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A phase 2 trial of pembrolizumab in metastatic NSCLC examining circulating tumor DNA levels as a surrogate biomarker of response

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10 August 2016	Revised in response to IRB comments Submitted to FDA as IND amendment (Serial No. 0001)
23 August 2017	Allow first line treatment for ≥ 50% PDL1-expressing tumors
26 June 2018	Allow squamous and optional repeat biopsy if tissue available
2 Dec 2019	Allow STAMP testing in lieu of tissue, allow 1% or higher PD-L1 first line, allow ECOG PS 0-1, schedule of events corrected
25 Jan 2021	Removed Dr. Sukhmani Padda as Co-PI

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PROTOCOL SYNOPSIS

TITLE	A Phase 2 trial of pembrolizumab in metastatic NSCLC examining circulating tumor DNA levels as a surrogate biomarker of response
STUDY PHASE	2
INDICATION	Metastatic NSCLC
INVESTIGATIONAL PRODUCT OR PROCEDURE	Pembrolizumab 200 mg intravenous every 3 weeks for up to 24 months of therapy
PRIMARY OBJECTIVE(S)	To correlate circulating tumor DNA (ctDNA) levels measured using Cancer Personalized Profiling by deep Sequencing (CAPP-Seq) with radiographic tumor assessments using RECIST v1.1 criteria in patients with metastatic NSCLC treated with pembrolizumab
SECONDARY OBJECTIVE(S)	• To determine the overall response rate (ORR) using RECIST v1.1 criteria in patients with metastatic NSCLC treated with pembrolizumab • To determine the progression-free survival (PFS) using RECIST v1.1 criteria in patients with metastatic NSCLC treated with pembrolizumab • To determine the safety and tolerability of pembrolizumab in patients with metastatic NSCLC
EXPLORATORY OBJECTIVES	• To correlate tumor immune leukocyte subpopulations identified using the CIBERSORT method alone and in conjunction with PD-L1 assessment on pre-treatment tumor samples with objective response using RECIST v1.1 criteria in patients with metastatic NSCLC treated with pembrolizumab

INCLUSION CRITERIA	In order to be eligible for participation in this trial, the patient must meet <u>*ALL*</u> of the following criteria (ie, mark "yes" to all criteria): 1. Has a pathologically proven recurrent or metastatic non-small cell lung cancer (non-squamous, squamous, mixed, and neuroendocrine histologies are allowed as of amendment 26 June 2018) 2. (a) Previously received at least one line of prior systemic therapy for metastatic disease. i. If the patient has a known sensitizing *EGFR* mutation or *ALK* rearrangement, the patient must have received at least one prior targeted therapy for metastatic disease (ie, EGFR TKI therapy or ALK TKI therapy, respectively). ii. There is no limit on prior therapies allowed. Patients must have completed previous treatment (including other investigational therapy) in greater than or equal to the following times prior to initiation of trial treatment: a. Anti-cancer monoclonal antibody (mAb) therapy must be completed ≥ 3 weeks prior to trial treatment b. Chemotherapy administered in a daily or weekly schedule must be completed ≥ 1 week prior to trial treatment c. Chemotherapy administered in an every 2-week schedule must be completed ≥ 2 weeks prior to trial treatment d. Chemotherapy administered in an every 3-week schedule must be completed ≥ 3 weeks prior to trial treatment e. Targeted small molecule therapy must be completed ≥ 1 week prior to trial treatment **<u>OR</u>** (b) Have not received prior systemic therapy for their cancer in recurrent or metastatic setting, AND have a tumor with Tumor Proportion Score (TPS) ≥ 1% as measured by PD-L1 IHC, AND no evidence of a sensitizing *EGFR* mutation or *ALK* rearrangement if adenocarcinoma histology (molecular testing not required for other histologies). 3. Prior radiation therapy allowed as long as completed in the following times prior to initiation of trial treatment: a. Definitive curative intent radiation ≥ 2 weeks prior to trial treatment b. Palliative body radiation ≥ 1 week prior to trial treatment c. Stereotactic brain radiation ≥ 1 week prior to trial treatment d. Whole brain radiation ≥ 2 weeks prior to trial treatment 4. Patients with previously treated (with radiation or surgery) brain metastases are allowed. Patients with untreated stable or

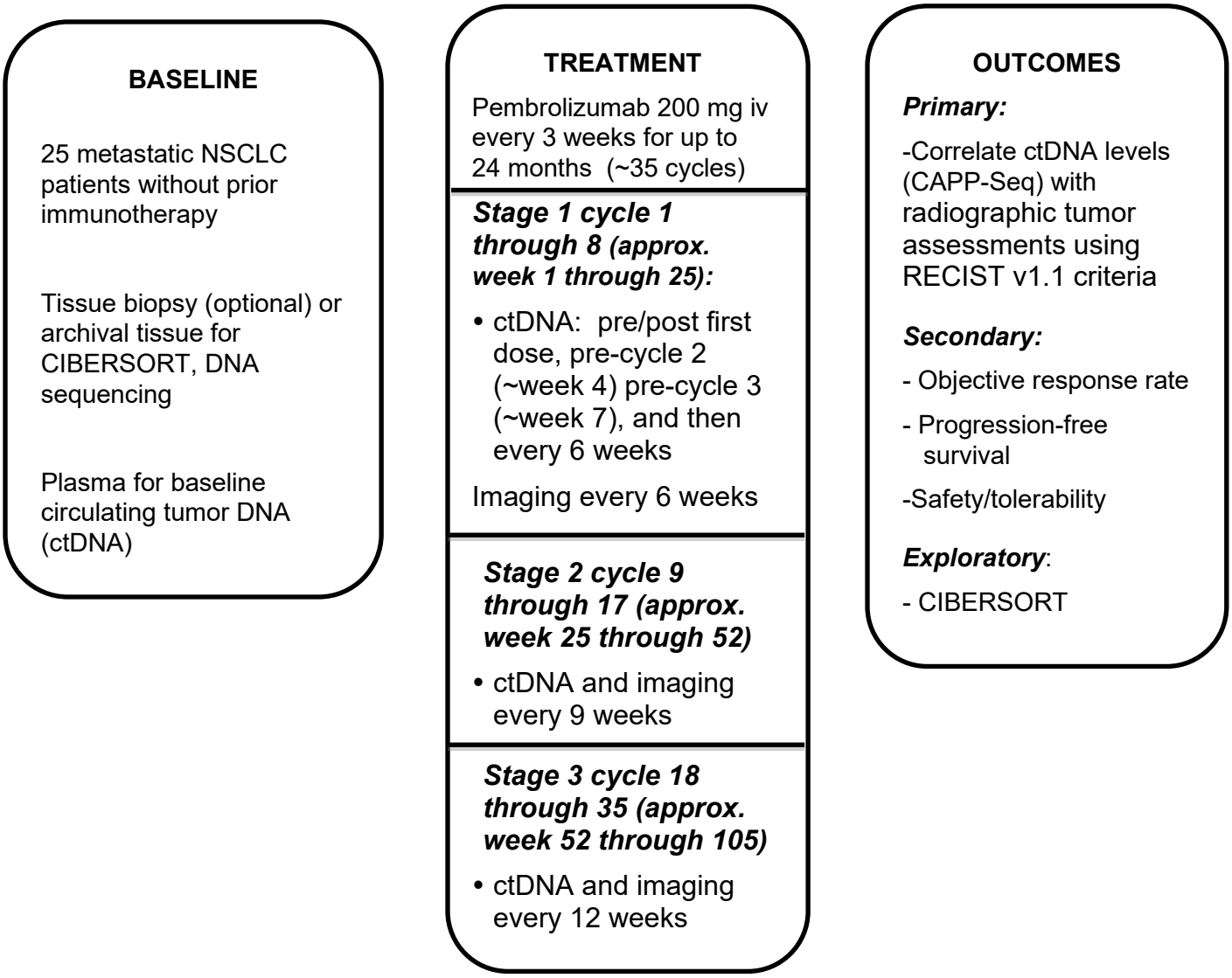
	progressing metastases must have metastases ≤ 1.5 cm, be asymptomatic, and either not be on steroids or be on 10 mg prednisone equivalent or less. 5. Has measurable disease based on RECIST v1.1 criteria. 6. Is medically able and willing to undergo needle biopsy of a tumor lesion, -OR- Has known tumor PD-L1 expression and believed to have sufficient archival tumor available for genomic analysis (block or 10 unstained slides or Stanford STAMP NGS testing previously resulted or in process) 7. Has life expectancy ≥ 3 months 8. Ability to understand and the willingness to sign a written informed consent document. 9. ≥ 18 years of age on day of signing informed consent 10. ECOG performance status of 0, 1, or 2 (Appendix A) 11. Adequate organ function: a. Absolute neutrophil count (ANC) ≥ 1,000/mcL b. Platelets ≥ 75,000/mcL c. Hemoglobin ≥ 8 g/dL d. Serum creatinine ≤ 1.5 X upper limit of normal (ULN) **OR** Measured or calculated creatinine clearance ≥ 30 mL/min for patient with creatinine levels > 1.5 X institutional ULN e. Serum total bilirubin ≤ 1.5 X ULN **OR** Direct bilirubin ≤ ULN for patients with total bilirubin levels > 1.5 ULN f. AST (SGOT) and ALT (SGPT) ≤ 3 X ULN **OR** ≤ 5 X ULN for patients with liver metastases 12. Female patients of childbearing potential must have a negative urine or serum pregnancy test prior to the first dose of trial treatment. They must also agree to acceptable contraceptive methods (ie, combined estrogen progesterone hormonal contraception, progesterone only hormonal contraception, intrauterine device, have history of bilateral tubal ligation, or two barrier method) or agree to abstain from heterosexual activity, for the course of the study through 120 days after the last dose of trial treatment. *-- Female patients are not considered to be of childbearing potential if (i) they have been surgically sterilized (such as hysterectomy plus bilateral salpingo-oophorectomy) or (ii) are free from menses for > 1 year (postmenopausal). Female patients who have not had> 1 year of amenorrhea may have confirmation of postmenopausal state with two FSH measurements in postmenopausal range and should not be on hormonal contraception or hormonal replacement therapy*

	during testing.
EXCLUSION CRITERIA	In order to be eligible for participation in this trial, the patient must <u>*NOT*</u> meet the following criteria (ie, mark "no" to all criteria): 1. Is currently receiving another investigational therapy 2. Has received prior anti-PD-1 or anti-PD-L1 therapy 3. Has clinically significant toxicities from previous anti-cancer therapy that have not resolved, or have not stabilized at a new baseline 4. Has undergone a surgical procedure involving general anesthesia within 2 weeks of starting trial treatment, or has inadequate healing or recovery from complications of surgery prior to starting trial treatment. This does *not* apply to low-risk procedures such as thoracentesis, paracentesis, chest tube/PleurX catheter placement, line placement, needle biopsy of tumor, and bronchoscopy. 5. Is receiving high dose systemic steroid therapy or other immunosuppressive therapy within 3 days of trial treatment. Topical and intraarticular steroid injections are allowed, as are physiologic doses of systemic steroids (≤ 10 mg of prednisone equivalent daily). 6. Has carcinomatous meningitis as determined by positive CSF cytology 7. Has known additional malignancy that is undergoing active treatment. 8. Has active autoimmune disease that has required systemic treatment in the past 2 years (ie, with use of disease modifying agents, supra-physiologic doses of systemic corticosteroids or immunosuppressive drugs). Replacement therapy (eg, thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment. Asthma, type I diabetes mellitus, hypothyroidism, and vitiligo are allowed. 9. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the patient's participation for the full duration of the trial, or is not in the best interest of the patient to participate, in the opinion of the treating investigator. This includes known active tuberculosis; Grade 3 active infection; history of allogeneic bone marrow transplant or solid organ transplant; known history of Human Immunodeficiency Virus (HIV); known active Hepatitis B (eg, Hep B DNA positive in prior 3 months) or known active Hepatitis C (eg, HCV RNA [qualitative] is detected in prior 3 months). 10. Known evidence of active, non-infectious, ≥ Grade 2

	pneumonitis (ie, medical intervention indicated) in the last 6 months 11. Is pregnant or breastfeeding, or expecting to conceive children within the projected duration of the trial, starting with the screening visit through 120 days after the last dose of trial treatment
TREATMENT SUMMARY	All patients will receive pembrolizumab, 200 mg fixed-dose, 30-minute intravenous infusion, every 3 weeks. Trial treatment will be continued for up to 24 months of therapy, or until disease progression without clinical benefit, unacceptable toxicity, treatment consent withdrawal, or initiation of other cancer therapy. Dose-delays are permitted per protocol.
SAMPLE SIZE	25 patients with metastatic NSCLC
STATISTICAL CONSIDERATIONS	**Statistical Plan** The primary explanatory variables are percent ctDNA and ctDNA titer (a continuous variable in units pg/mL). The correlation of percent ctDNA (and DNA titer) with sum of longest diameter of tumor target lesions (continuous variable in mm or cm, RECIST v1.1). Correlations of two continuous variables will be made using Kendall correlation. **Sample size justification:** Clinically validated differences in ctDNA have not been established to date, but the variability over time does appear to closely follow tumor volume as demonstrated by previously published data[1]. This may vary across tumor types. 25 patients would provide 80% power to detect a correlation of 0.64 with ctDNA and radiographic RECIST v1.1-based tumor assessments when using a Kendall correlation.

SCHEMA

This is a prospective, open-label, non-randomized single-arm study that will enroll approximately 25 patients with metastatic NSCLC.



LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse event
BID	Twice daily
BSA	Body surface area
CBC	Complete blood count
CI	Confidence interval
CNS	Central nervous system
CRF	Case report/Record form
CR	Complete response
CTCAE	Common Terminology Criteria for Adverse Events
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
HIV	Human Immunodeficiency Virus
HTN	Hypertensions
IRB	Institutional Review Board
IV	Intravenous
LLN	Lower limit of normal
OS	Overall survival
PD	Progressive disease
PFS	Progression-free survival
PR	Partial response
RECIST	Response evaluation criteria in solid tumors
RR	Response rate
SAE	Serious adverse event
SD	Stable disease
ULN	Upper limit of normal
WBC	White blood cell

1. OBJECTIVES

1.1 Primary Objective

To correlate circulating tumor DNA (ctDNA) levels measured using Cancer Personalized Profiling by deep Sequencing (CAPP-Seq) with radiographic tumor assessments using RECIST v1.1 criteria in patients with metastatic NSCLC treated with pembrolizumab

- ***Hypothesis:*** **Circulating tumor DNA quantitative levels will serve as a surrogate biomarker of radiographic tumor measurements made using RECIST v1.1 criteria**.

1.2 Secondary Objectives

- To determine the overall response rate (ORR) using RECIST v1.1 criteria in patients with metastatic NSCLC treated with pembrolizumab
- To determine the progression-free survival (PFS) using RECIST v1.1 in patients with metastatic NSCLC treated with pembrolizumab
- To determine the safety and tolerability of pembrolizumab in patients with metastatic NSCLC

1.3 Exploratory Objectives

- To correlate tumor immune leukocyte subpopulations identified using the CIBERSORT method alone and in conjunction with PD-L1 assessment on pre-treatment tumor samples with overall response rate using RECIST v1.1 criteria in patients with metastatic NSCLC treated with pembrolizumab

2. BACKGROUND

2.1 Study Disease

Background Lung Cancer and Immunotherapeutic Approaches

In the United States, lung cancer is the number one cause of cancer-related deaths, with an estimated 221,200 new cases of lung cancer and 158,040 lung cancer-related deaths in 2015.[2] Non-small cell lung cancer (NSCLC) comprises 85% of new cases of lung cancer, and includes adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. In the first line setting, platinum-based chemotherapy improves overall survival when compared to best supportive care.[3-7] Second line chemotherapeutics have more limited survival times, including single agent pemetrexed,[8] docetaxel,[8-11] and erlotinib.[12] There has been significant progress in lung cancer care in the last two decades, with personalized treatment based on histology and genetic driver mutations such as *EGFR* and *ALK*.[13-16] Chemotherapies and targeted therapies in the form of monoclonal antibodies and small molecule inhibitors have become the standard therapeutic classes used for the treatment of lung cancer.

Historically, lung cancer was considered to be a non-immunogenic tumor, with a lack of clinical activity seen with previously attempted immunotherapeutic approaches such as vaccines and biologics (ie, interferon, tumor necrosis-factor-alpha). However, as of March 4 2015, immunotherapy has become a new standard therapeutic approach for the treatment of lung cancer. Nivolumab, a monoclonal antibody to immune checkpoint program death receptor-1 (PD-1), marked the first FDA approval of immunotherapy for lung cancer. The PD-L1/PD-1 pathway is hijacked by the tumor and the interaction between the PD-L1 ligand on the tumor and PD-1 on the immune cell, inactivates the immune cell, resulting in tumor immune evasion, growth, and metastases. Nivolumab outperformed docetaxel in a randomized phase 3 study in squamous cell NSCLC after first-line platinum-based chemotherapy (CHECKMATE-017), with an improvement in median overall survival of 9.2 months (95% CI, 7.3 to 13.3) vs 6.0 months (95% CI, 5.1 to 7.3), respectively (HR 0.59, 95% CI 0.44 to 0.79, $P < 0.001$).[17] The randomized phase 3 study of nivolumab vs docetaxel in NSCLC of the lung after the first-line setting was also a positive trial, with a median improvement in overall survival of 12.2 vs 9.4 months, respectively (HR 0.73, 95% CI 0.59 to 0.89, $P = 0.0015$).[18] Pembrolizumab was the 2nd FDA-approved PD-1 inhibitor. The clinical activity of pembrolizumab, the PD-1 inhibitor selected for examination in this trial, is described in *Section 2.2*. There are several PD-1/PD-L1 inhibitors in development for the treatment of NSCLC, all of which have demonstrated clinical activity. In this time durvalumab (PD-L1) has been approved as a consolidation therapy after concurrent chemotherapy and radiation for stage III NSCLC[19] and atezolizumab (PD-L1) has been approved in the second line setting also outperforming docetaxel in survival.[20] **Despite responses seen in 15 to 20% of NSCLC patients with all PD-1/PD-L1 inhibitors, a robust predictive biomarker for response is not available.**

Pembrolizumab

2.2.1 Pembrolizumab Pharmaceutical and Therapeutic Background

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors.

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 CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is 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 (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

2.2.2 Pembrolizumab Preclinical and Clinical Trial Data

2.2.2.1 Mechanism of Action

The programmed cell death 1 (PD-1) pathway represents a major immune control switch, which may be engaged by tumor cells to overcome active T-cell immune surveillance.

Pembrolizumab (KEYTRUDA, MK-3475; previously known as SCH 900475 and ORG 307488-0) is a potent and highly selective humanized monoclonal antibody (mAb) of the immunoglobulin G4 (IgG4)/kappa isotype designed to directly block the interaction between PD-1 and its ligands, programmed cell death ligand 1 (PD-L1) and programmed cell death ligand 2 (PD-L2). This blockade enhances functional activity of the target lymphocytes to facilitate tumor regression and ultimately immune rejection. 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 because of its mechanism of action to bind the PD-1 receptor on the T cell. Keytruda (pembrolizumab) has been approved for lung cancer in the following indications: (i) single agent for first line treatment of patients with metastatic NSCLC whose tumors have high PD-L1 expression [tumor proportion score ≥ 50%] as determined by an FDA-approved test with no *EGFR* or *ALK* genomic tumor aberrations; (ii) single agent for treatment of patients with metastatic NSCLC whose tumors express PD-L1 [(tumor proportion score ≥ 1%] as determined by an FDA-approved test, with disease progression on or after platinum-containing chemotherapy (patients with *EGFR* or *ALK* genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving pembrolizumab) (iii) in combination with pemetrexed and carboplatin, as first-line treatment of patients with metastatic NSCLC (accelerated approval based on response rate and progression-free survival). For more details on specific indications refer to the most up to date Investigator Brochure.

2.2.2.2 <u>Animal Studies-</u> Please refer to most up to date Investigator Brochure.

2.2.2.2.1 *Nonclinical Pharmacology*- Please refer to most up to date Investigator Brochure.

Pembrolizumab binds to human and Cynomolgus monkey PD-1 with comparable affinity and blocks the binding of human and Cynomolgus monkey PD-1 to PD-L1 and PD-L2 with comparable potency. Pembrolizumab does not cross-react with dog, rat, or mouse PD-1. Pembrolizumab does not bind immunoglobulin superfamily members cluster of differentiation 28 (CD28), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), or inducible T-cell costimulator (ICOS).

Pembrolizumab strongly enhances T-lymphocyte immune responses in cultured blood cells from healthy human donors, cancer subjects, and nonhuman primates. In T-cell activation assays using human donor blood cells, the half-maximal effective concentration (EC50) has been approximately 0.1 to 0.3 nM. In addition to interleukin-2 (IL-2), tumor necrosis factor alpha (TNFα), interferon gamma (IFNγ), and levels of other cytokines were found to be modulated by pembrolizumab. The antibody potentiates existing immune responses only in the presence of antigen and does not nonspecifically activate T-cells. In the in vitro peripheral blood mononuclear cell (PBMC) and whole blood cytokine release assays, the cytokine levels induced by pembrolizumab were low and comparable to those induced by trastuzumab. Pembrolizumab does not induce antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC).

Using anti–murine PD-1 surrogate antibodies, PD-1 blockade has been shown to significantly inhibit tumor growth in a variety of syngeneic murine tumor models. In these experiments in mice, anti–PD-1 therapy is synergistic with chemotherapeutic agents such as gemcitabine and 5-fluorouracil (5-FU), and combination therapy results in increased complete tumor regression

rates in vivo. Studies also revealed that immunosuppressive doses of dexamethasone included in combination with agents used in standard-of-care treatment for NSCLC do not reduce the anti-tumor efficacy of an anti–murine PD-1 surrogate antibody.

2.2.2.2.2 *Nonclinical Pharmacokinetics*- Please refer to most up to date Investigator Brochure.

The pharmacokinetics (PK) of pembrolizumab were evaluated in a non–Good Laboratory Practices (GLP) single-dose PK study and two GLP repeat-dose toxicokinetic (TK) studies (1 month and 6 month) in Cynomolgus monkeys. Pembrolizumab stability as a modified IgG4 molecule was evaluated in vivo in mice.

After single-dose IV administration at 0.3; 3; or 30 mg/kg in Cynomolgus monkeys, decline of serum concentration followed multiphasic kinetics. Anti–drug antibodies (ADAs) were detected in most of the treated animals. Clearance (CL) and terminal half-life (t1/2) appeared to be dose-dependent in the dose range tested with t1/2 varying from 4 to 10 days. In the 1-month repeat-dose (once weekly) GLP toxicity study at 6, 40, or 200 mg/kg in Cynomolgus monkeys, ADAs were detected in most of the low-dose (6 mg/kg) treated animals. The systemic exposure over the 7-day dosing interval (AUC0-7 days) was sex-independent and increased with increasing dose. The mean t1/2 values in individual ADA-negative animals ranged from 15.7 to 22.3 days across doses.

In the 6-month repeat-dose (every other week) GLP toxicity study at 6, 40, or 200 mg/kg in Cynomolgus monkeys, ADAs were detected in most of the low-dose (6 mg/kg) treated animals. The systemic exposure to pembrolizumab was independent of sex and was approximately dose-proportional across all doses. The mean t1/2 values in individual ADA-negative animals ranged from 21 to 22 days across doses.

IgG4 wild-type molecule can undergo in vivo molecular rearrangement called Fab-arm (or half molecule) exchange by swapping their half molecule with other IgG4 half molecules, thereby generating bispecific or hybrid antibodies. Pembrolizumab is a hinge mutated IgG4 (S228P), which prevents in vivo half-molecule swap (formation of hybrid). An *in vivo* mice experiment has demonstrated that pembrolizumab did not form hybrid antibody with another wild-type IgG4 molecule.

2.2.2.2.3 *Safety Pharmacology/Toxicology* Please refer to most up to date Investigator Brochure.

The safety of pembrolizumab was characterized in the 1-month repeat-dose toxicity study in Cynomolgus monkeys when administered as IV doses of 6, 40, or 200 mg/kg once-a-week (total of 5 doses) and in the 6-month repeat-dose toxicity study in Cynomolgus monkeys when administered as IV doses of 6, 40 or 200 mg/kg every other week (total of 12 doses). Pembrolizumab was well tolerated in Cynomolgus monkeys with a systemic exposure ($AUC_{0\text{-}7\ days}$) of up to approximately 170,000 μg day/mL over the course of the 1-month study, and with a systemic exposure ($AUC_{0\text{-}14days}$) of up to approximately 67,500 μg day/mL over the course of the 6-month study.

No findings of toxicological significance were observed in either the 1-month or 6-month toxicity studies with pembrolizumab and the no observed adverse effect level (NOAEL) was ≥ 200 mg/kg (the highest dose evaluated). In addition, no findings of toxicological relevance were observed in the in vitro tissue cross-reactivity studies with pembrolizumab using human and Cynomolgus monkey tissues or in immunotoxicology evaluation using the T-cell-dependent antibody response (TDAR) study in mice with antimurine PD-1 surrogate antibody muDX400.

The nonclinical profile supports the safe clinical use of pembrolizumab at the proposed doses.

2.2.2.3 Clinical Activity in Non-Small Cell Lung Cancer

•*Single Agent Activity*

Pembrolizumab, the PD-1 inhibitor used in this study, received initially FDA-accelerated approved in October 2015 followed by full approval as single agent for treatment of patients with metastatic NSCLC whose tumors express PD-L1 [tumor proportion score ≥ 1%] as determined by an FDA-approved test, with disease progression on or after platinum-containing chemotherapy (patients with *EGFR* or *ALK* genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving pembrolizumab).[22]

In the phase 1 study of pembrolizumab in 495 heavily pretreated patients with lung cancer, there was a response rate of 19.4%; median duration of response (DOR) of 12.5 months; median PFS of 3.7 months; and median OS of 12.0 months. In updated results from the KEYNOTE-001 phase 1 study of *treatment-naïve* NSCLC patients administered pembrolizumab as first-line therapy, the ORR was 26.7% (95% CI 18.4 to 36.5), including one complete response, disease control rate (DCR) of 67.3% (95% CI 57.3 to 76.3), median PFS of 6.1 months (95% CI 4.1 to 9.1), and median OS not reached (95% CI 16.2 months to NR).[23] Based on this study, there were studies of pembrolizumab as first-line therapy in non-small cell lung cancer (NSCLC) including phase 3 KEYNOTE-024[24] and KEYNOTE-042[25] studies, the former of which has been resulted and is described below.[26] Pembrolizumab is also noted to have activity in brain metastases of patients with NSCLC.[27] Of the 11 patients treated and evaluable, there was one complete response and 4 partial responses in the brain for an overall RR of 45%. Intracranial response duration for the 5 responders was between 12+ to 22+ weeks.

The KEYNOTE-010 study, a randomized, open label, phase 2/3 study of pembrolizumab 2 mg/kg, pembrolizumab 10 mg/kg, or docetaxel 75 mg/m² every 3 weeks in advanced non-small cell lung cancer patients with PDL-1 positivity was reported.[28] The median overall survival was 10.4 months (95% CI 9.4 to 11.9) with pembrolizumab 2 mg/kg, 12.7 months (95% CI 10.0 to 17.3) with pembrolizumab 10 mg/kg, and 8.5 months (95% CI 7.5 to 9.8) with docetaxel. Overall survival was significantly longer for pembrolizumab at 2 mg/kg and at 10 mg/kg vs docetaxel (HR 0.71, 95% CI 0.58 to 0.88, P = 0.0008 and HR 0.61, 95% CI 0.49 to 0.75, P < 0.0001, respectively). Median progression free survival was 3.9 months (95% CI 3.1 to 4.1) with pembrolizumab 2 mg/kg, 4.0 months (95% CI 2.7 to 4.3) with pembrolizumab 10 mg/kg, and 4.0 months (95% CI 3.1 to 4.2) with docetaxel, with no significant difference for pembrolizumab 2 mg/kg vs docetaxel (HR 0.88, 95% CI 0.74 to 1.05, P = 0.07) or for pembrolizumab 10 mg/kg vs docetaxel (HR 0.79, 95% CI 0.66 to 0.94, P = 0.004.) The overall survival between pembrolizumab 2 mg/kg and 10 mg/kg was similar in the overall population (HR 1.17, 95% CI 0.94 to 1.45). The progression-free survival was also similar at each pembrolizumab dose (HR 1.09, 95% CI 0.92 to 1.30). There was a response rate of 18% (N = 62/344) in the 2 mg/kg pembrolizumab group, 18% (N = 64/346) in the pembrolizumab 10 mg/kg group, and 9% (N = 32/343) in the docetaxel group. All responses were partial, and median time to response was 9 weeks. The response rate was statistically significantly higher for either pembrolizumab dose vs docetaxel. The median duration of response was also longer in the pembrolizumab arm vs docetaxel, not reached vs 8 months, respectively.

Pembrolizumab has also been approved as a single agent for first line treatment of patients with metastatic NSCLC whose tumors have high PD-L1 expression [tumor proportion score ≥ 50%]

as determined by an FDA-approved test with no *EGFR* or *ALK* genomic tumor aberrations. This was based on the KEYNOTE-024[26] study (NCT02142738), which was a randomized, multicenter, open-label, active-controlled trial in patients with metastatic NSCLC, whose tumors had high PD-L1 expression [tumor proportion score (TPS) of 50% or greater] as determined by PD-L1 IHC 22C3 pharmDx Kit immunohistochemistry assay, and had not received prior systemic treatment for metastatic NSCLC. Patients with *EGFR* or *ALK* genomic tumor aberrations; autoimmune disease that required systemic therapy within 2 years of treatment; a medical condition that required immunosuppression; or who had received more than 30 Gy of radiation in the thoracic region within the prior 26 weeks of initiation of study were ineligible. Randomization was stratified by ECOG performance status (0 vs 1); histology (squamous vs nonsquamous); and geographic region (East Asia vs non-East Asia). Patients were randomized 1:1 to receive pembrolizumab 200 mg intravenously every 3 weeks or investigator's choice of multiple platinum-containing chemotherapy regimens for 4 to 6 cycles followed by optional pemetrexed maintenance (for nonsquamous histologies). Treatment with pembrolizumab continued until RECIST v1.1-defined progression of disease as determined by an independent radiology committee; unacceptable toxicity; or for up to 24 months. Treatment could continue beyond disease progression if the patient was clinically stable and was considered by the investigator to be deriving clinical benefit. Patients randomized to chemotherapy were offered pembrolizumab at the time of disease progression. Assessment of tumor status was performed every 9 weeks. The main efficacy outcome measure was PFS as assessed by a blinded independent central radiologists' (BICR) review according to RECIST v1.1. Additional efficacy outcome measures were OS and ORR as assessed by the BICR according to RECIST v1.1.

A total of 305 patients were randomized: 154 patients to the pembrolizumab arm and 151 to the chemotherapy arm. The study population characteristics were: median age of 65 years (range: 33 to 90); 54% age 65 or older; 61% male; 82% white and 15% Asian; 65% ECOG performance status of 1; 18% with squamous and 82% with nonsquamous histology; and 9% with history of brain metastases. A total of 66 patients in the chemotherapy arm received pembrolizumab at the time of disease progression. The trial demonstrated a statistically-significant improvement in PFS for patients randomized to pembrolizumab as compared with chemotherapy (10.3 months vs 6 months respectively; HR 0.50; 95% CI 0.37 to 0.68; $p < 0.001$). Additionally, a pre-specified interim OS analysis at 108 events (64% of the events needed for final analysis) also demonstrated statistically-significant improvement of OS for patients randomized to pembrolizumab as compared with chemotherapy (70% vs 54% of patients alive at 12 months, respectively; HR 0.60; 95% CI 0.41 to 0.89; $p = 0.005$).

•*Combinations clinical activity*

Pembrolizumab is also being developed in combination strategies in NSCLC including in combination with chemotherapies, targeted therapies, radiation, and other immunotherapeutic approaches. Most recently, pembrolizumab has received accelerated approval in combination with pemetrexed and carboplatin, as first-line treatment of patients with metastatic NSCLC (accelerated approval based on response rate and progression-free survival). This was based on data from the randomized phase 2 cohort G of the Keynote 21 study performed in the US and Taiwan.[29] Patients were eligible for this study if they had no prior treatment for non-squamous stage IIIB or IV non-small cell lung cancer and no evidence of *EGFR* or *ALK* alteration; ECOG

performance status 0-1; measurable disease per RECIST v 1.1; and tumor biopsy sample available for assessment of PD-L1 expression. Pertinent exclusion criteria included receiving more than 30 Gy of radiation to the lungs in the prior 6 months; autoimmune disease requiring systemic treatment in the previous 2 years; untreated brain metastases (although stable treated metastases were allowed); or active interstitial lung disease or pneumonitis that required steroids. In the pembrolizumab plus chemotherapy group, pembrolizumab 200 mg IV, pemetrexed 500 mg/m², and carboplatin AUC 5 were given IV every 3 weeks followed by 24 months of pembrolizumab and optional pemetrexed maintenance chemotherapy indefinitely. The chemotherapy group received carboplatin and pemetrexed with optional pemetrexed maintenance indefinitely. Crossover was allowed for patients on the carboplatin and pemetrexed only group to pembrolizumab monotherapy. Treatment was discontinued for intolerable toxicity or disease progression; however, patients could be treated beyond disease progression if ongoing clinic benefit. Tumor imaging by CT or MRI was done at baseline and every 6 weeks the first 18 weeks, then every 9 weeks for the first 12 months and every 12 weeks thereafter. The primary endpoint was objective response rate as defined by RECIST version 1.1).

There were 123 patients randomized, 60 patients in the pembrolizumab plus chemotherapy arm vs 63 patients in the chemotherapy-alone arm. Overall, baseline characteristics were well balanced, although there was a higher proportion of adenocarcinoma, never smokers, and stable brain metastases in the pembrolizumab plus chemotherapy arm. Also, pemetrexed maintenance was given more commonly in the pembrolizumab plus chemotherapy arm vs the chemotherapy-alone arm (85% vs 69%, respectively). The distribution of PD-L1 expression in the pembrolizumab plus chemotherapy arm was approximately one third each of the following tumor proportion score categories $< 1\%$; 1 to 49%, and $\geq 50\%$. The study met its primary endpoint as there was a statistically significant improvement in objective response rate with the pembrolizumab plus chemotherapy arm versus chemotherapy-alone arm: 55% (95% CI 42 to 68; n = 33/60) vs 29% (95% CI 18 to 41; n = 18/63), respectively, p = 0.0016. The median time to response was also faster in the pembrolizumab plus chemotherapy arm: 1.5 months (IQR 1.4 to 2.8) vs 2.7 months (IQR 1.4 to 2.8), respectively. The median duration of response in both arms was not reached. There was also less progressive disease seen in the pembrolizumab plus chemotherapy arm: 3% vs 17%, respectively. 32% of the 62 patients in the chemotherapy crossed over to pembrolizumab monotherapy. The responses appeared to be independent of PD-L1 expression in the pembrolizumab plus chemotherapy arm, as the PD-L1 $< 1\%$ group had a response rate of 57% while the PD-L1 $\geq 1\%$ group had a response rate of 54%. However, in the PD-L1 high-expressers ($\geq 50\%$), the objective response rate was 80% (n = 16/20). Progression-free survival was also longer with the pembrolizumab plus chemotherapy arm compared to the chemotherapy-alone arm [median 13.0 months (95% CI 8.3 to NR) vs 8.9 months (4.4 to 10.3), respectively; HR 0.53, 95% CI 0.31 to 0.91; p = 0.010). Most recent reports of overall survival show trend for benefit and the KEYNOTE189 randomized phase 3 study has been reported as positive. The rate of treatment related adverse events were similar in both arms. The most common treatment-related events of any grade in the pembrolizumab plus chemotherapy arm were fatigue (64%), nausea (58%), and anemia (32%). Rash (27%) and alopecia (14%) were more common in the pembrolizumab plus chemotherapy arm compared to the chemotherapy alone arm by greater than 10%. Immune-related adverse events were of course higher in the pembrolizumab plus chemotherapy arm at 22% vs 11% in the chemotherapy arm. The most common immune-mediate adverse events were hypothyroidism (15%),

hypothyroidism (8%), pneumonitis (5%). These results were subsequently confirmed by data from the larger KEYNOTE-189 study in a similar patient population.[30]

2.2.2.4 Clinical Pharmacokinetics – Please refer to most up to date Investigator Brochure and/or most up to date Package Insert.

The pharmacokinetic profile of pembrolizumab, with low clearance and limited volume of distribution is typical for therapeutic antibodies. Exposure to pembrolizumab is approximately linear in the dose range of clinical relevance (1 to 10 mg/kg). Furthermore, pembrolizumab has a low potential of eliciting the formation of anti-drug antibodies. No formal pharmacokinetic drug interaction studies have been conducted.

The pharmacokinetics (PK) of pembrolizumab was characterized using a population PK analysis with concentration data collected from 2993 patients with various cancers who received pembrolizumab doses of 1 to 10 mg/kg every 2 weeks, 2 to 10 mg/kg every 3 weeks, or 200 mg every 3 weeks. Pembrolizumab clearance (CV%) is approximately 23% lower [geometric mean, 195 mL/day (40%)] at steady state than that after the first dose [252 mL/day (37%)]; this decrease in clearance with time is not considered clinically important. The geometric mean value (CV%) for volume of distribution at steady state is 6.0 L (20%) and for terminal half-life ($t_{1/2}$) is 22 days (32%). Steady-state concentrations of pembrolizumab were reached by 16 weeks of repeated dosing with an every 3-week regimen and the systemic accumulation was 2.1-fold. The peak concentration (Cmax), trough concentration (Cmin), and area under the plasma concentration versus time curve at steady state (AUCss) of pembrolizumab increased dose proportionally in the dose range of 2 to 10 mg/kg every 3 weeks.

Specific Populations: The following factors had no clinically important effect on the CL of pembrolizumab: age (range: 15 to 94 years); sex; race (89% White); renal impairment (eGFR greater than or equal to 15 mL/min/1.73 m^2); mild hepatic impairment (total bilirubin less than or equal to upper limit of normal (ULN) and AST greater than ULN or total bilirubin between 1 and 1.5 times ULN and any AST); or tumor burden. There is insufficient information to determine whether there are clinically important differences in the CL of pembrolizumab in patients with moderate or severe hepatic impairment.

2.2.2.5 Clinical Safety- Please refer to most up to date Investigator brochure.

Safety data are presented for a total of 2799 subjects in ongoing, Merck-sponsored clinical trials. Tables from the most recent Investigator Brochure are provided below for reference:

Table: Most Frequently Reported (≥ 5%) Adverse Events Presented by Decreasing Frequency and Considered Drug-Related by the Investigator in Subjects Treated with Pembrolizumab (ASaT Population)

Preferred Term	Reference Safety Dataset for Pembrolizumab[a]	
	n	(%)
Subjects in population	2799	
Fatigue	678	(24.2)
Pruritus	467	(16.7)
Rash	386	(13.8)
Diarrhoea	343	(12.3)
Nausea	304	(10.9)
Arthralgia	281	(10.0)
Decreased appetite	255	(9.1)
Asthenia	218	(7.8)
Hypothyroidism	213	(7.6)
Vitiligo	159	(5.7)
Myalgia	146	(5.2)

Every subject is counted a single time for each applicable row and column.
MedDRA version used is 18.1.
[a] Includes all subjects who received at least one dose of MK-3475 in KN001 Part B1, B2, B3, D, C, F1, F2, F3; KN002 (original phase), KN006, and KN010.
(KN001 Database Cutoff Date for Melanoma: 18APR2014).
(KN001 Database Cutoff Date for Lung Cancer: 23JAN2015).
(KN002 Database Cutoff Date: 28FEB2015).
(KN006 Database Cutoff Date: 03MAR2015).
(KN010 Database Cutoff Date: 30SEP2015).

Table: Most Frequently Reported (≥0.2%) Serious Adverse Events Presented by Decreasing Frequency and Considered Drug-Related by the Investigator in Subjects Treated with Pembrolizumab (ASaT Population)

Preferred Term	Reference Safety Dataset for Pembrolizumab[a]	
	n	(%)
Subjects in population	2799	
Pneumonitis	44	(1.6)
Colitis	25	(0.9)
Diarrhoea	17	(0.6)
Pyrexia	10	(0.4)
Autoimmune hepatitis	8	(0.3)
Pneumonia	8	(0.3)
Adrenal insufficiency	7	(0.3)
Hyponatraemia	7	(0.3)
Dyspnoea	6	(0.2)
Hyperthyroidism	6	(0.2)
Nausea	6	(0.2)

Every subject is counted a single time for each applicable row and column.
MedDRA version used is 18.1.
[a] Includes all subjects who received at least one dose of MK-3475 in KN001 Part B1, B2, B3, D, C, F1, F2, F3; KN002 (original phase), KN006, and KN010.
(KN001 Database Cutoff Date for Melanoma: 18APR2014).
(KN001 Database Cutoff Date for Lung Cancer: 23JAN2015).
(KN002 Database Cutoff Date: 28FEB2015).
(KN006 Database Cutoff Date: 03MAR2015).
(KN010 Database Cutoff Date: 30SEP2015).

2.2.3 Rationale for Dose Selection/Regimen/Modification

The planned dose of pembrolizumab for this study is 200 mg every 3 weeks (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 mg/kg Q3W versus 10 mg/kg Q2W (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.

For clinicaltrials.gov compliance

An IND application was submitted because patients being treated are not necessarily same as the same FDA approved indication.

2.3 Rationale

2.3.1 Rationale for the Trial and Selected Subject Population

Rationale for Selected Subject Population

PD-1 and PD-L1 inhibition is an active therapy in lung cancer. Pembrolizumab, the PD-1 inhibitor in this study, is FDA approved for lung cancer as a monotherapy in the following indications: (i) single agent for first-line treatment of patients with metastatic NSCLC whose tumors have high PD-L1 expression [tumor proportion score ≥ 50%] as determined by an FDA-approved test with no *EGFR* or *ALK* genomic tumor aberrations; (ii) single agent for treatment of patients with metastatic NSCLC whose tumors express PD-L1 [(tumor proportion score ≥ 1%] as determined by an FDA-approved test, with disease progression on or after platinum-containing chemotherapy (patients with *EGFR* or *ALK* genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving pembrolizumab). As described in Section *2.2.2.3*. For this study, we plan to enroll patients in the approved frontline monotherapy indication for pembrolizumab (KEYNOTE-024 study)[26], and expand this slightly to include 1% or greater based on the KEYNOTE-042 data

(Lopes, ASCO 2018 LBA4). However, in the second line setting and beyond we will enroll patients irrespective of expression of PD-L1. The rationale for that is detailed below since responses have been in NSCLC even in absence of PD-L1 expression.

In the 495 patients reported from the phase 1 study, there were a total of 401 (81%) non-squamous; 81 squamous; 7 adenosquamous; and 2 unknown histologies.[33] The ORR in patients with squamous histology was 23.5% (N=20/85) and was similar to that in patients with non-squamous histology, 18.7% (N=75/401). Within the patients with squamous histology, there was a higher response rate in patients with the highest PD-L1 proportion score (PS ≥ 50%, PS1 to 49%, PS < 1%): 64.3% (N = 9/14) > 22.7% (N = 5/22) > 0% (N = 0/8). In the non-squamous histologies, there was also a higher response rate in patients with the highest PD-L1 proportion score: 37.7% (N = 23/61) > 14.9% (N = 13/87) > 13.6% (N = 3/22). The non-squamous histology cohort included in this report included patients that were naïve to systemic therapy and patients who had progressed after ≥ 2 previous regimens. Patients had received a variety of prior treatments: 19% were treatment naïve, 14.9% had received 1 prior systemic therapy, 24% had received 2, 21.4% had received 3, and 20.6% had received ≥ 4. In patients that were previously treated, there was a higher ORR in patients with high PD-L1 proportion scores: 43.9% (N = 25/57) > 15.6% (N = 12/77) > 9.1% (N = 24/22). In patients that were treatment naïve, this trend for improved ORR was also true in patients with high PD-L1 proportion scores (PS ≥ 50%, PS1 to 49%, PS < 1%): 50% (N = 8/16) > 19.2% (N = 5/26) > 16.7% (N = 1/6). PD-L1 proportion score ≥ 50% was seen in equal prevalence in patients who were previously treated or treatment naïve, 22.7% (N = 146/643) and 24.9% (N = 45/181).

Both treatment-naïve (PD-L1 high-expressers with absence of ALK/EGFR alteration) and previously-treated (irrespective of PD-L1 expression) metastatic NSCLC patients will be enrolled in this trial.

Rationale for The Trial

Despite clinical activity seen with PD-1/PD-L1 inhibition, there is no robust predictive biomarker at this time. Described below are the biomarkers currently being studied, including PD-L1 protein expression by immunohistochemistry, gene expression signatures, T-cell receptor repertoire, tumor-infiltrating lymphocytes, cytokines, and mutational burden. This is not a comprehensive list, as this field is evolving rapidly.

The purpose of our study is to examine potential surrogate and predictive biomarkers of response to PD-1 therapy, pembrolizumab, in metastatic NSCLC using Stanford technologies, including circulating tumor DNA (CAPP-Seq)[1] and as an exploratory endpoint gene expression immune signatures (CIBERSORT)[34].

Biomarkers

PD-L1 Protein Expression by Immunohistochemistry

Many biomarkers are being developed to determine the subgroup of patients who benefit from these therapies since collectively, PD-1/PD-L1 inhibitors only appear to results in response in 15 to 20% of patients with previously-treated unselected NSCLC and up to 50% in patients with high PD-L1 expression. PD-L1 protein expression as determined by immunohistochemistry is emerging as the primary predictor of response. In a study examining 7 clinical trials in 511 patients treated with PD-1/PD-L1 inhibition in lung cancer, the ORR was 23.2% (95% CI, 18.5 to 27.9) for PD-L1 positive patients and 14.5% (95% CI, 9.6-19.4) for PD-L1 negative

patients.[35] In the KEYNOTE-001 pembrolizumab data published for NSCLC, using a definition of PD-L1 positivity of expression in at least 50% of tumor cells, patients with PD-L1 positive tumors had a higher response rate at 45.2%, longer median PFS of 6.3 months, and median overall survival has not been reached.[22] In updated results from the KEYNOTE-001 study, PD-L1 expression and its association with clinical benefit was evaluated in 91 patients who were treatment naïve and given pembrolizumab as first-line therapy.[23] Of 91 patients with evaluable PD-L1 expression, 29.7% had a proportion score of ≥ 50%, 57.1% had a proportion score (PS) 1 to 49%, and 13.2% had a proportion score < 1%. The clinical activity of the groups were as follows based on PD-L1 expression: 1) PS ≥ 50%, ORR 51.9% / DCR 77.8% / mPFS 12.5 months / mOS not reached; 2) PS1-49%, ORR 17.3% / DCR 63.5% / mPFS 4.2 months / mOS 16.2 months; and 3) PS < 1%, ORR 8.3% / DCR 66.7% / mPFS 3.5 months / mOS 10.4 months, respectively. There is also a confirmatory phase 3 study (KEYNOTE-024) of pembrolizumab frontline for patients with PD-L1 PS ≥ 50% where the response rate was 45%.[26]

The *prototype* PD-L1 immunohistochemistry assay of Merck PD-1 compound, pembrolizumab, was developed and validated at a single CLIA-certified laboratory site (QualTek Goleta, California).[36] The 22C3 monoclonal mouse anti-human PD-L1 antibody clone was used along with commercially available reagents from the DAKO EnVision FLEX+ HRP-Polymer Kit (DAKO K8012, Carpinteria, California, USA). The *clinical trial* immunohistochemistry assay has been developed as a companion diagnostic assay using a modified version of the prototype assay. There were 4 standard scoring methods evaluated with the clinical trial assay: 1) proportion score PS 0 to 100, the percentage of cells staining at any intensity; 2) proportion moderate/strong score P2S, 0-100, the percentage of cells staining at moderate or strong intensity; 3) proportion strong score, P3S, 0-100, the percentage of cells staining at strong intensity; 4) H-score, HS, sum of PS, P2S, and P3S. In order to be an adequate sample in the prototype assay, ≥ 50 viable tumor cells or ≥ 5 viable PD-L1+ cells had to be present, and in the clinical trial assay, ≥ 100 viable tumor cells had to be present. In the prototype assay, scoring accounted for *tumor and immune cells* demonstrating partial or complete membrane staining and in the clinical trial assay, *only tumor cells* were counted that demonstrated partial or complete membrane staining. It is known that samples greater than 6 months resulted in deterioration PD-L1 expression by immunohistochemistry and the samples were excluded. In the training set of 182 patients by all 4 scoring methods, the area under the ROC was similar, 0.74-0.77. The cut off for the PS score chosen was 50%. PD-L1 positivity based on this score resulted in an association with a significantly longer progression free survival by immune-related response criteria and a higher number of responders (odds ratio of 8.93).[36]

The PD-L1 IHC 22C3 pharmDx is currently FDA-approved (BLA 125514, supplement 5, see package insert http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/125514s005lbl.pdf) for pembrolizumab.

Many other biomarkers are being examined since not all patients with PD-L1 expression have a response to these therapies, and responses are also seen in those patients without PD-L1 expression. There are also several variables for the PD-L1 biomarker, including the definition of positivity from threshold percentage of cells staining positive to the types of cells staining (ie, tumor cells, tumor infiltrating immune cells, stroma). The assays used also differ per company including the antibodies used for staining and also the IHC platform used. The predictive ability of PD-L1 as a biomarker may also vary by line of

therapy.[37] Tumor PD-L1 testing will be performed using standard of care FDA-approved PD-L1 testing for this trial.

Biomarkers of PD-1/PD-L1 therapies

Listed below are examples of biomarkers being explored for immunotherapy. This is not an updated or comprehensive list, as this is a rapidly evolving field.

Gene expression signatures

Other biomarkers are being examined to help predict clinical activity to anti-PD-1/PD-L1 therapy, and include RNA expression gene signatures. For example, using the NanoString counter system, at least one of the following gene signatures that was enriched for interferon-gamma (6-gene), T-cell receptor (TCR) signaling (13-gene), expanded immune (18-gene), and de novo (33-gene) were predictive of response to pembrolizumab in gastric cancer[38], bladder cancer[39], and head and neck cancer[40]. In melanoma patients treated with pembrolizumab, there were several immune-related gene signatures that were identified that were associated with clinical benefit to PD-1 therapy, including signatures describing interferon gamma signaling, antigen presentation, and T-cell specific markers.[41] For example, there was a trend for improved response with the preliminary interferon gamma 10-gene signature and the preliminary expanded immune 28-gene signature in pembrolizumab-treated melanoma patients. For another inhibitor targeting the PD-1/PD-L1 axis, atezolizumab, T-helper type 1 (T_H1) gene expression, CTLA4 expression and the absence of fractalkine (CX3CL1) in baseline specimens was associated with response.[42]

T-cell receptor (TCR) repertoire, $CD8^+$ T-cells, Cytokines, Others

The heterogeneity of tumor infiltrating T-cell repertoire is also being examined as a potential predictor of clinical activity or lack thereof to PD-1 therapy.[43] The TCR repertoire and cytokines have been explored in clinical trials with pembrolizumab in metastatic melanoma.[44] Although there was no net expansion in unique TCR repertoire seen in patients treated with pembrolizumab, responders overall had more unique TCRs than nonresponders. A more limited TCR repertoire in baseline tumor samples, suggestive of a clonal population of T cells, has also been associated with improved response.[45] There was also a decrease in certain cytokines seen in responders but not in nonresponders with PD-1 therapy, including MCP-1, FGF-2, and IL-8.[43] Even peripheral blood count eosinophils have been correlated with long-term disease control in metastatic melanoma patients treated with pembrolizumab.[46] In a study of 46 melanoma patients treated with pembrolizumab, it was suggested that an immunocompetent tumor, with pre-existing $CD8^+$ T-cells, is required to achieve benefit to PD-1 therapy.[45] The presence of $CD8^+$ T cells at the invasive margin in baseline tumor samples was associated with response to pembrolizumab, and an increase in $CD8^+$ T-cells at the invasive margin or in the center of the tumor was also associated with improved clinical benefit. Overall, there were significantly higher numbers of $CD8^+$; $PD\text{-}1^+$; $PD\text{-}L1^+$ cells at both the invasive margin and the tumor center in responders compared to nonresponders, suggesting an immunocompetent phenotype, is required for response to PD-1 therapy.

Mutational Burden/Neoantigens

It has been hypothesized that high somatic mutation burden with subsequent expression of immunogenic neoantigens explains why the main tumors (which are induced by mutagenic carcinogens, ie, UV light and cigarette smoking), melanoma and lung cancer, respond to immune

checkpoint inhibitors. This hypothesis was corroborated by recent data in metastatic melanoma patients treated with ipilimumab, where whole exome sequencing was performed on tumors with matched blood samples.[47] Mutational load was associated with clinical benefit in this study, but was not a sufficient predictive factor for benefit. Identification of certain neoantigens via whole exome sequencing has also been reported as a potential predictor of response to immunotherapies in melanoma.

Tumor mutation burden is being used to as a biomarker in ongoing large randomized studies with immunotherapy in lung cancer. Somatic mutation burden and identification of neoantigens have also been associated with durable clinical benefit (DCB) in patients treated with pembrolizumab in metastatic NSCLC.[48] Lung cancer is a notoriously heterogeneous group, with a large ranging mutation burden, particularly because some lung cancers are caused by cigarette smoke and others are not. Whole exome sequencing was performed on two independent cohorts treated with pembrolizumab (n = 16 and n = 18, respectively). There was a median of 200 non-synonymous mutations per sample (11-1192) and a median number of 327 exonic mutations per sample (45-1732). The number of nonsynonymous mutations correlated with DCB in 16 patients in the discovery cohort; for example, there were 302 mutations in patients with DCB and 148 in those without DCB (P=0.02). Using a median of 209 mutations, patients whose tumor fell below the median had a 13% rate of DCB and those that fell above the median had a 73% rate of DCB (P = 0.04). This differential benefit of high mutation burden was also seen for other clinical endpoints, including objective response rate (63% vs 0%, P = 0.03) and PFS (14.5 vs 3.7 months, P = 0.01, HR 0.19, 95% CI 0.05 to 0.70). These findings were also seen in the validation cohort of 18 patients, who had similar baseline clinical characteristics (AUC 87%). Not surprisingly, since smoking increases the nonsynonymous mutation burden rate, smoking history is also associated with clinical benefit to pembrolizumab. A molecular smoking signature, which is transversion high (TH) C-A, was associated with the highest overall response rate (56% vs 17% in transversion low (TL), P = 0.03) and the longest progression free survival (median not reached vs 3.5 months in TL, P = 0.0001). In this study, neoantigens were also examined, with the quantity of neoantigens correlating with mutation burden of the tumor, and higher candidate neoantigen burden associated with DCB. The predictive benefit of mutational burden also potentially explains why patients with *EGFR* mutations do not seem to derive as much benefit with the PD-1/PD-L1 inhibition, demonstrated across several large randomized studies.[18]

2.4 Study Design

- This is a prospective open-label non-randomized single-arm treatment trial of pembrolizumab in metastatic NSCLC patients, with a primary endpoint of evaluation of pharmacodynamics minimally invasive surrogate biomarker of response, circulating tumor DNA.

2.5 Correlative Studies Background

2.5.1 Detecting Tumor Mutations and Circulating Tumor DNA using Capp-Seq

Beyond RECIST: Using circulating tumor DNA (ctDNA) levels as a surrogate biomarker of response to pembrolizumab

While immune-related radiographic response criteria (ie, RECIST)[49] have been developed for immunotherapy clinical trials, which allow for some degree of growth and the development of

new lesions, these criteria have not been fully validated and frequently are erroneous in separating responders from non-responders in real-time.[50,51] Peripheral blood monitoring of cancer while on treatment is attractive. **CA**ncer **P**ersonalized **P**rofiling by Deep **Seq**uencing (CAPP-Seq) is a method to measure ctDNA using a selector of biotinylated DNA oligonucleotides that detect recurrent mutations in cancers.[1] The initial design of the CAPP-Seq selector was created for NSCLC. The initial selector targeted 521 exons and 13 introns from a total of 139 mutated genes, and could identify 96% of patients with NSCLC. Using CAPP-Seq for noninvasive monitoring of cancer in the initial publication, had an impressive receiving operating characteristic (AUC 0.95), with a sensitivity of 85% and specificity of 96%. The sensitivity was higher in tumors of more advanced stage; reaching 100% in Stage II-IV tumors. Newman et al (Diehn/Alizadeh labs, Stanford University, Stanford, CA) were able to correlate ctDNA levels with tumor volumes determined radiographically by PET and/or CT ($R^2 = 0.89$, $P = 0.0002$). ctDNA levels were also shown to fall dramatically in patients responding to chemotherapy and targeted therapeutics. ctDNA was also helpful in determining if a patient had progressed after receiving definitive radiotherapy due to the difficulty in interpreting CT imaging after radiotherapy, and was also useful in surveillance monitoring after curative intent therapy for early-stage NSCLC. Circulating tumor DNA as measured by CAPP-Seq has also been shown to identify molecular residual disease after definitive radiation treatment of locally advanced lung cancer: (i) ctDNA was detectable in the first post-treatment blood sample in 94% of patients who subsequently experienced recurrence and (ii) ctDNA detected recurrence prior to radiographic progression in 72% of patients by a median of 5.2 months.[52]

ctDNA as measured by CAPP-Seq technology may serve as a useful surrogate biomarker of clinical activity to pembrolizumab using baseline levels of ctDNA or change in levels of ctDNA longitudinally. CAPP-Seq will be performed on baseline tumor samples when available to identify mutations. In this trial, we will measure ctDNA levels as a surrogate biomarker of response, and correlate levels with radiographic response kinetics. ctDNA may be superior to radiographic assessments in identifying responders to pembrolizumab from non-responders at an earlier time point.

2.5.2 PD-L1 IHC

PD-L1 immunohistochemistry will be performed using standard of care FDA-approved PD-L1 testing and is performed as part of standard of care. The *prototype* PD-L1 immunohistochemistry assay of Merck PD-1 compound, pembrolizumab, was developed and validated at a single CLIA-certified laboratory site (QualTek; Goleta, California).[36] The 22C3 monoclonal mouse anti-human PDL 1 antibody clone was used along with commercially available reagents from the DAKO EnVision FLEX+ HRP-Polymer Kit (DAKO K8012, Carpinteria, California, USA). The *clinical trial* immunohistochemistry assay has been developed as a companion diagnostic assay using a modified version of the prototype assay. The PD-L1 IHC 22C3 pharmDx is currently FDA-approved for pembrolizumab as a qualitative immunohistochemical assay using Monoclonal Mouse Anti-PD-L1, Clone 22C3 intended for use in the detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) tissue using EnVision FLEX visualization system on Autostainer Link 48. PD-L1 protein expression is determined by using Tumor Proportion Score (TPS), which is the percentage of viable tumor cells showing partial or complete membrane staining. The specimen is considered PD-L1 positive in the frontline setting if TPS $\geq$ 50% of the viable tumor cells exhibit membrane staining at any intensity. PD-L1 IHC 22C3 pharmDx is indicated as an aid in

identifying NSCLC patients for treatment with pembrolizumab (http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm301431.htm). PD-L1 expression has been correlated with response and other clinical outcomes as described in Section 2.3.1.

2.5.3 Immune gene expression signature using CIBERSORT

Beyond PD-L1 protein expression: Using CIBERSORT as a predictive biomarker of response to pembrolizumab

While some studies have demonstrated a high proportion of response in patients with PD-L1 expression on tumors and tumor-infiltrating cells[35,53], there are still responses observed with pembrolizumab in PD-L1 negative patients. The simple presence or absence of certain tumor-infiltrating immune cell subsets may also contribute to the likelihood of response.[45] In solid tumors, it is challenging to profile individual immune cell infiltrates since methodologies such as FACS require large numbers of cells and dissolution of tumor stromal architecture. CIBERSORT has been used to illustrate the diversity of tumor immune cell infiltrate subsets in triple negative breast cancer[54] and also overall survival outcomes of 18,000 human tumors across 39 malignancies.[55] In the triple-negative breast cancer cohort, following whole tumor gene expression microarray analysis, the Alizadeh/Diehn labs generated an algorithm (termed CIBERSORT) to identify individual infiltrative immune cell populations, and established an "immune score" based on this algorithm.[54] The overall immune score and presence of activated $CD4^+$ memory T cells in tumors both strongly correlated with pathological complete response (pCR) to chemotherapy plus a PARP inhibitor in breast cancer patients.

CIBERSORT will be used in an exploratory analysis to examine baseline lung tumor tissue (when available) prior to anti-PD-1 treatment to identify the tumor infiltrating immune cell subtypes that may correlate with response to pembrolizumab. This technique is potentially superior to conventional flow cytometry since it requires only a small amount of frozen tissue from biopsy as opposed to a larger amount of tissue with subsequent dissociation and manipulation.

3. PARTICIPANT SELECTION AND ENROLLMENT PROCEDURES

3.1 Inclusion Criteria

In order to be eligible for participation in this trial, the patient must meet *ALL* of the following criteria (ie, mark "yes" to all criteria):

1. Has a pathologically-proven recurrent or metastatic non-small cell lung cancer (non-squamous; squamous; mixed; and neuroendocrine histologies are allowed as of amendment 26 June 2018)
2. Previously received at least one line of prior systemic therapy for metastatic disease.
   a. If the patient has a known sensitizing *EGFR* mutation or *ALK* rearrangement, the patient must have received at least 1 prior targeted therapy for metastatic disease (ie, EGFR TKI therapy or ALK TKI therapy, respectively).
   b. There is no limit on prior therapies allowed. Patients must have completed previous treatment (including other investigational therapy) in greater than or equal to the following times prior to initiation of trial treatment:
      i. Anti-cancer monoclonal antibody (mAb) therapy must be completed ≥ 3 weeks prior to trial treatment
      ii. Chemotherapy administered in a daily or weekly schedule must be completed ≥ 1 week prior to trial treatment
      iii. Chemotherapy administered in an every 2-week schedule must be completed ≥ 2 weeks prior to trial treatment
      iv. Chemotherapy administered in an every 3-week schedule must be completed ≥ 3 weeks prior to trial treatment
      v. Targeted small molecule therapy must be completed ≥ 1 week prior to trial treatment

   OR

   Has not received prior systemic therapy for their cancer in recurrent or metastatic setting, AND have a tumor with Tumor Proportion Score (TPS) ≥ 1% as measured by PD-L1 IHC, AND no evidence of a sensitizing *EGFR* mutation or *ALK* rearrangement if adenocarcinoma histology (molecular testing not required for other histologies).
3. Prior radiation therapy allowed as long as completed in the following times prior to initiation of trial treatment:
   a. Definitive curative intent radiation ≥ 2 weeks prior to trial treatment
   b. Palliative body radiation ≥ 1 week prior to trial treatment
   c. Stereotactic brain radiation ≥ 1 week prior to trial treatment
   d. Whole brain radiation ≥ 2 weeks prior to trial treatment
4. Patients with previously-treated (with radiation or surgery) brain metastases are allowed. Patients with untreated stable or progressing metastases must have metastases ≤ 1.5 cm; be asymptomatic; and either not be on steroids or be on 10 mg prednisone equivalent or less.
5. Has measurable disease based on RECIST v1.1 criteria

6. Is medically able and willing to undergo needle biopsy of a tumor lesion,
   OR
   Has known tumor PD-L1 expression and believed to have sufficient archival tumor available for genomic analysis (block or 10 unstained slides or Stanford STAMP NGS testing previously resulted or in process)
7. Has life expectancy ≥ 3 months
8. Ability to understand and the willingness to sign a written informed consent document.
9. ≥ 18 years of age on day of signing informed consent
10. ECOG performance status of 0, 1, or 2 (Appendix A)
11. Adequate organ function:
    a. Absolute neutrophil count (ANC) ≥ 1,000/mcL
    b. Platelets ≥ 75,000/mcL
    c. Hemoglobin ≥ 8 g/dL
    d. Serum creatinine ≤ 1.5 x upper limit of normal (ULN) **OR** measured or calculated creatinine clearance ≥ 30 mL/min for patient with creatinine levels > 1.5 x institutional ULN
    e. Serum total bilirubin ≤ 1.5 x ULN **OR** Direct bilirubin ≤ ULN for patients with total bilirubin levels > 1.5 ULN
    f. AST (SGOT) and ALT (SGPT) ≤ 3 x ULN **OR ≤** 5 x ULN for patients with liver metastases
12. Female patients of childbearing potential must have a negative urine or serum pregnancy test prior to the first dose of trial treatment. They must also agree to acceptable contraceptive methods (ie, combined estrogen progesterone hormonal contraception; progesterone-only hormonal contraception; intrauterine device; have history of bilateral tubal ligation; or 2-barrier method), or agree to abstain from heterosexual activity, for the course of the study through 120 days after the last dose of trial treatment.

    Female patients are not considered to be of childbearing potential if (i) they have been surgically-sterilized (such as hysterectomy plus bilateral salpingo-oophorectomy) or (ii) are free from menses for > 1 year (postmenopausal). Female patients who have not had > 1 year of amenorrhea may have confirmation of postmenopausal state with 2 FSH measurements in postmenopausal range and should not be on hormonal contraception or hormonal replacement therapy during testing.

3.2 Exclusion Criteria

In order to be eligible for participation in this trial, the patient must *NOT* meet the following criteria (ie, mark "no" to all criteria):

1. Is currently receiving another investigational therapy
2. Has received prior anti-PD-1 or anti-PD-L1 therapy
3. Has clinically-significant toxicities from previous anti-cancer therapy that have not resolved, or have not stabilized at a new baseline
4. Has undergone a surgical procedure involving general anesthesia within 2 weeks of starting trial treatment, or has inadequate healing or recovery from complications of surgery prior to starting trial treatment. This does *not* apply to low-risk procedures such

as thoracentesis; paracentesis; chest tube/PleurX catheter placement; line placement; needle biopsy of tumor; and bronchoscopy.

5. Is receiving high-dose systemic steroid therapy or other immunosuppressive therapy within 3 days of trial treatment. Topical and intraarticular steroid injections are allowed, as are physiologic doses of systemic steroids (≤ 10 mg of prednisone equivalent daily).
6. Has carcinomatous meningitis as determined by positive CSF cytology
7. Has known additional malignancy that is undergoing active treatment.
8. Has active autoimmune disease that has required systemic treatment in the past 2 years (ie, with use of disease-modifying agents, supra-physiologic doses of systemic corticosteroids or immunosuppressive drugs). Replacement therapy (eg, thyroxine, insulin; or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc) is not considered a form of systemic treatment. Asthma; type I diabetes mellitus; hypothyroidism; and vitiligo are allowed.
9. Has a history or current evidence of any condition; therapy; or laboratory abnormality that might confound the results of the trial, interfere with the patient's participation for the full duration of the trial, or is not in the best interest of the patient to participate, in the opinion of the treating investigator. This includes known active tuberculosis; Grade 3 active infection; history of allogeneic bone marrow transplant or solid organ transplant; known history of human immunodeficiency virus (HIV); known active Hepatitis B (eg, Hep B DNA-positive in prior 3 months) or known active Hepatitis C (eg, HCV RNA [qualitative] is detected in prior 3 months).
10. Known current (non-infectious) pneumonitis or history of (non-infectious) pneumonitis that required oral steroids.
11. Is pregnant or breastfeeding, or expecting to conceive children within the projected duration of the trial, starting with the screening visit through 120 days after the last dose of trial treatment

Statement of Eligibility

By signing this form of this trial I verify that this patient is [☐ **eligible** / ☐ **ineligible**] for participation in the study. This study is approved by the Stanford Cancer Institute Scientific Review Committee, the Stanford IRB, and has finalized financial and contractual agreements as required by Stanford School of Medicine's Research Management Group (RMG).

Treating Physician Signature:	Date:
Printed Name:	
Secondary Reviewer Signature:	Date:
Printed Name:	
Study Coordinator Signature:	Date:
Printed Name:	

3.3 Informed Consent Process

All participants must be provided a consent form describing the study with sufficient information for participants to make an informed decision regarding their participation. Participants must sign the IRB approved informed consent prior to participation in any study specific procedure. The participant must receive a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

3.4 Randomization Procedures

Patients in this study will not be randomized.

3.5 Study Timeline

Primary Completion:

The study will reach primary completion 24 months from the time the study opens to accrual.

Study Completion:

The study will reach study completion 48 months from the time the study opens to accrual.

4. TREATMENT PLAN

Patients will initially be screened for eligibility. If they are eligible for the trial and have signed informed consent, they will receive pembrolizumab. There are 3 stages during the treatment phase of the study: Stage I Cycle 1 through 8 (approximately Week 1 to 25), Stage II Cycle 9 through 17 (approximately Week 25 to 52), and Stage III Cycle 18 through 35 (approximately Week 52 through 105). The frequency of tumor imaging and correlative studies blood collection differs per stage of the treatment phase. Screening, study visits, end of treatment visit, and follow-up will be conducted as outlined on the Study Calendar (Section 9).

Details on Pembrolizumab Treatment:

Dose Selection

The rationale for selection of doses to be used in this trial is provided in Section 2.2.3 Background and Rationale. Details on preparation and administration of pembrolizumab are provided in the Pharmacy Manual.

Timing of Dose Administration

Trial treatment should be administered on Day 1 of each cycle after all procedures/assessments have been completed as detailed on the Study Calendar (Section 9) unless otherwise specified. Trial treatment may be administered up to 3 days before or after the scheduled Day 1 of each cycle.

Pembrolizumab 200 mg will be administered as a 30 minute intravenous infusion every 3 weeks. Sites should make every effort to target infusion timing to be as close 30 minutes as possible. However, given the variability of infusion pumps, a window is permitted (ie, infusion time is 30 minutes: -5 min / +60 min).

The Pharmacy Manual contains specific instructions for the preparation of the pembrolizumab infusion fluid and administration of infusion solution.

Management of Infusion Reactions

If an infusion reaction occurs, 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.

Table 1 below shows treatment guidelines for patients who experience an infusion reaction associated with administration of pembrolizumab.

Table 1. Treatment Guidelines for Infusion Reactions Associated with Administration of Pembrolizumab

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 patient is deemed medically stable	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (eg, antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for ≤ 24 hrs	**Stop Infusion and monitor symptoms.** 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 patient is deemed medically stable. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (eg, from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the patient should be premedicated for the next scheduled dose. **For subjects who develop grade 2 toxicity despite adequate premedication, it is strongly recommended that the subject is permanently discontinued from further trial treatment administration. In certain circumstances, rechallenge of drug may be allowed only with approval from the study PI**	Subject may be premedicated prior to infusion of pembrolizumab with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500 to 1000 mg po (or equivalent dose of antipyretic).
Grade 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 Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine** in cases of anaphylaxis, use promptly Increase monitoring of vital signs as medically indicated until the patient is deemed medically stable. Hospitalization may be indicated. **It is strongly recommended that the subject is permanently discontinued from further trial treatment administration. In certain circumstances, rechallenge of drug may be allowed only with approval from the study PI**	No subsequent dosing

4.1 General Concomitant Medication and Supportive Care Guidelines

Medications specifically prohibited in the exclusion criteria (with the exception of palliative radiation) are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications specifically prohibited during the trial, discontinuation from trial therapy is usually appropriate, but exceptions can be granted with approval from the study PI.

4.1.1 Acceptable Concomitant Medications and other Supportive Therapies

All treatments that the investigator considers necessary for a patient's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care.

Palliative radiation is allowed at the investigator's discretion.

4.1.2 Prohibited Concomitant Medications and other Supportive Therapies

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase of this trial:

- Antineoplastic systemic anti-cancer chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Systemic glucocorticoids (prednisone equivalent dose > 10 mg) for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology, unless permission is given from the study PI. The use of physiologic doses of corticosteroids (prednisone equivalent dose ≤ 10 mg) is permitted throughout the study.
- Patients who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from trial treatment but should continue in the follow-up period if progression without clinical benefit has not yet occurred.

  There are no prohibited therapies during post-treatment follow-up.

4.1.3 Blood draws

The risks of blood draws include pain, bruising at the site where the blood is taken, redness and swelling of the vein and infection, and rare risk of fainting due to vasovagal syncope. During the study, extra blood will be taken for correlative studies (ie, ctDNA).

4.1.4 Tumor Biopsy

This clinical trial has an optional tumor biopsy prior to enrollment. The risks of the biopsy vary depending on the location of the biopsy or the procedure that is required to do the biopsy. The risk of a lung biopsy includes some discomfort at the biopsy needle site, minor bleeding at the puncture site, hemoptysis, pneumothorax which may require placement of a chest tube; uncommon risks include infection and air embolism; rare risks include need for emergency surgery due to complications with procedure and death.

4.1.5 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Non-pregnant, non-breast-feeding women of childbearing potential may be enrolled if they are willing to use acceptable birth control (guidance below) or are women NOT of childbearing potential. The following definitions are considered women NOT of childbearing potential1) surgically-sterilized, including one of the following: hysterectomy, bilateral salpingectomy, or bilateral oophorectomy; 2) postmenopausal, including a woman who has not had menses for greater than 1 year OR in absence of 12 months of amenorrhea, a high follicle stimulating hormone level in postmenopausal range on two FSH measurements as long as woman is not using hormonal

contraception or hormonal replacement therapy; or 3) not heterosexually active for the specified duration of the study.

The following are considered highly effective contraceptive methods that are user dependent (f*ailure rate of <1% per year when used consistently and correctly*): 1) combined (estrogen- and progesterone- containing) hormonal contraception (i.e. oral, intravaginal, transdermal, injectable); and 2) progesterone-only hormonal contraception (i.e. oral, injectable). The following are considered highly effective contraceptive methods that have lower user dependency (f*ailure rate of <1% per year when used consistently and correctly*): 1) progesterone- only contraceptive implant; 2) intrauterine hormone-releasing system (IUS); 3) intrauterine device (IUD); 4) bilateral tubal occlusion; 5) vasectomized partner (i.e. provided that the partner is the sole male sexual partner of the women of childbearing potential and the absence of sperm has been confirmed; if not, an additional method of contraception should be used); 6) abstinence from heterosexual intercourse. The acceptable alternative for the above highly effective contraceptive methods is 2 barrier methods including cervical cap, diaphragm; sponge, condom (including by the partner); or spermicide; or alternatively complete abstinence.

Women subjects 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 they must adhere to the contraception requirement **for the duration of the study and through 120 days after the last dose of trial treatment.**

There are no specific contraception requirements for male subjects with female partners of childbearing potential.

4.2 Criteria for Removal from Trial Treatment

A patient must be discontinued from trial treatment for any of the following reasons:

- The patient withdraws consent (see below Section 4.2.1 for further details on type of consent withdrawn).
- Confirmed radiographic disease progression (RECIST v1.1) without clinical benefit as determined by investigator
    - If the patient is having clinical benefit, patients may continue on therapy post-progression following approval from the PI before the second post-progression dose of therapy.
- Unacceptable toxicity, including that which prevents further administration of trial treatment, or results in interruption of trial treatment greater than 12 weeks from the date of the scheduled interrupted dose
- Reason other than treatment-related toxicity that results in interruption of trial treatment greater than 8 weeks from date of the scheduled interrupted dose.
- Intercurrent illness that prevents further administration of trial treatment
- Investigator's decision to withdraw the patient
- Pregnancy
- Death

- Consistent and significant noncompliance with trial treatment or protocol-mandated procedures
- The patient is lost to follow-up
  - *Study personnel may use public records to check for mortality for any patients considered "lost to follow-up," if permitted by applicable laws or regulations*.
- Completed 24 months of treatment (approximately 35 treatments) with pembrolizumab
- Administrative reasons
- The study is terminated by the investigator or Merck.
  - If the study is terminated by Merck, patients who are receiving clinical benefit from their participation in this study may continue to receive pembrolizumab from Merck.

4.2.1 Withdrawal

Patients may choose to discontinue the trial at any time, for any reason, and without prejudice to further treatment. A patient who withdraws consent either in part or in whole of study participation will be asked to state the type (s) of withdrawal.

- **Treatment**: Withdrawal from *treatment* only; in this case, correlative research studies may be performed and follow up and outcomes data will continue as planned.
- **Treatment and correlative research**: Withdrawal from *treatment* with no further measurement of correlative research (note this will affect the primary endpoint but will not affect correlative research studies that have already been collected).
- **Treatment, correlative research, and follow-up**: Withdrawal from treatment, correlative research, and *follow-up* period (allowing no collection of off-study data, such as subsequent anti-cancer therapy, progression if not determined during study treatment, and survival).

In the event of withdrawal of consent, the study staff and/or investigator must make every effort to ascertain the types(s) of consent withdrawn. Full withdrawal of consent would include all 3 types (treatment, correlative research, and follow up), and will be the default withdrawal unless otherwise affirmed by the subject.

After discontinuation of *treatment* (unless there is full withdrawal of consent), the End of Treatment and Follow-up visit procedures should continue as described in Section 9 (Study Calendar). These patients will be followed up for efficacy outcomes until the end of the study. They should be followed for safety until resolution or permanent sequelae of all toxicities attributable to the study drug.

4.2.2 Discontinuation of Study Therapy after CR

Discontinuation of study treatment may be considered for patients who have attained a confirmed CR that have been treated for at least 24 weeks with pembrolizumab and had at least 2 treatments with pembrolizumab beyond the date when the initial CR was declared. Future restarting of pembrolizumab would be expected to be administered as standard-of-care treatment, since pembrolizumab is FDA-approved for NSCLC. Assessments should proceed per section 9.

5. INVESTIGATIONAL AGENT INFORMATION (Pembrolizumab)

5.1 Investigational Agent/Device/Procedure

Refer to the most up-to-date Investigator's Brochure (IB)/approved labeling for detailed background information on Pembrolizumab and see Section 2.2 for summary.

Pembrolizumab 200 mg will be administered as a 30-minute intravenous infusion every 3 weeks for up to 24 months of therapy until disease progression without clinical benefit or unacceptable toxicity.

For each individual trial, clinical supplies are to be stored in accordance with specific instructions on the label.

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 investigational product in accordance with the protocol and any applicable laws and regulations.

Pembrolizumab will be provided by Merck as summarized in Table 2.

Table 2 Product Descriptions

Product Name & Potency	Dosage Form
Pembrolizumab 100 mg/ 4 mL	Solution for Injection

Packaging and Labeling Information

Supplies will be labeled in accordance with regulatory requirements.

Clinical Supplies Disclosure

This trial is open-label; therefore, the patient, the trial site personnel, the Sponsor and/or designee are not blinded to treatment. Drug identity (name, strength) is included in the label text; random code/disclosure envelopes or lists are not provided.

5.2 Availability

Merck & Co (Kenilworth, NJ) is providing pembrolizumab.

5.3 Agent Ordering

Refer to Merck order form for instructions to order drug or contact:

Dana Gardener, Merck Research Laboratories
351 N Sumneytown Pike UG2CD-30
North Wales, PA 19454-2505
Phone: 267-305-5403 | Fax: 267-305 6534 | Email: dana_gardner@merck.com.

5.4 Agent Accountability

Storage and Handling Requirements

Clinical supplies will be stored in a secure, limited-access location under the storage conditions specified on the label. Receipt and dispensing of trial medication will be recorded by an

authorized person at the trial site. Clinical supplies may not be used for any purpose other than that stated in the protocol.

Returns and Reconciliation

The investigator is responsible for keeping accurate records of the clinical supplies received from Merck or designee, the amount dispensed to and returned by the patients and the amount remaining at the conclusion of the trial.

Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. It is the *Investigator's responsibility* to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

6. DOSE MODIFICATIONS AND TOXICITY MANAGEMENT

6.1 ADVERSE EVENT MANAGEMENT

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the 1st dose or several months after the last dose of treatment and may affect more than one body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes (i.e. disease progression or bacterial/viral infection). Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. It is generally recommended to involve other specialists for grade 3 and 4 events. 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. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab are provided in Table 3 of section 6.2.

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. ***Suggested*** supportive care measures for the management of adverse events with potential immunologic etiology are outlined below. Where appropriate, these guidelines include the use of oral or intravenous 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. The treatment guidelines are intended to be applied when the investigator determines the events to be *related* to pembrolizumab. In cases in which intravenous steroids are suggested below, oral steroids may be administered instead, if clinically appropriate. Occasionally, more powerful immunosuppressants such as infliximab or mycophenolate mofetil may be necessary to control immune-related adverse events.

6.2 Dose Modification Guidelines for Drug-Related Adverse Events

Pembrolizumab should be withheld for severe or life-threatening AEs as per Table 3 below. **These are considered guidelines, but drug administration can also be held for events not described below at the treating physician's discretion. If the recommendation is for trial treatment to be permanently discontinued, this should be strongly considered, but drug rechallenge may be allowed with prior approval from the PI.**

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 the date the scheduled dose was missed or corticosteroids cannot be reduced to ≤ 10 mg prednisone during this same timeframe.
3. For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids.

Immune-related AEs	Toxicity grade or conditions (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 to 2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment • Add prophylactic antibiotics for opportunistic infections
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus). • Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.
	Grade 4	Permanently discontinue		
AST / ALT elevation or Increased	Grade 2	Withhold	• Administer corticosteroids (initial dose of 0.5 to 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is

Immune-related AEs	Toxicity grade or conditions (CTCAE v4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
bilirubin	Grade 3 or 4	Permanently discontinue	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	stable
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.
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		

Immune-related AEs	Toxicity grade or conditions (CTCAE v4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
All other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	• Based on type and severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Gullain-Barre Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		

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).

7. ADVERSE EVENTS AND REPORTING PROCEDURES

7.1 Potential Adverse Events

Refer to the most up-to-date Investigator's Brochure (IB)/approved labeling for additional adverse event information on Pembrolizumab.

Safety

Refer to Section **2.2.2.5 Clinical Safety for further details.**

Important Safety Considerations- Refer to the most up-to-date Investigator's Brochure (IB)

There are no specific safety concerns based on the results of nonclinical studies. Pembrolizumab has the same mechanism of action as other anti–PD-1 monoclonal antibodies. Preclinical studies have suggested similar potency, and pharmacokinetic (PK) modeling has suggested similar human PK in the class. Accordingly, the AEs observed with other anti–PD-1 antibodies may serve as an indicator for the AEs to expect in cancer subjects. Furthermore, AEs from other immunotherapies for cancer were considered in the Pembrolizumab Program (MK-3475) Event of Clinical Interest Guidance Document.

Pembrolizumab is generally well tolerated and demonstrates a favorable safety profile in comparison to chemotherapy. Pembrolizumab is an immunomodulatory agent, and based on this mechanism of action, immune mediated adverse events are of primary concern. Important identified risks for pembrolizumab are of an immune mediate nature, including: pneumonitis; colitis; hepatitis; nephritis; endocrinopathies that include hypophysitis (including hypopituitarism and secondary adrenal insufficiency); thyroid disorder (hypothyroidism, hyperthyroidism); and Type I diabetes mellitus; uveitis; myositis; Guillain-Barré syndrome; pancreatitis; myocarditis; severe skin reactions including Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), some with fatal outcome; and "solid organ transplant rejection following pembrolizumab treatment in donor organ recipients" (risk applicable to postmarketing setting only, as such patients are currently excluded from Merck clinical trials with pembrolizumab). The risk profile for pembrolizumab also included 2 important potential risks – ie, myasthenic syndrome and increased risk of severe complications (such as early severe graft versus host disease and veno- occlusive disease) of allogeneic transplant in patients with hematologic malignancies who have previously been treated with PD-1 inhibitors. Two new important identified risks also included "encephalitis" and "sarcoidosis.' The majority of immunemediated adverse events were mild to moderate in severity, were manageable with appropriate care, and rarely required discontinuation of therapy. Further details around frequency, reporting, and management of immune-related adverse events (irAEs) are described in other section of protocol and the most up-to-date Investigator's Brochure. In addition to the previously noted identified risks, infusion-related reactions are a risk but are not considered immune mediated; these are further described below.

Infusion Reactions

Infusion reactions have been reported with pembrolizumab at a rate of 2.5%; these were generally Grade 1 and 2 and the majority were considered related by the Investigator. One event of Grade 4 anaphylaxis has been reported. Infusion reactions may present as allergic reaction, serum sickness, infusion reaction, cytokine release syndrome, or anaphylaxis. Mild infusion reactions can generally be treated with interruption of the infusion and medical intervention including IV fluids, antihistamines, nonsteroidal anti-inflammatory drugs, acetaminophen, and

narcotics as needed. More severe or life threatening reactions may require pressors, corticosteroids, and epinephrine**.** Please see Table 1 of Section 4: Treatment Guidelines for Infusion Reactions Associated with Administration of Pembrolizumab.

7.1.1 Assessing and Recording Adverse Events

An adverse event is defined as any untoward medical occurrence in a patient administered trial treatment and which does *not* necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (ie, any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of trial treatment, may also be considered an adverse event.

Changes resulting from normal growth and development that do not vary significantly in frequency or severity from expected levels are not to be considered adverse events. Examples of this may include onset of menopause occurring at a physiologically appropriate time.

Adverse events may occur during the course of the use of Merck product in clinical trials, or as prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

Progression of the cancer under study is not considered an adverse event.

Adverse events may occur during the course of the use of trial treatment or within the follow-up period specified by the protocol.

Adverse events may also occur in screened patients during any ***pre-allocation baseline period as a result of a protocol-specified intervention***, including washout or discontinuation of usual therapy or a procedure.

All adverse events should be **recorded from the time the consent form is signed through the end of treatment, and at each examination on the Adverse Event CRFs**. The reporting timeframe for adverse events meeting any serious criteria is described in Section 7.2. The investigator may follow subjects with non-serious adverse events for outcome.

Adverse events may not be collected for subjects during the pre-screening period (for determination of archival tissue status) as long as that subject has not undergone any protocol-specified procedure or intervention. If the subject requires a blood draw, fresh tumor biopsy, etc, the subject is first required to provide consent to the main study and AEs should be captured according to guidelines for standard AE reporting.

7.1.2 Evaluating Adverse Events

Adverse events will be graded according to CTCAE v4.03. Both Serious and Non-Serious Adverse Events will be clearly noted in source documentation and listed on study specific Case Report Forms (CRFs). The Protocol Director (PD) or designee will assess each Adverse Event (AE) to determine whether it is unexpected according to the Informed Consent, Protocol Document, or Investigator's Brochure, and related to the investigation. **Toxicities should be characterized in terms regarding seriousness, causality, toxicity grading, duration, and action taken with regard to trial treatment.** All AEs of unknown etiology associated with pembrolizumab exposure

should be evaluated to determine if it is possibly an event of clinical interest (ECI) of a potentially immunologic etiology (termed immune-related adverse events, or irAEs).

Patients with a trial treatment-related AE of Grade ≥ 1 and SAEs should be followed until the resolution of the AE to Grade 0 to 1, 30 days from last trial treatment, or until the beginning of a new anti-neoplastic therapy, whichever occurs first.

7.1.2.2 Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor and to Merck

For purposes of this trial, an overdose of pembrolizumab is considered as any dose of 1,000 mg or greater (≥ 5-fold the indicated dose). No specific information is available on the treatment of overdose of pembrolizumab. Appropriate supportive treatment should be provided if clinically indicated. In the event of overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an adverse event(s) is associated with ("results from") the overdose of a Merck product, the adverse event(s) may be reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of Merck's product meeting the protocol definition of **overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose may be reported as a non-serious Event of Clinical Interest (ECI),** using the terminology "accidental or intentional overdose without adverse effect."

A report of overdose with and without an adverse event should be reported to the Sponsor and to Merck Global Safety (Attn: Worldwide Product Safety; FAX 215-661-6229).

7.1.2.3 Reporting of Pregnancy and Lactation to the Sponsor and to Merck

Although pregnancy and infant exposure during breast feeding are not considered adverse events, the investigators or their designees should report any pregnancy or lactation in a subject (spontaneously reported to them) that occurs during the trial.

Pregnancies and lactations that occur after the consent form is signed but before treatment allocation should be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy or a procedure.

Pregnancies and infant exposure during breast feeding that occur from the time of treatment allocation through the duration of trial treatment should be reported by the investigator. **All reported pregnancies may 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 should be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) may also be reported.**

Such events should be reported to the Sponsor and to Merck Global Safety
(Attn: Worldwide Product Safety; FAX 215-661-6229)

7.1.3 Serious Adverse Events and Events of clinical interest

A serious adverse event is any adverse event occurring at any dose or during any use of pembrolizumab that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization; Hospitalizations for elective medical or surgical procedures or treatments planned before enrollment in the treatment plan or routine check-ups are not SAEs by this criterion. Admission to a palliative unit or hospice care facility is not considered to be a hospitalization.
- Is a congenital anomaly/birth defect;
- Is medically significant, may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed previously
- **Note:** In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, may be reportable to the Merck as SAEs to meet certain local requirements.
    - Is a new cancer (that is not a condition of the study);
    - Is associated with an overdose.

Events of clinical interest (ECI) for this trial include:

1. An overdose of Merck product, as defined in Section 7.1.2.2 - *Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor*, 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 3 X ULN and an elevated total bilirubin lab value that is greater than or equal to 2 X ULN and, at the same time, an alkaline phosphatase lab value that is less than 2 X ULN, 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.

Stanford Specific Reporting

All Serious Adverse Events (SAEs) should be tracked until resolution of the SAE to Grade 0 to 1; until 30 after the last dose of the study treatment; or until the beginning of a new anti-neoplastic therapy, whichever comes earlier. SAEs CTCAE Grade 3 and above (defined above), and all subsequent follow-up reports will be reported to the Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) using the study specific CRF **regardless of the event's relatedness to the investigation**. Following review by the DSMC, events meeting the IRB definition of 'Unanticipated Problem' will be reported to the IRB using eProtocol **within 10 working days** of DSMC review, **or within 5 working days** for deaths or life-threatening experiences.

Merck Specific Reporting

For the time period beginning when the consent form is signed until treatment allocation, any serious adverse event or ECI, or follow-up to a serious adverse event or ECI, including death due to any cause other than progression of the cancer under study, that occurs to any subject should be reported to the Sponsor and to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy or a procedure.

For the time period beginning at treatment allocation through 30 days following cessation of treatment, any serious adverse event or ECI, or follow up to a serious adverse event or ECI including death due to any cause other than progression of the cancer under study whether or not related to the Merck product, should be reported to the Sponsor and Merck Global Safety.

Any serious adverse event must be reported within 24 hours from the time the investigator is notified to the Sponsor and within 2 working days to Merck Global Safety.

SAE reports should be forwarded to the Merck Global Safety facsimile number: +1- 215-661-6229

A copy of all 15-Day Reports and Annual Progress Reports is submitted as required by FDA, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local regulators. Investigators may cross reference this submission according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally investigators should submit a copy of these reports to Merck & Co., Inc (Attn: Worldwide Product Safety; FAX **+1-215-661-6229**) at the time of submission to FDA.

8. CORRELATIVE/SPECIAL STUDIES

Additional/alternative correlative research may be performed pending development of new technology and scientific advances.

8.1 OPTIONAL TUMOR BIOPSY for Correlative Studies (CAPP-Seq, PD-L1 IHC, CIBERSORT)

8.1.1 Collection of Specimen(s)

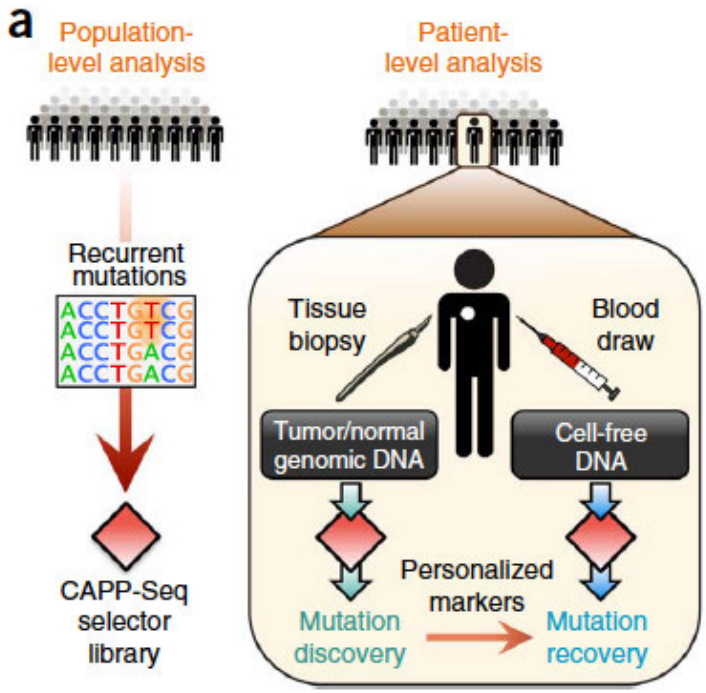
Patients may undergo an optional fresh tumor tissue biopsy prior to trial treatment to perform correlative studies including CAPP-Seq and CIBERSORT. Additional/alternative correlative research may be performed pending development of new technology and scientific advances. If patient agrees to an optional fresh tumor biopsy, core biopsies (generally CT-guided or bronchoscopic) are preferred- if the patient needs a surgical approach or excisional approach for a standard of care related reason, this tissue can be used as long as the patient has already consented for the study.

Fine needle aspiration, thoracentesis, or other specimens without tissue architecture preservation are generally not preferred but may be used if a core needle biopsy is contraindicated.

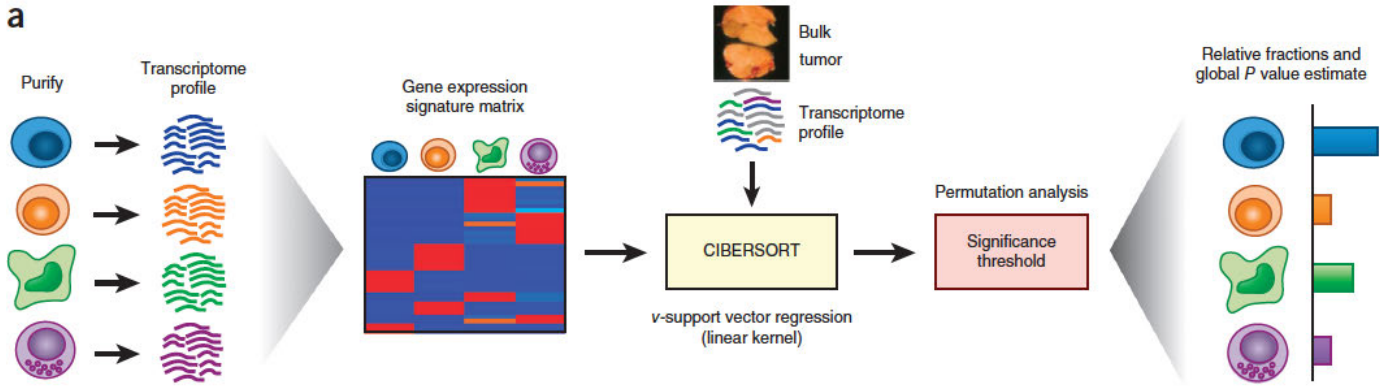
At the point of the optional biopsy, a goal of at least 4 cores should be obtained once tumor yield is identified by a cytotechnologist. The proceduralist may take additional cores if it is not felt to significantly increase the risk to the patient. It is estimated that 2 cores will be snap-frozen,

separately, and 2 cores will be sent to pathology for formalin fixation and later recovery for correlative work. If a patient undergoes an optional tumor biopsy, the following will be planned:

- **CAPP-Seq:** Paraffin-embedded tissue will be sent for DNA sequencing to Stanford Molecular Pathology Laboratory (STAMP, Solid Tumor Actionable Panel, Stanford Hospital) or Diehn/Alizadeh labs (Stanford Campus) to establish patient specific mutational markers (> 95% of NSCLC patients have an identifiable marker in the panel). These can include single nucleotide variants and rearrangements such as ALK, ROS1, and RET.



- **CIBERSORT**[34]: Fresh or snap frozen tissue will be sent to Alizadeh/Diehn labs (Stanford Campus) to undergo total RNA extraction and reverse transcription, then analyzed by microarray. The data will be processed and scored using the CIBERSORT algorithm (CIBERSORT.stanford.edu). A lung cancer population CIBERSORT algorithm will be established[54], as the CIBERSORT algorithm can robustly identify an "unknown" new tumor type. Individual populations of different subtypes of immune cell infiltrate subsets will be quantitated and an overall "immune score" will be generated.



- **PD-L1 IHC[33]:** Paraffin embedded tissue may be sent per standard protocol as standard of care FDA approved test if not already done. This test does not have to be sent on the optional tumor biopsy as part of this study but could have been sent on archival tissue as per standard of care.

8.1.2 Handling of Specimens(s)

- Once the biopsy is obtained it will be placed in a container with dry ice and transported to the Alizadeh/Diehn labs on the same day. Part of the tissue will be sent to the Stanford Pathology Histology section in formalin for paraffin embedding. PD-L1 IHC should have been sent previously on archival tumor as part of standard of care. The rest of the tissue will be directly processed by Diehn/Alizadeh laboratories for Capp-Seq, CIBERSORT, and other studies that do not require paraffin embedding. The samples will be stored in Diehn/Alizadeh laboratories.

8.1.3 Shipping of Specimen(s)

- Biopsy will be transported on ice to the Diehn/Alizadeh lab on the same day.
- PD-L1 testing will be performed per standard of care FDA approved test and may have been sent previously on archival tumor as part of standard of care.

8.1.4 Site(s) Performing Correlative Study

Diehn/Alizadeh lab (Stanford Campus) will be performing CAPP-Seq and CIBERSORT. If additional correlative work is proposed pending scientific advances, this may require the cooperation of other laboratories.

8.1.5 Coding of specimens for privacy protection

All specimens will be de-identified with a unique code identifier.

8.2 BLOOD SAMPLES for Correlative Studies (CAPP-Seq ctDNA)

8.2.1 Collection of Specimen(s)

Blood will be drawn (goal of 30 to 50 mL whole blood at each timepoint) at the timepoints listed in Section 9 Study Calendar.

The blood will be brought to the Diehn/Alizadeh labs as soon as possible, but always within 4 hours of collection, for further processing.

8.2.2 Handling of Specimens(s)

Blood samples will be hand-transported on ice to the Diehn/Alizadeh lab within 4 hours of draw.

8.2.3 Shipping of Specimen(s)

We do not anticipate shipping of specimens.

8.2.4 Site(s) Performing Correlative Study

CAPP-Seq will be performed by Diehn/Alizadeh labs (Stanford Campus). CAPP-Seq result will be reported as circulating tumor DNA (ctDNA) as a percentage of total circulating free DNA and also as ctDNA titer (a continuous variable in units pg/mL). The assay can identify less than 0.005% ctDNA, though many advanced NSCLC tumors have greater than 1% ctDNA, resulting in a wide dynamic range for analysis. If additional correlative work is proposed pending scientific advances, this may require the cooperation of other laboratories.

8.2.5 Coding of specimens for privacy protection

All specimens will be de-identified with a unique code identifier.

9. STUDY CALENDAR (Table 4)

Trial Period:		Treatment Phase [a]															End of Treatment [t]	Follow-Up [s] Every 12 weeks
		Stage I (cycles 1-8) [a]								Stage II (cycles 9-17) [a]						Stage III [a]		
Visit/Treatment Cycle:	Screening [b]	1	2	3	4	5	6	7	8	9	10	11	12	13	14+ (14-17)	18+ (18-35)	Dsc'n	Follow-Up
Scheduling Window (Days):	-28 to 1	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 14
Informed Consent [c]	X																	
Inclusion/Exclusion Criteria	X																	
Demographics and Medical History [d]	X																	
Prior and Concomitant Medication Review [e]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pembrolizumab Administration [f]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Review Adverse Events [g]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Examination [h]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs and Weight [i]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ECOG Performance Status	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy Test – Urine or Serum β-HCG [k]	X																	
CBC with Differential [m]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Comprehensive Metabolic Panel [m]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
TSH with reflex T4 [n]	X			X		X		X		X								
Body Tumor Imaging [o]	X			X		X		X		X			X			X	X	X
Brain Imaging [p] (+ pts with known untreated brain mets)	X			X+		X+		X+		X+			X+			X+	X+	X+
Fresh tumor biopsy (optional) [q]	X																X*	
Correlative Studies Blood Collection [r]		X	X	X		X		X		X			X			X	X	X
Post-study anticancer therapy status [s]																		X
Survival Status [s]																		X

Footnotes

a In general, assessments/procedures are to be performed on Day 1 and **prior** to the pembrolizumab treatment for each cycle unless otherwise specified. Treatment cycles are 3 weeks (21-days). In general, the window for each cycle is ± 3 days unless otherwise specified. There are 3 stages during the treatment phase of the study: Stage I Cycle 1 through 8 (approximately Week 1-25), Stage II Cycle 9 through 17 (approximately Week 25 to 52), and Stage III Cycle 18 through 35 (approximately Week 52 through 105). The frequency of tumor imaging and correlative studies blood collection differs per stage of the treatment phase (see separate bullet points for tumor imaging and correlative studies blood collection for details).

- *Treatment-Related Toxicity Delay*: If a patient has a treatment delay due to treatment-related adverse event, the frequency of tumor imaging and correlative studies blood collection should continue to be performed at the frequency according to the stage of the study in which the **cycle** was interrupted, when feasible. A dosing interruption for a treatment-related adverse event is allowed for 12 weeks from the date the scheduled dose was missed. After approximately 21 days from date the scheduled dose was missed, the cycle should be recorded as completed at the initial "ideal" date and the next cycle initiated, even if drug is held and assessments were not performed to allow for ongoing scheduled correlatives.
- *Other delay*: While the routine window for cycle is ± 3 days, a scheduled cycle (including drug administration, imaging, and correlative blood draw) may be additionally delayed for up to 21 days due to patient/physician preference or in the case of medical / surgical events or logistical reasons not related to trial treatment (eg, elective surgery, unrelated medical events, patient vacation, and/or holidays). An interruption in this scenario is allowed for 21 days from date the scheduled cycle was missed. Delay of cycles within Stage 1 of the trial is allowed but discouraged.
- In order to minimize blood draws, if the patient had a dose interruption for any reason, the frequency of tumor imaging and correlative studies blood collection can be extended up to 21 days to allow these to coincide with other associated visit assessments/procedures. This window can be further shortened/extended for unusual circumstances after discussion with the PI/co-PI.

b Screening: Results of a test performed prior to the patient signing consent as part of routine clinical management are acceptable in lieu of a screening test if performed within the specified time frame (28 days). Screening tests are allowable on day of first trial treatment *as long as performed prior* to trial treatment.

c Written informed consent should be obtained prior to any study-specific procedure.

d Medical history should be taken including details of prior cancer treatment.

e The investigator or qualified designee should review medication use at screening, including any protocol-specified washout requirement, and should record medication, if any, taken by the patient during the trial.

f Pembrolizumab 200 mg should be administered as a 30 minute IV infusion (± 3 days). Trial treatment should be administered on Day 1 of each cycle after all procedures/assessments have been completed as detailed on the Study Calendar, except where noted.

g The investigator or qualified designee should assess each patient to evaluate for potential new or worsening AEs at each cycle and more frequently if clinically indicated. Adverse events should be graded and recorded throughout the study according to NCI CTCAE v4.03.

h The investigator or qualified designee should perform a physical exam at screening and prior to trial treatment administration.

i Oxygen saturation should be measured at least at screening.

k Perform either urine or serum pregnancy test on women of childbearing potential prior to first dose of trial treatment.

m Safety Laboratory: After Cycle 1, safety lab samples (ie, CBC+diff, complete metabolic panel) can be collected up to 3 days prior to the scheduled time point. Laboratory results should be known and acceptable prior to dosing (except for thyroid function tests, *eg*, T3, T4, TSH). Complete blood count with differential (eg, Platelet, hemoglobin, white blood cell count and differential). Chemistries include albumin; alkaline phosphatase; alanine aminotransferase; aspartate aminotransferase; carbon dioxide/bicarbonate; calcium; creatinine; chloride; glucose; potassium; sodium; total bilirubin; total protein.

n Thyroid function tests should be performed at screening and then every 2 cycles (6 weeks) starting with Cycle 3 through Cycle 9. They can be repeated as clinically indicated.

o Body tumor imaging should be performed with computed tomography, with IV contrast when possible. The initial tumor imaging should be performed within 30 days prior to the first dose of trial treatment. 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 trial treatment. The same method of assessment should be used to characterize *each identified and reported lesion at baseline and during follow-up*. For Stage I of treatment phase (Cycle 1 through 8), tumor imaging should be performed *every 6 weeks (± 7 days*). For Stage II of treatment phase (Cycle 9 through 17), tumor imaging should be performed *every 9 weeks (± 7 days)*. For Stage III of treatment phase (≥ Cycle 18), tumor imaging should be performed *every 12 weeks (± 7 days)*. It is at the discretion of the investigator or qualified designee should more frequent imaging be clinically necessary. Local reading (investigator assessment) should be used to determine eligibility and for patient management. After the first documentation of progression (if the patient has clinical benefit) per RECIST v1.1, repeat imaging for confirmation is suggested given possibility of pseudo-progression. Confirmatory imaging may be performed as early as 28 days later; alternately, the scan performed at the next scheduled timepoint may be used as confirmation.

p All patients must have a brain MRI (preferred) or head CT with contrast at baseline **within 12 weeks prior to enrollment**. Patients with known brain metastases at baseline must have a brain MRI (preferred) or head CT within 28 days prior to enrollment. Patients with known untreated brain metastases at baseline should have repeat imaging at the same interval as listed in *footnote q* for body tumor imaging.

q If the patient has consented to the optional tumor biopsy, this should be performed prior to first trial treatment. Archival tissue may be retrieved when available. Needle biopsy is preferred; cytology and fine needle aspiration is generally not sufficient but may be used. Patients who initially receive clinical benefit from pembrolizumab but then come off study for progression may be offered a biopsy at the time of progression. If the patient does consent to repeat biopsy at progression, this should ideally be performed within 30 days of the end of treatment visit and prior to initiation of new anti-cancer systemic therapy.

r Correlative blood study collection should be obtained in the following schedule (± 3 day window except where noted): prior to first dose of trial treatment; within 30 minutes (± 10 minutes) after completion of first dose of trial treatment; pretreatment at Cycle 2 (~Week 4); and Cycle 3 (~Week 7). For the remaining part of Stage I of treatment phase, correlative studies blood collection should be collected every 6 weeks (± 3 days). For Stage II of treatment phase (Cycle 9 through 17), correlative blood studies should be collected every 9 weeks (± 3 days). For Stage III of treatment phase (≥ Cycle 18), correlative blood studies should be collected every 12 weeks (± 3 days). Correlative blood studies should be collected at the end of treatment visit (± 3 days). ***All efforts should be made to have the correlative studies blood collection be performed at a similar time to imaging studies.***

s During the follow-up phase, *all* patients should be contacted every 12 weeks (± 2 weeks) to evaluate survival status from the time of the end of treatment visit until death, withdrawal of follow-up consent, or the end of the study, whichever occurs first. For patients who came off-study for reasons other than disease progression, the following assessments/procedures should be strongly recommended every 12 weeks (± 2 weeks) until disease progression, initiating a cancer treatment, withdrawing consent or becoming lost to follow-up: tumor imaging and correlative studies blood collection. In addition, anticancer therapy status, concomitant medication review, review of adverse events, directed physical exam, vital signs and weight, ECOG performance status may be performed if possible.

t. End of treatment visit will be marked by confirmed radiographic disease progression without clinical benefit; unacceptable adverse events; completion of 24 months of therapy; or other event requiring trial treatment discontinuation. Repeat imaging is recommended if patient has end of treatment visit for reason other than progression if imaging performed ≥ 6 weeks prior.

10. MEASUREMENTS

Primary Outcome Measure: To correlate circulating tumor DNA (ctDNA) levels measured using Cancer Personalized Profiling by deep Sequencing (CAPP-Seq) with radiographic tumor assessments using RECIST v1.1 criteria in patients with metastatic NSCLC treated with pembrolizumab

Title: Circulating tumor DNA (CAPP-Seq)

Time Frame: See Study Calendar (Section 9)

Safety Issue: Is this outcome measure assessing a safety issue? **No**

10.1 Primary and Secondary Outcome measures

10.1.1 Primary Outcome

- To correlate circulating tumor DNA (ctDNA) levels measured using Cancer Personalized Profiling by deep Sequencing (CAPP-Seq) with radiographic tumor assessments using RECIST v1.1 criteria in patients with metastatic NSCLC treated with pembrolizumab
- *Relevant Subset*: All patients treated with at least one dose of pembrolizumab, have at least one evaluable ctDNA measurement, and at least one follow-up imaging.
- *Measurement Definition*
  - ctDNA measured as percentage of total circulating free DNA and also as ctDNA titer (continuous variable in units pg/mL)
  - Radiographic tumor assessments using RECIST v1.1 criteria (sum of longest diameters (SLD) of up to 5 target lesions, measured in mm or cm)
- Measurement Methods
  - ctDNA levels measured using Cancer Personalized Profiling by deep Sequencing (CAPP-Seq)
  - Radiographic tumor assessments using RECIST v1.1 criteria (Appendix B)[56]
- Measurement Timepoints
  - Refer to Study Calendar Section 9
  - Response Review
    - Investigators on the trial will perform tumor measurements based on RECIST v1.1 criteria. There will be no independent review.

10.2.1 Secondary Outcome

10.2.1.1 To determine the overall response rate using RECIST v1.1 criteria in patients with metastatic NSCLC treated with pembrolizumab

- Relevant Subset: All patients treated with at least one dose of pembrolizumab.
- Measurement Definition
  - Proportion of complete responses + partial responses as determined by RECIST v1.1 criteria
- Measurement Methods
  - RECIST version 1.1 criteria (Appendix B)

- Measurement Timepoints
  - Refer to Study Calendar Section 9 for frequency of imaging

10.2.1.2 To determine the progression-free survival using RECIST v1.1 criteria in patients with metastatic NSCLC treated with pembrolizumab

- Relevant Subset: All patients treated with at least one dose of pembrolizumab.
- Measurement Definition
  - Progression free survival measured from the time of first treatment with pembrolizumab to the time of radiographic progression, unequivocal clinical progression, or death from any cause, whichever comes earlier
- Measurement Methods
  - RECIST v1.1 criteria (Appendix B)[56]
- Measurement Timepoints
  - Refer to Study Calendar Section 9 for frequency of imaging

10.2.1.3 To determine the safety and tolerability of pembrolizumab in patients with metastatic NSCLC

- Relevant Subset: All patients enrolled on study who received trial treatment.
- Measurement Definition
  - Incidence of adverse events
- Measurement Methods
  - Common Terminology Criteria for Adverse Events (CTCAE v4.03)
- Measurement Timepoints
  - From time of enrollment through the end-of-treatment visit. Some patients will have adverse event collection during follow-up phase.

11. REGULATORY CONSIDERATIONS

Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the Sponsor of the trial is solely responsible for determining whether the trial and its results are patient to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. Information posted will allow patients to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

11.1 Institutional Review of Protocol

The protocol, the proposed informed consent and all forms of participant information related to the study (eg, advertisements used to recruit participants) will be reviewed and approved by the Stanford IRB and Stanford Cancer Institute Scientific Review Committee (SRC). Any changes made to the protocol will be submitted as a modification and will be approved by the IRB prior to implementation. The Protocol Director will disseminate the protocol amendment information to all participating investigators.

11.2 Data and Safety Monitoring Plan*:*

The Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) will be the monitoring entity for this study. The DSMC will audit study-related activities to determine whether the study has been conducted in accordance with the protocol, local standard operating procedures, FDA regulations, and Good Clinical Practice (GCP). This may include review of the following types of documents participating in the study: regulatory binders, case report forms, eligibility checklists, and source documents. In addition, the DSMC will regularly review serious adverse events and protocol deviations associated with the research to ensure the protection of human patients. Results of the DSMC audit will be communicated to the IRB and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, as needed.

11.3 Data Management Plan

Case Report Forms (CRFs) are printed or electronic documents designed to record all protocol-related information on each trial participant. CRFs should summarize the clinical findings and observations necessary to ensure safety of participants on the study, and to document the study outcomes. CRFs are required by the SRC for all Interventional studies. CRF design and creation must be completed prior to enrollment of the first participant. The Protocol Director, or his/her designee, will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study. Study specific Case Report Forms (CRFs) will document treatment outcomes for data analysis. Case report forms will be developed using the ONCORE_ database system and will be maintained by the research team. CRFs will be kept in a locked office, only accessible to the research team.

12. STATISTICAL CONSIDERATIONS

12.1 Statistical Analysis Plan Summary

This is an open-label non-randomized single-arm treatment trial of pembrolizumab in NSCLC patients, with a primary endpoint of evaluation of pharmacodynamics biomarker, circulating tumor DNA and its correlation with radiographic tumor assessments using RECIST v1.1 criteria. There is no statistical stopping rule is planned.

12.2 Primary Analysis and Sample size justification

The primary analysis will correlate circulating tumor DNA (ctDNA) levels measured using Cancer Personalized Profiling by deep Sequencing (CAPP-Seq) with radiographic tumor assessments using RECIST v1.1 criteria in patients with metastatic NSCLC treated with pembrolizumab. The primary analysis population will include all patients treated with at least one dose of pembrolizumab, have at least one evaluable ctDNA measurement, and at least one follow-up imaging. The primary explanatory variables are percent ctDNA and ctDNA titer (a continuous variable in units pg/mL). The correlation of percent ctDNA (and DNA titer) with sum of longest diameter of tumor target lesions (continuous variable in mm or cm, RECIST 1.1) will be calculated using Kendall correlation. The correlation of ctDNA (and DNA titer) with overall response category (RECIST v1.1) will be calculated using the Wilcoxon rank sum test. P-values will be reported without correction for multiple testing, however the number of statistical tests planned and/or performed will be included in reports of this study.

12.2.1 Sample Size

25 NSCLC patients
Accrual estimate: We estimate that we will enroll 25 eligible and evaluable metastatic NSCLC patients within an 18-month period.

12.2.2 Sample size justification

Clinically validated differences in ctDNA have not been established to date, but the variability over time does appear to closely follow tumor volume as demonstrated by previously-published data[1]. This may vary across tumor types. 25 patients would provide 80% power to detect a correlation of 0.64 with ctDNA and radiographic RECIST 1.1-based tumor assessments when using a Kendall correlation.

12.2.3 Effect size justification

Clinically validated differences in ctDNA have not been established to date. Previous studies have shown pretreatment ctDNA levels correlation with CT imaging in 9 patients ($R^2 = 0.89$, $p = 0.0002$) and ctDNA levels correlation with CT imaging during therapy ($R^2 = 0.95$ in a single patient and $R^2 = 0.85$ in a single patient).[1] Here, we aim for a slightly lower correlation given the larger sample size.

12.3 Secondary Analysis Statistical Plan:

12.3.1 Summary of Objectives

- To determine the overall response rate using RECIST v1.1 criteria in patients with metastatic NSCLC treated with pembrolizumab
- To determine the progression-free survival using RECIST v1.1 criteria in patients with metastatic NSCLC treated with pembrolizumab

- To determine the safety and tolerability of pembrolizumab in patients with metastatic NSCLC

12.3.2 Populations Defined

- See section 10.1.1 for primary endpoint population.
- **The safety population** includes all patients enrolled on the study who receive pembrolizumab.
- **The efficacy population** includes all patients treated with at least one dose of study drug.
- **The correlative study population** (CIBERSORT, etc) includes all patients treated with at least one dose of pembrolizumab, have at least one evaluable correlative study assessment (ie, undergone optional tumor biopsy), and at least one follow-up imaging study.

12.3.3 Statistics Defined (Descriptive and for Secondary Objectives)

Demographic variables (such as age) and baseline tumor and treatment characteristics will be summarized by descriptive statistics. The correlation of dichotomies will be assessed using Fisher's exact test. The correlation of continuous or ordinal variables with a dichotomy will be made using Wilcoxon rank sum test. The correlations of two continuous variables will be performed using Kendall correlation. Adverse events will be tabulated by category and severity. Time-to-event categories will be calculated with Kaplan-Meier method. Correlation involving a time-to-event variable with a dichotomy will be made using the log-rank test and displayed using Kaplan-Meier. All analyses will use a two-sided significance level of 5%. No adjustment for multiplicity will be carried out at this stage.

12.4 Exploratory Objective

•To correlate tumor immune leukocyte subpopulations identified using the CIBERSORT method alone and in conjunction with PD-L1 assessment on pre-treatment tumor samples with objective response using RECIST v1.1 criteria in patients with metastatic NSCLC treated with pembrolizumab.

For the exploratory data analysis that involves tumor immune leukocyte subpopulations identified by CIBERSORT, the data will be processed and scored using the CIBERSORT algorithm (CIBERSORT.stanford.edu). A lung population CIBERSORT algorithm will be determined to generate an overall "immune score," either as a categorical variable or a continuous variable. This data may be correlated with response and other clinical outcome data. The correlation of CIBERSORT (or CIBERSORT in conjunction with PD-L1 ie, immune score high/PD-L1 positive) with response may be assessed using Fisher's exact test. The correlations of continuous or ordinal variables with a dichotomy (ie, high immune score vs low score or high immune score/PD-L1 positive vs high immune score/PD-L1 negative) may be made using Wilcoxon rank sum test. The correlations of two continuous variables will be performed using Kendall correlations.

12.4 Criteria for future studies

If ctDNA appears promising as a surrogate biomarker of response to immunotherapy, we could design a trial stratified by this biomarker for further clinical validation.

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Appendix A: ECOG Performance Status

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

* As published in Am. J Clin Oncol.: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.* The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

Appendix B: RECIST Version 1.1 Criteria
Response Evaluation Criteria in Solid Tumors (RECIST) Version 1.1 for Evaluating Response in Solid Tumors

RECIST version 1.1* will be used in this study for assessment of tumor response. While either CT or MRI may be utilized, as per RECIST 1.1, CT is the preferred imaging technique in this study.

* As published in the European Journal of Cancer:

E.A. Eisenhauer, P. Therasse, J. Bogaerts, L.H. Schwartz, D. Sargent, R. Ford, J. Dancey, S. Arbuck, S. Gwyther, M. Mooney, L. Rubinstein, L. Shankar, L. Dodd, R. Kaplan, D. Lacombe, J. Verweij. New response evaluation criteria in solid tumors: Revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009 Jan;45(2):228-47.

At baseline, tumor lesions/lymph nodes will be categorized measurable or non-measurable as follows:

Measurable:

- Tumor lesions: Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:
  - 10 mm by CT, MRI or PET scan (slice thickness no greater than 5 mm)
  - 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable)
  - 20 mm by chest X-ray
- Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT, MRI or PET scan (slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable:

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

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

- Clinical lesions: Clinical lesions will only be considered measurable when they are superficial and ≥ 10mm diameter as assessed using calipers (eg, skin nodules). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is suggested. As noted above, when lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study.

- Chest X-ray: Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.
- CT, MRI: CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5mm or less.
- Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement.
- Endoscopy, laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised.
- Tumor markers: Tumor markers alone cannot be used to assess objective tumor response. If markers are initially above the upper normal limit, however, they must normalize for a patient to be considered in complete response.
- Cytology, histology: These techniques can be used to differentiate between PR and CR in rare cases if required by protocol.

Tumor Response Evaluation

Baseline documentation of 'target' and 'non-target' lesions

- When more than one measurable lesion is present at baseline all lesions up to a maximum of 5 lesions total (and a maximum of 2 lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline.
- Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements.
- A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then as noted above, only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Systemic Response Criteria

Complete Response (CR) requires ALL of the following:

- Disappearance of all target and non-target lesions
- All pathological lymph nodes, whether target or non-target, must have reduction in short axis to < 10 mm.
- No new lesion

Partial Response (PR) requires ALL of the following:

- At least 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum of diameters.
- No unequivocal progression of existing non-target lesions
- No new lesion

Progression of Disease (PD) requires ANY of the following:

- At least 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm.
- Unequivocal progression of existing non-target lesions
- Appearance of one or more new lesions

Stable Disease (SD) requires ALL of the following:

- Not CR
- Not PR
- Not PD