medical occurrence that, at any dose:
a. Results in death
b. Is life-threatening ● The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
c. Requires inpatient hospitalization or prolongation of existing hospitalization ● Hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization for an elective procedure to treat a pre-existing condition that has not worsened is not an SAE. A pre-existing condition is a clinical condition that is diagnosed prior to the use of an MSD product and is documented in the patient's medical history.
d. Results in persistent or significant disability/incapacity ● The term disability means a substantial disruption of a person's ability to conduct normal life functions. ● This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

- in offspring of participant taking the product regardless of time to diagnosis

f. Other important medical events:

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

  Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Additional Events Reported in the Same Manner as SAE

Additional events which require reporting in the same manner as SAE

- In addition to the above criteria, AEs meeting either of the below criteria, although not serious per ICH definition, are reportable to the Sponsor in the same time frame as SAEs to meet certain local requirements. Therefore, these events are considered serious by the Sponsor for collection purposes.
- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose.

Recording AE and SAE

AE and SAE recording

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory, and diagnostics reports) related to the event.
- The investigator will record all relevant AE/SAE information on the AE CRFs/worksheets at each examination.
- It is **not** acceptable for the investigator to send photocopies of the participant's medical records to the Sponsor in lieu of completion of the AE CRF page.
- There may be instances when copies of medical records for certain cases are requested by the Sponsor. In this case, all participant identifiers, with the exception of the participant number, will be blinded on the copies of the medical records before submission to the Sponsor.

- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of intensity

- An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.
- The investigator will make an assessment of intensity for each AE and SAE (and other reportable safety event) according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 4.0. Any AE which changes CTCAE grade over the course of a given episode will have each change of grade recorded on the AE CRFs/worksheets.
  - Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
  - Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL).
  - Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
  - Grade 4: Life threatening consequences; urgent intervention indicated.
  - Grade 5: Death related to AE.

Assessment of causality

- Did the Sponsor's product cause the AE?
  - The determination of the likelihood that the Sponsor's product caused the AE will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test product and the AE based upon the available information
  - **The following components are to be used to assess the relationship between the Sponsor's product and the AE;** the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely the Sponsor's product caused the AE:
    - **Exposure:** Is there evidence that the participant was actually exposed to the Sponsor's product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?

- **Time Course:** Did the AE follow in a reasonable temporal sequence from administration of the Sponsor's product? Is the time of onset of the AE compatible with a drug-induced effect (applies to studies with investigational medicinal product)?
- **Likely Cause:** Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors
- **Dechallenge:** Was the Sponsor's product discontinued or dose/exposure/frequency reduced?
  - If yes, did the AE resolve or improve?
    - If yes, this is a positive dechallenge.
  - If no, this is a negative dechallenge.

  (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the Sponsor's product; (3) the study is a single-dose drug study); or (4) Sponsor's product(s) is/are only used one time.)
- **Rechallenge:** Was the participant re-exposed to the Sponsor's product in this study?
  - If yes, did the AE recur or worsen?
    - If yes, this is a positive rechallenge.
  - If no, this is a negative rechallenge.

  (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the study is a single-dose drug study); or (3) Sponsor's product(s) is/are used only one time.)

NOTE: IF A RECHALLENGE IS PLANNED FOR AN AE WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY THE SPONSOR'S PRODUCT, OR IF RE-EXPOSURE TO THE SPONSOR'S PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE PARTICIPANT THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE SPONSOR CLINICAL DIRECTOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL, AND IF REQUIRED, THE INIRB/IEC.

- **Consistency with Study treatment Profile:** Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding the Sponsor's product or drug class pharmacology or toxicology?

- The assessment of relationship will be reported on the CRFs/worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.
- Use the following scale of criteria as guidance (not all criteria must be present to be indicative of a Sponsor's product relationship).

- Yes, there is a reasonable possibility of Sponsor's product relationship: There is evidence of exposure to the Sponsor's product. The temporal sequence of the AE onset relative to the administration of the Sponsor's product is reasonable. The AE is more likely explained by the Sponsor's product than by another cause.
- No, there is not a reasonable possibility of Sponsor's product relationship: Participant did not receive the Sponsor's product OR temporal sequence of the AE onset relative to administration of the Sponsor's product is not reasonable OR the AE is more likely explained by another cause than the Sponsor's product. (Also entered for a participant with overdose without an associated AE.)

- For each AE/SAE, the investigator must document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to the Sponsor. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to the Sponsor.
- The investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements

Follow-up of AE and SAE

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by Sponsor to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- New or updated information will be recorded in the CRF.
- The investigator will submit any updated SAE data to the Sponsor within 24 hours of receipt of the information.

Reporting of AEs, SAEs, and Other Reportable Safety Events to the Sponsor

AE, SAE, and other reportable safety event reporting to Sponsor via electronic data collection tool

● The primary mechanism for reporting to the Sponsor will be the electronic data collection (EDC) tool.
  ● Electronic reporting procedures can be found in the EDC data entry guidelines (or equivalent).
  ● If the electronic system is unavailable for more than 24 hours, then the site will use the paper AE Reporting form.
  ● Reference Section 8.4.1 for reporting time requirements
● The site will enter the SAE data into the electronic system as soon as it becomes available.
● After the study is completed at a given site, the EDC tool will be taken off-line to prevent the entry of new data or changes to existing data.
● If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the EDC tool has been taken off-line, then the site can report this information on a paper SAE form or by telephone (see next section).
● Contacts for SAE reporting can be found in the Investigator Trial File Binder (or equivalent).

SAE reporting to the Sponsor via paper CRF
● If the EDC tool is not operational, facsimile transmission or secure e-mail of the SAE paper CRF is the preferred method to transmit this information to the Sponsor. ● In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service. ● Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE CRF pages within the designated reporting time frames. ● Contacts and instructions for SAE reporting and paper reporting procedures can be found in the Investigator Trial File Binder (or equivalent).

10.5 Appendix 5: Clinical Laboratory Tests

- The tests detailed in Table 13 will be performed by the local laboratory.
- Protocol-specific requirements for inclusion or exclusion of participants are detailed in Section 5 of the protocol.
- Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.

Table 13 Protocol-required Safety Laboratory Assessments

Laboratory Assessments	Parameters			
Hematology	Platelet Count RBC Count Hemoglobin Hematocrit	RBC Indices: MCV MCH %Reticulocytes	WBC count with Differential: Neutrophils Lymphocytes Monocytes Eosinophils Basophils	
Chemistry	Blood Urea Nitrogen (BUN)	Potassium	Aspartate aminotransferase (AST)/ Serum glutamic-oxaloacetic transaminase (SGOT)	Total bilirubin (and direct bilirubin, if total bilirubin is elevated above the upper limit of normal)
	Albumin	Bicarbonate	Chloride	Phosphorous
	Creatinine	Sodium	Alanine aminotransferase (ALT)/ Serum glutamic-pyruvic transaminase (SGPT)	Total protein
	Glucose [fasting]	Calcium	Alkaline phosphatase	Magnesium
	Uric acid	Thyroid stimulating hormone (TSH)	Triiodothyronine (T3)	Free thyroxine (FT4)
Routine Urinalysis	• Specific gravity • pH, glucose, protein, blood, ketones, bilirubin, urobilinogen, nitrite, leukocyte esterase by dipstick • Microscopic examination (if blood or protein is abnormal)			

Laboratory Assessments	Parameters
Other Screening Tests	• Follicle-stimulating hormone and estradiol (as needed in women of nonchildbearing potential only) • Serum or urine β human chorionic gonadotropin (β hCG) pregnancy test (as needed for WOCBP) • Serology (HIV antibody, hepatitis B surface antigen [HBsAg], and hepatitis C virus antibody) if applicable • Prothrombin time (PT), International normalized ratio (INR), activated partial thromboplastin time (aPTT) • BRAF mutational analysis • Lactate dehydrogenase • Blood for future biomedical research and planned genetic analysis.
Central Laboratory Samples	• All study-required laboratory assessments will be performed by the local laboratory, with the exception of: • • Blood for PBMCs • Blood for all correlative studies
NOTES: • GFR (measured or calculated) or creatinine clearance (CrCl) can be used in place of creatinine • T3 is preferred but, if not available, free T3 may be tested.	

Investigators must document their review of each laboratory safety report.

10.6 Appendix 6: 12.5 Description of the iRECIST Process for Assessment of Disease Progression

Assessment at Screening and Prior to RECIST 1.1 Progression

Until radiographic disease progression based on RECIST 1.1, there is no distinct iRECIST assessment.

Assessment and Decision at RECIST 1.1 Progression

For participants who show evidence of radiological PD by RECIST 1.1 as determined by the Investigator, the Investigator will decide whether to continue a participant on study treatment until repeat imaging is obtained (using iRECIST for participant management (see Table 5 and Figure 2). This decision by the Investigator should be based on the participant's overall clinical condition.

Clinical stability is defined as the following:

- Absence of symptoms and signs indicating clinically significant progression of disease
- No decline in ECOG performance status
- No requirements for intensified management, including increased analgesia, radiation, or other palliative care

Any participant deemed **clinically unstable** should be discontinued from study treatment at site-assessed first radiologic evidence of PD, and is not required to have repeat tumor imaging for confirmation of PD by iRECIST.

If the Investigator decides to continue treatment, the participant may continue to receive study treatment and the tumor assessment should be repeated 4 to 8 weeks later to confirm PD by iRECIST, per Investigator assessment.

Tumor flare may manifest as any factor causing radiographic progression per RECIST 1.1, including:

- Increase in the sum of diameters of target lesion(s) identified at baseline to ≥20% and ≥5 mm from nadir
    - Note: the iRECIST publication uses the terminology "sum of measurements", but "sum of diameters" will be used in this protocol, consistent with the original RECIST 1.1 terminology.
- Unequivocal progression of non-target lesion(s) identified at baseline
- Development of new lesion(s)

iRECIST defines new response categories, including iUPD (unconfirmed progressive disease) and iCPD (confirmed progressive disease). For purposes of iRECIST assessment, the first visit showing progression according to RECIST 1.1 will be assigned a visit (overall) response of iUPD, regardless of which factors caused the progression.

At this visit, target and non-target lesions identified at baseline by RECIST 1.1 will be assessed as usual.

New lesions will be classified as measurable or non-measurable, using the same size thresholds and rules as for baseline lesion assessment in RECIST 1.1. From measurable new lesions, up to 5 lesions total (up to 2 per organ), may be selected as New Lesions – Target. The sum of diameters of these lesions will be calculated, and kept distinct from the sum of diameters for target lesions at baseline. All other new lesions will be followed qualitatively as New Lesions – Non-target.

Assessment at the Confirmatory Imaging

On the confirmatory imaging, the participant will be classified as progression confirmed (with an overall response of iCPD), or as showing persistent unconfirmed progression (with an overall response of iUPD), or as showing disease stability or response (iSD/iPR/iCR).

Confirmation of Progression

Progression is considered confirmed, and the overall response will be iCPD, if ANY of the following occurs:

- Any of the factors that were the basis for the initial iUPD show worsening
  - For target lesions, worsening is a further increase in the sum of diameters of ≥5 mm, compared to any prior iUPD time point
  - For non-target lesions, worsening is any significant growth in lesions overall, compared to a prior iUPD time point; this does not have to meet the "unequivocal" standard of RECIST 1.1
  - For new lesions, worsening is any of these:
    - An increase in the new lesion sum of diameters by ≥5 mm from a prior iUPD time point
    - Visible growth of new non-target lesions
    - The appearance of additional new lesions
- Any new factor appears that would have triggered PD by RECIST 1.1

Persistent iUPD

Progression is considered not confirmed, and the overall response remains iUPD, if:

- None of the progression-confirming factors identified above occurs AND
- The target lesion sum of diameters (initial target lesions) remains above the initial PD threshold (by RECIST 1.1)

Additional imaging for confirmation should be scheduled 4 to 8 weeks from the imaging on which iUPD is seen. This may correspond to the next visit in the original visit schedule. The assessment of the subsequent confirmation imaging proceeds in an identical manner, with possible outcomes of iCPD, iUPD, and iSD/iPR/iCR.

Resolution of iUPD

Progression is considered not confirmed, and the overall response becomes iSD/iPR/iCR, if:

- None of the progression-confirming factors identified above occurs, AND
- The target lesion sum of diameters (initial target lesions) is not above the initial PD threshold.

The response is classified as iSD or iPR (depending on the sum of diameters of the target lesions), or iCR if all lesions resolve.

In this case, the initial iUPD is considered to be pseudo-progression, and the level of suspicion for progression is "reset". This means that the next visit that shows radiographic progression, whenever it occurs, is again classified as iUPD by iRECIST, and the confirmation process is repeated before a response of iCPD can be assigned.

Management Following the Confirmatory Imaging

If repeat imaging does not confirm PD per iRECIST, as assessed by the Investigator, and the participant continues to be clinically stable, study treatment may continue and follow the regular imaging schedule. If PD is confirmed, participants will be discontinued from study treatment.

NOTE: If a participant has confirmed radiographic progression (iCPD) as defined above, but the participant is achieving a clinically meaningful benefit, an exception to continue study treatment may be considered following consultation with the Sponsor. In this case, if study treatment is continued, tumor imaging should continue to be performed following the intervals as outlined in Section 6.

Detection of Progression at Visits After Pseudo-progression Resolves

After resolution of pseudo-progression (ie, achievement of iSD/iPR/iCR), iUPD is indicated by any of the following events:

- Target lesions
  - Sum of diameters reaches the PD threshold (≥20% and ≥5 mm increase from nadir) either for the first time, or after resolution of previous pseudo-progression. The nadir is always the smallest sum of diameters seen during the entire trial, either before or after an instance of pseudo-progression.
- Non-target lesions
  - If non-target lesions have never shown unequivocal progression, their doing so for the first time results in iUPD.
  - If non-target lesions have shown previous unequivocal progression, and this progression has not resolved, iUPD results from any significant further growth of non-target lesions, taken as a whole.
- New lesions
  - New lesions appear for the first time

- Additional new lesions appear
- Previously identified new target lesions show an increase of ≥5 mm in the new lesion sum of diameters, from the nadir value of that sum
- Previously identified non-target lesions show any significant growth

If any of the events above occur, the overall response for that visit is iUPD, and the iUPD evaluation process (see Assessment at the Confirmatory Imaging above) is repeated. Progression must be confirmed before iCPD can occur.

The decision process is identical to the iUPD confirmation process for the initial PD, with one exception: if new lesions occurred at a prior instance of iUPD, and at the confirmatory imaging the burden of new lesions has increased from its smallest value (for new target lesions, the sum of diameters is ≥5 mm increased from its nadir), then iUPD cannot resolve to iSD or iPR. It will remain iUPD until either a decrease in the new lesion burden allows resolution to iSD or iPR, or until a confirmatory factor causes iCPD.

Additional details about iRECIST are provided in the iRECIST publication [Seymour, L., et al 2017].

10.7 Appendix 7: Abbreviations

Abbreviation	Definition
AE	Adverse Event
APaT	All Participants as Treated
CD	Cluster of Differentiation
CI	Confidence Interval
CNS	Central Nervous System
CR	Complete Response
CRF	Case Report Form
CSR	Clinical Study Report
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTLA-4	Cytotoxic T-lymphocyte-associated Antigen 4
CyTOF	Time of Flight Mass Cytometry
CytS	Cytotoxic Score
ECG	Electrocardiogram
ECI	Events of Clinical Interest
ECOG	Eastern Cooperative Oncology Group
EDC	Electronic Data Capture
ERC	Ethics Review Committee
FCT	fraction of cytotoxic tumor-infiltrating T-lymphocytes
GCP	Good Clinical Practice
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
IB	Investigator's Brochure
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
Ig	Immunoglobulin
IHC	Immunohistochemistry
irAE	Immune-related Adverse Event
IRB	Institutional Review Board
iRECIST	Modified Response Evaluation Criteria in Solid Tumors 1.1 for Immune-based Therapeutics
IV	Intravenous(ly)
KCO	Klarman Cell Observatory
KN	KEYNOTE
LC MS/MS	Liquid chromatography tandem mass spectrometry
mAb	Monoclonal Antibody
MRI	Magnetic Resonance Imaging
NCI	National Cancer Institute
NSCLC	Non-small Cell Lung Cancer
ORR	Overall Response Rate
OS	overall survival
PBMCs	Peripheral Blood Mononuclear Cells

Abbreviation	Definition
PBPK	Physiologically-based Pharmacokinetic
PD	progressive disease
PD-1	Programmed Cell Death 1
PD-L1	Programmed Cell Death 1 Ligand 1
PD-L2	Programmed Cell Death 1 Ligand 2
PFS	Progression-free Survival
PK	Pharmacokinetics
PR	Partial Response
Q2W	Every 2 Weeks
Q3W	Every 3 Weeks
QC	Quality Control
RECIST	Response Evaluation Criteria In Solid Tumors
RNASeq	RNA Sequencing
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
scRNAseq	Single Cell RNA-sequencing
SD	Stable Disease
sSAP	Supplemental Statistical Analysis Plan
TCR	T-cell Receptor
Teff	Effector T-lymphocytes
Tregs	Regulatory T-cells
UMI	Unique Molecular Identifiers
WOCBP	Woman of Childbearing Potential

11. References

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