Official Title:	Moving PD-1 Blockade with Pembrolizumab into Concurrent Chemoradiation for Locally Advanced Non-Small Cell Lung Cancer (Multi-Center)
NCT number:	NCT02621398
Document Type:	Study Protocol and Statistical Analysis study protocol SAP 2
Date of the Document:	10/21/21

[3]Pathology report confirming diagnosis of NSCLC must be obtained and reviewed by the treating physician before registration to the study

[4]Mandatory submission of unstained slides from an archived tumor block for PD-L1 analysis. Sites must verify that an archived tumor block is available before registration on the trial. Specimen can be obtained 70 days before initiation of chemoradiation treatment.

[5] For women of childbearing potential only. This must be completed and negative within 7 days of registration for protocol therapy.

[6] Correlative blood samples during radiation therapy on weeks 1, 3, 6 for CD8+ Tcells and Tregulatory cells.

[7] First Chest CT scan should be day 15 after the end of chemoradiation +/- 2 weeks. Following assessment with CT Chest every 9 weeks +/-2 weeks during pembrolizumab therapy.

[8] For patients starting pembrolizumab 2 weeks before end of CRT or at the start of CRT.

[9] Correlative samples will be taken during cycles 1, 2, 3 of pembrolizumab

[10] Thyroid screening will occur during day 22+7 days of chemoradiation when subject receives pemrolizumab concurrent with chemoradiation.

[11] Every 12 weeks ±14 days for the first year and every 16 weeks ± 14 days for the second year after treatment completes and every 6 months for years 3-5 and yearly thereafter. PET-CT may be done in place of regular CT at the discretion of the treating investigator.

[12]Serious events and events of clinical interest will be collected for 90 days after the end of treatment with pembrolizumab

[13]Treatment-related adverse events will be followed until resolution, return to baseline, or deemed clinically insignificant. Assessment of AEs includes events of clinical interest and immune related adverse events.

[14]May be done within 3 days of chemotherapy.

7.0 TRIAL PROCEDURES

7.1 Trial Procedures

The Trial Flow Chart - Section 6.0 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

Furthermore, additional evaluations/testing may be deemed necessary by the Sponsor and/or Merck for reasons related to subject safety. In some cases, such evaluation/testing may be potentially sensitive in nature (e.g., HIV, Hepatitis C, etc.), and thus local regulations may require that additional informed consent be obtained from the subject. In these cases, such evaluations/testing will be performed in accordance with those regulations.

7.1.1 Administrative Procedures

7.1.1.1 Informed Consent

The Investigator must obtain documented consent from each potential subject prior to participating in a clinical trial.

7.1.1.1.1 General Informed Consent

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

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

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

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

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

7.1.1.2 Inclusion/Exclusion Criteria

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

7.1.1.3 Medical History

A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the Investigator. Details regarding the disease for which the subject has enrolled in this study will be recorded separately and not listed as medical history.

7.1.1.4 Prior and Concomitant Medications Review

7.1.1.4.1 Prior Medications

The investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the subject within 28 days before starting the trial. Treatment for the disease for which the subject has enrolled in this study will be recorded separately and not listed as a prior medication.

7.1.1.4.2 Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the subject during the trial. All medications related to reportable SAEs and ECIs should be recorded as defined in Section 7.2.

7.1.1.5 Disease Details and Treatments

7.1.1.5.1 Disease Details

The investigator or qualified designee will obtain prior and current details regarding disease status.

7.1.1.5.2 Prior Treatment Details

The investigator or qualified designee will review all prior cancer treatments including systemic treatments, radiation and surgeries.

7.1.1.5.3 Subsequent Anti-Cancer Therapy Status

The investigator or qualified designee will review all new anti-neoplastic therapy initiated after the last dose of trial treatment. If a subject initiates a new anti-cancer therapy within 30 days

after the last dose of trial treatment, the 30 day Safety Follow-up visit must occur before the first dose of the new therapy. Once new anti-cancer therapy has been initiated the subject will move into survival follow-up.

7.1.1.6 Assignment of Subject Study Identification Number

A copy of the institution's IRB-approved informed consent document and written justification for any changes made to the informed consent for this protocol will be on file at F[redacted] bef[redacted]

Sites will contact the [redacted] OHRS Registration Desk ([redacted] to register subjects. Registration/Enrollment process:

- Registration: Any subject that has signed the consent will be assigned a unique patient study number. Site will contact OHRS and send a copy of the de-identified consent. OHRS will provide site with the subject study number. The patient will not be identified by name.

- Enrollment: Once eligibility has been confirmed, the completed, signed and dated eligibility checklist will be sent to OHRS with additional source documents, if requested. OHRS Registration Desk will enroll the subject to the study and send a confirmatory email to the site. This is the point that the patient is considered on study.

- **Patients will <u>not</u> start protocol treatment prior to enrollment.**

7.1.1.7 Trial Compliance (Medication/Diet/Activity/Other)

Interruptions from the treatment plan for >12 weeks between pembrolizumab doses require consultation between the investigator and Sponsor and written documentation of the collaborative decision on the management of the subject.

Administration of the pembrolizumab will be witnessed by the investigator and/or trial staff. The total volume of pembrolizumab infused will be compared to the total volume prepared to determine compliance to each dose administered.

7.1.2 Clinical Procedures/Assessments

7.1.2.1 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 4.0 (see Section 11.2). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

For subjects receiving treatment with pembrolizumab 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): see the separate ECI guidance document regarding the identification, evaluation and management of potential irAEs.

Please refer to section 7.2 for detailed information regarding the assessment and recording of AEs.

7.1.2.2 Full Physical Exam

The investigator or qualified designee will perform a complete physical exam during the screening period. Clinically significant abnormal findings should be recorded as medical history. A full physical exam should be performed during screening.

7.1.2.3 Directed Physical Exam

For cycles that do not require a full physical exam per the Trial Flow Chart, the investigator or qualified designee will perform a directed physical exam as clinically indicated prior to trial treatment administration.

7.1.2.4 Vital Signs

The investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment and at treatment discontinuation as specified in the Trial Flow Chart (Section 6.0). Vital signs should include temperature, pulse, respiratory rate, pulse oximetry both walking and resting, weight and blood pressure. Height will be measured at screening only.

7.1.2.5 Eastern Cooperative Oncology Group (ECOG) Performance Scale

The investigator or qualified designee will assess ECOG status (see Section 11.1) at screening, prior to the administration of each dose of trial treatment and discontinuation of trial treatment as specified in the Trial Flow Chart.

7.1.2.6 Tumor Imaging and Assessment of Disease

Response assessments will be made both using the Immune Related Response Criteria, and using RECIST v1.1, allowing additional comparisons among these criteria for disease response assessment. The same measurable and non-measurable lesions will be followed by both RECIST v1.1 & irRC. RECIST 1.1 will be used to determine the primary objective of the trial and RECIST 1.1 and Immune Related Response Criteria will be used in secondary objective of PFS.

7.1.2.6.1 Immune Related Response Criteria:

This study will use the Immune Related Response Criteria (irRC). These response criteria were developed to overcome the variable and unusual patterns of response to immunotherapeutic agents, in particular, ipilimumab [24]. The development of the guidelines were prompted by observations, mostly in patients with metastatic melanoma, of initial disease progression followed by later response, late responses, and mixed responses with an overall decrease in tumor burden.

7.1.2.6.2 Antitumor response based on total measurable tumor burden

For the irRC, only index and measurable new lesions are taken into account (in contrast to conventional WHO criteria, which do not require the measurement of new lesions, nor do they include new lesion measurements in the characterization of evolving tumor burden). At the baseline tumor assessment, the sum of the products of the two largest perpendicular diameters (SPD) of all index lesions (five lesions per organ, up to 10 visceral lesions) is calculated. At each subsequent tumor assessment, the SPD of the index lesions and of new, measurable lesions (up to 5 new lesions per organ; 10 visceral lesions) are added together to provide the total tumor burden: Tumor Burden = SPDindex lesions + SPDnew, measurable lesions.

Table 7: Comparison between WHO criteria and the irRC criteria

	WHO	irRC
New, measurable lesions (ie ≥5 x 5 mm)	Always represent PD	Incorporated into tumor burden
New, nonmeasurable lesions (ie < 5x 5 mm)	Always represent PD	Do not define progression (but preclude irCR)
Non-index lesions	Changes contribute to defining BOR of CR, PR, SD, and PD	Contribute to defining irCR (complete disappearance required)
CR	Disappearance of all lesions in two consecutive observations not less than 4 wk apart	Disappearance of all lesions in two consecutive observations not less than 4 wk apart
PR	≥50% decrease in SPD of all index lesions compared with	≥50% decrease in tumor burden compared with

	baseline in two observations at least 4 wk apart, in absence of new lesions or unequivocal progression of non-index lesions	baseline in two observations at least 4 wk apart
SD	50% decrease in SPD compared with baseline cannot be established nor 25% increase compared with nadir, in absence of new lesions or unequivocal progression of non-index lesions	50% decrease in tumor burden compared with baseline cannot be established nor 25% increase compared with nadir
PD	At least 25% increase in SPD compared with nadir and/or unequivocal progression of non-index lesions and/or appearance of new lesions (at any single time point	At least 25% increase in tumor burden compared with nadir (at any single time point) in two consecutive observations at least 4 wk apart

7.1.2.6.3 Time-point response assessment using irRC

Percentage changes in tumor burden per assessment time point describe the size and growth kinetics of both conventional and new, measurable lesions as they appear. At each tumor assessment, the response in index and new, measurable lesions is defined based on the change in tumor burden (after ruling out irPD). Decreases in tumor burden must be assessed relative to baseline measurements (i.e., the SPD of all index lesions at screening). The irRC were derived from WHO criteria and, therefore, the thresholds of response remain the same. However, the irRC response categories have been modified from those of WHO criteria as detailed.

7.1.2.6.4 Overall response using the irRC

The sum of the products of diameters at tumor assessment using the immune-related response criteria (irRC) for progressive disease incorporates the contribution of new measurable lesions. Each net Percentage Change in Tumor Burden per assessment using irRC criteria accounts for the size and growth kinetics of both old and new lesions as they appear.

Definition of Index Lesions Response Using irRC

- **irComplete Response (irCR):** Complete disappearance of all *index* lesions. This category encompasses exactly the same subjects as "CR" by the mWHO criteria

- **irPartial Response (irPR):** Decrease, relative to baseline, of 50% or greater in the sum of the products of the two largest perpendicular diameters of all *index* and all new measurable lesions (ie., Percentage Change in Tumor Burden). Note: the appearance of new measurable lesions is factored into the overall tumor burden, but does not automatically qualify as progressive disease until the SPD increases by ≥25% when compared to SPD at nadir.
- **irStable Disease (irSD):** Does not meet criteria for irCR or irPR, in the absence of progressive disease.
- **irProgressive Disease (irPD):** At least 25% increase Percentage Change in Tumor Burden (i.e., taking sum of the products of all *index* lesions and any new lesions) when compared to SPD at nadir.

Definition of Non-Index Lesions Response Using irRC

- **irComplete Response (irCR):** Complete disappearance of all *non-index* lesions. This category encompasses exactly the same subjects as "CR" by the mWHO criteria.
- **irPartial Response (irPR) or irStable Disease (irSD):** *non-index* lesion(s) are not considered in the definition of PR, these terms do not apply.
- **irProgressive Disease (irPD):** Increases in number or size of *non-index* lesion(s) does not constitute progressive disease unless/until the Percentage Change in Tumor Burden increases by 25% (i.e., the SPD at nadir of the index lesions increases by the required amount).

Impact of New Lesions on irRC

New lesions in and by themselves do not qualify as progressive disease. However their contribution to total tumor burden is included in the SPD which in turn feeds into the irRC criteria for tumor response. Therefore, new non-measurable lesions will not discontinue any subject from the study.

7.1.2.6.5 Definition of Overall Response Using irRC

Overall response using irRC will be based on these criteria (see Table 9):

- **Immune-Related Complete Response (irCR):** Complete disappearance of *all* tumor lesions (index and nonindex together with no new measurable/unmeasurable lesions) for at least 4 weeks from the date of documentation of complete response.
- **Immune-Related Partial Response (irPR):** The sum of the products of the two largest perpendicular diameters of all index lesions is measured and captured as the SPD baseline. At each subsequent tumor assessment, the sum of the products of the two largest perpendicular diameters of all index lesions and of new measurable lesions are added together to provide the Immune Response Sum of Product Diameters (irSPD). A decrease, relative to baseline of the irSPD compared to the previous SPD baseline, of 50% or greater is considered an immune Partial Response

(irPR).

- **Immune-Related Stable Disease (irSD):** irSD is defined as the failure to meet criteria for immune complete response or immune partial response, in the absence of progressive disease.
- **Immune-Related Progressive Disease (irPD):** It is recommended in difficult cases to confirm PD by serial imaging. Any of the following will constitute progressive disease:
- At least 25% increase in the sum of the products of all index lesions over nadir SPD calculated for the index lesions.
- At least a 25% increase in the sum of the products of all index lesions and new measurable lesions (irSPD) over the baseline SPD calculated for the index lesions.

Immune-Related Best Overall Response Using irRC (irBOR)

irBOR is the best confirmed irRC overall response over the study as a whole, recorded between the date of first dose until the last tumor assessment before subsequent therapy (except for local palliative radiotherapy for painful bone lesions) for the individual subject in the study. For the assessment of irBOR, all available assessments per subject are considered. irCR or irPR determinations included in the irBOR assessment must be confirmed by a second (confirmatory) evaluation meeting the criteria for response and performed no less than 4 weeks after the criteria for response are first met.

Table 8: Derivation of irRC overall responses

Measurable response	Nonmeasurable response	Nonmeasurable response	Overall response
Index and new, measurable lesions (tumor burden)*,%	Non-index lesions	New, nonmeasurable lesions	Using irRC
↓100	Absent	Absent	irCR†
↓100	Stable	Any	irPR†
↓100	Unequivocal progression	Any	irPR†
↓≥50	Absent/stable	Any	irPR†
↓≥50	Unequivocal progression	Any	irPR†

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IRB ID:
Approval Date:
Expiration Date:

↓<50 to <25↑	Absent/stable	Any	irSD
↓<50 to <25↑	Unequivocal progression	Any	irSD
≥25	Any	Any	irPD†

*Decreases assessed relative to baseline, including measurable lesions only (>5 × 5 mm).

†Assuming response (irCR) and progression (irPD) are confirmed by a second, consecutive assessment at least 4 wk apart.

7.1.2.6.6 Method of assessment

The same method of assessment and the same technique will be used whenever possible to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment.

Chest x-ray- Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI- These techniques will be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT will be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis.
Ultrasound (US)- Because one of the endpoints of the study is objective response evaluation, US will not be used to measure tumor lesions. US might be used, at the discretion of the investigator, to confirm the complete disappearance of superficial lesions assessed by clinical examination.

Cytology, histology- These techniques may be used to differentiate between partial responses (PR) and complete responses (CR) if necessary and determined by the investigator. Cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

7.1.2.6.7 Definitions Associated with Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1

Measurable disease
The presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

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

Non-measurable lesions
All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥ 10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.
Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

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

Malignant lymph nodes
To be considered pathologically enlarged and measurable, a lymph node must be >15 mm in short axis when assessed by CT scan (CT 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.

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

7.1.2.6.8 Evaluation of Target Lesions: Table 9

Complete Response (CR):	Disappearance of all target lesions
Partial Response (PR):	At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD.
Progressive Disease (PD):	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.
Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started.

7.1.2.6.9 Evaluation of Non-Target Lesions: Table 10

Complete Response (CR):	Disappearance of all non-target lesions and *normalization of tumor marker level.
Incomplete Response/ Stable Disease (SD):	Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.
Progressive Disease (PD):	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions.
Although a clear progression of "non-target" lesions only is exceptional, in such circumstances the opinion of the investigator will prevail.	
*Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.	

7.1.2.6.10 Evaluation of Best Overall Response: Table 11

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

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Notes:

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration." Every effort will be made to document the objective progression.

In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, the residual lesion will be investigated (fine needle aspirate/biopsy if possible) before confirming the complete response status.

7.1.2.7 Tumor Tissue Collection and Correlative Studies Blood Sampling

Mandatory submission of unstained slides from an archived tumor block for PD-L1 analysis. Sites must verify that an archived tumor block is available to obtain unstained slides from prior to registration to the trial. Unstained slides submitted for PD-L1 staining must be cut and sent within 7 days of analysis. A fresh biopsy is NOT mandatory to participate in this trial.

A fine needle aspirate, frozen sample, plastic embedded sample, cell block, clot, bone, bone marrow or cytologic specimen are not be acceptable for PD-L1 analysis.
Please refer to the Study Procedures Manual for processing, labeling and shipping instructions.

7.1.3 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below. Laboratory Safety Evaluations (Hematology, Chemistry and Urinalysis)

Laboratory tests for hematology, chemistry, and others are specified in Table 5.

Table 12 Laboratory Tests

Hematology	Chemistry		Other
Hematocrit	Albumin		Serum β-human chorionic gonadotropin†
Hemoglobin	Alkaline phosphatase		(β-hCG)†
Platelet count	Alanine aminotransferase (ALT)		PT (INR)
WBC (total and differential)	Aspartate aminotransferase (AST)		aPTT
Red Blood Cell Count	Lactate dehydrogenase (LDH)		Total thriiodothyronine (T3)
Absolute Neutrophil Count	Carbon Dioxide ‡		Free tyroxine (T4)
Absolute Lymphocyte Count	*(CO_2 or biocarbonate)*	Urine or serum pregnancy test †	Thyroid stimulating hormone (TSH)
	Uric Acid		PK
	Calcium		
	Chloride		Blood for correlative studies
	Glucose		
	Phosphorus		
	Potassium		
	Sodium		
	Magnesium		
	Total Bilirubin		
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*		
	Total protein		
	Blood Urea Nitrogen		

† Perform on women of childbearing potential only.

‡ If considered standard of care in your region.

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Approval Date:
Expiration Date:

Laboratory tests for screening or entry into the Second Course Phase should be performed within 10 days prior to the first dose of treatment. After Cycle 1, pre-dose laboratory procedures can be conducted up to 72 hours prior to dosing. Results must be reviewed by the investigator or qualified designee and found to be acceptable prior to each dose of trial treatment.

7.1.3.1 Pharmacokinetic/Pharmacodynamic Evaluations

As PK data have previously been examined in other clinical trials, we do not anticipate the need to perform PK testing unless otherwise directed by the Sponsor.

7.1.3.1.1 Blood Collection for Serum Pembrolizumab

Sample collection, storage and shipment instructions for serum samples will be provided in the Laboratory Manual.

The timepoints for PK blood sampling are described in Section 6 – Trial Flow Chart.

7.1.3.1.2 Blood Collection for Anti-Pembrolizumab Antibodies

Sample collection, storage and shipment instructions for blood samples will be provided in the Laboratory Manual.

7.1.4 Other Procedures

7.1.4.1 Withdrawal/Discontinuation

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 7.2 - Assessing and Recording Adverse Events. Subjects who a) attain a CR or b) complete 36 doses of treatment with pembrolizumab may discontinue treatment with the option of restarting treatment if they meet the criteria specified in Section 7.1.5.5. After discontinuing treatment following assessment of CR, these subjects should return to the site for a Safety Follow-up Visit (described in Section 7.1.5.3.1) and then proceed to the Follow-Up Period of the study (described in Section 7.1.5.4).

7.1.4.2 Blinding/Unblinding

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

7.1.5 Visit Requirements

Visit requirements are outlined in Section 6.0 - Trial Flow Chart. Specific procedure-related details are provided above in Section 7.1 - Trial Procedures.

7.1.5.1 Screening

Approximately 28 days before beginning treatment, potential subjects will be evaluated to determine if they fulfill the eligibility criteria. Tests performed before the subject signs consent are permitted as long as they are performed within the appropriate time frame as per the trail flow chart. Subjects may be rescreened after failing to meet the inclusion/exclusion criteria. Please seen the trial flow chart is in Section 6.1.

7.1.5.1.1 Screening Period

The screening period may last from 1-28 days as long as the subject is able to have necessary testing completed in the appropriate time frames.

7.1.5.2 Treatment Period

Section 6.1 details the treatment period, consisting of 6 weeks of chemoradiation therapy and then initiation of the pembroliumab every 3 weeks for up to 18 cycles. Pembrolizumab will be incorporated during chemoradiation by the phase I trial.

7.1.5.3 Post-Treatment Visits

7.1.5.3.1 Safety Follow-Up Visit

The mandatory Safety Follow-Up Visit should be conducted approximately 30 days after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever comes first. All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Subjects with an AE of Grade > 1 will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-neoplastic therapy, whichever occurs first. SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded. Subjects who are eligible for retreatment with pembrolizumab (as described in Section 7.1.5.5) may have up to two safety follow-up visits, one after the Treatment Period and one after the Second Course Phase.

7.1.5.4 Follow-up Visits

Subjects who discontinue trial treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed every 12 weeks (± 2 weeks) by radiologic imaging to monitor disease status. After 1 year, the imaging time point will occur every 16 weeks (± 2 weeks). Every effort should be made to collect information regarding disease status until the start of new anti-neoplastic therapy, disease progression, death, end of the study or if the subject begins retreatment with pembrolizumab as detailed in Section 7.1.5.5. Information regarding post-study anti-neoplastic treatment will be collected if new treatment is initiated.

Subjects who are eligible to receive retreatment with pembrolizumab according to the criteria in Section 7.1.5.5 will move from the follow-up phase to the Second Course Phase when they

experience disease progression. Details are provided in Section 7.1.5.5 – and the Study Flow Chart (Table 6) for Retreatment.

7.1.5.4.1 Survival Follow-up

Once a subject experiences confirmed disease progression or starts a new anti-cancer therapy, the subject moves into the survival follow-up phase and should be contacted by telephone every 12 weeks to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.

7.1.5.5 Second Course Phase (Retreatment Period)

Subjects who stop pembrolizumab with SD or better may be eligible for up to one year of additional pembrolizumab therapy if they progress after stopping study treatment. This retreatment is termed the Second Course Phase of this study and is only available if the study remains open and the subject meets the following conditions:

- **Either**
  - Stopped initial treatment with pembrolizumab after attaining an investigator-determined confirmed CR according to RECIST 1.1, and
    - Was treated for at least 24 weeks with pembrolizumab before discontinuing therapy
    - Received at least two treatments with pembrolizumab beyond the date when the initial CR was declared

      OR
  - Had SD, PR or CR and stopped pembrolizumab treatment after 18 doses of study therapy for reasons other than disease progression or intolerability

AND

- Experienced an investigator-determined confirmed radiographic disease progression after stopping their initial treatment with pembrolizumab
- Did not receive any anti-cancer treatment since the last dose of pembrolizumab
- Has a performance status of 0 or 1 on the ECOG Performance Scale
- Demonstrates adequate organ function as detailed in Section 5.1.2

- Female subject of childbearing potential should have a negative serum or urine pregnancy test within 72 hours prior to receiving retreatment with study medication.
- Female subject of childbearing potential should be willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication (Reference Section 5.7.2). Subjects of child bearing potential are those who have not been surgically sterilized or have been free from menses for > 1 year.
- Male subject should agree to use an adequate method of contraception starting with the first dose of study therapy through 120 days after the last dose of study therapy.
- Does not have a history or current evidence of any condition, therapy, or laboratory abnormality that might interfere with the subject's participation for the full duration of the trial or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

Subjects who restart treatment will be retreated at the same dose and dose interval as when they last received pembrolizumab. Treatment will be administered for up to one additional year.

Visit requirements are outlined in Section 6.0 – Trial Flow Chart.

7.2 Assessing and Recording Adverse Events

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product 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 (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the Merck's product, is also 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, but are not limited to, teething, typical crying in infants and children and onset of menses or menopause occurring at a physiologically appropriate time.

[redacted] protocol-specified procedure, whether investigational (including placebo or active comparator medication) or marketed, manufactured by, licensed by, provided by or distributed by [redacted] human use.

[redacted] IRB
IRB ID:
Approval Date:
Expiration Date:

Adverse events may occur during the course of the use of [redacted] product in clinical trials or within the follow-up period specified by the protocol, or prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

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

Progression of the cancer under study is not considered an adverse event unless it is considered to be drug related by the investigator.

All adverse events will be recorded from the time the consent form is signed through 30 days following cessation of treatment and at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in section 7.2.3.1.

7.2.1 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 will be defined as any dose of 1,000 mg or greater (≥5 times 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) is reported as a serious adverse event, even if no other seriousness criteria are met.

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

All reports of overdose with and without an adverse event must be reported within 24 hours to the Sponsor and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

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

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them), including the pregnancy of a male subject's female partner that occurs during the trial or within 120 days of completing the trial completing the trial, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever [redacted]

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

Such events must be reported within 24 hours to the Sponsor and within 2 working days to [redacted]

7.2.3 Immediate Reporting of Adverse Events to the Sponsor and to Merck

7.2.3.1 Serious Adverse Events

A serious adverse event is any adverse event occurring at any dose or during any use of Merck's product that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose;
- Is an other important medical event

Refer to Table 6 for additional details regarding each of the above criteria.

Progression of the cancer under study is not considered an adverse event unless it results in hospitalization or death.

Any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any subject from the time the consent is signed through 90 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to [redacted] must be reported within 24 hours to the Sponsor-Investigator (report [redacted] with MedWatch 3500A form attached, please see DCP for additional reporting direction). The Sponsor-Investigator will report event to [redacted] within 24 hours of becoming aware of event.

Non-serious Events of Clinical Interest will be forwarded to [redacted] and will be handled in the same manner as SAEs.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to [redacted] that is brought to the attention of the investigator at

[redacted]

any time outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor and to [redacted]

SAE reports and any other relevant safety information are to be forwarded to the [redacted] Global Safety facsimile number: [redacted]

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 will cross reference this submission according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally investigators will submit a copy of these reports to [redacted] at the time of submission to FDA. Sponsor-Investigator is responsible for communication to the FDA.

All subjects with serious adverse events must be followed up for outcome.

7.2.3.2 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be recorded as such on the Adverse Event case report forms/worksheets and reported within 24 hours to the Sponsor-Investigator (report to RCINJ via OnCore with MedWatch 3500A form attached, please see DCP for additional reporting direction). The Sponsor-Investigator will report event to [redacted] hours of becoming aware of event(At [redacted] ts of clinical interest for this trial inclu[redacted]

1. An overdose of [redacted] uct, as defined in Section 7.2.1 - 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 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

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

3. Additional adverse events:

A separate guidance document has been provided entitled "Event of Clinical Interest Guidance Document" (previously entitled, "Event of Clinical Interest and Immune-Related Adverse Event Guidance Document"). This document provides guidance regarding identification, evaluation and management of ECIs and irAEs.

ECIs (both non-serious and serious adverse events) identified in this guidance document from the date of first dose through 90 days following cessation of treatment, or 30 days after the initiation of a new anticancer therapy, whichever is earlier, need to be reported within 24 hours to the Sponsor and within 2 working days to [redacted]), regardless of attribution to study treatment, consistent with standard SAE reporting guidelines.

Subjects should be assessed for possible ECIs prior to each dose. Lab results should be evaluated and subjects should be asked for signs and symptoms suggestive of an immune-related event. Subjects who develop an ECI thought to be immune-related should have additional testing to rule out other etiologic causes. If lab results or symptoms indicate a possible immune-related ECI, then additional testing should be performed to rule out other etiologic causes. If no other cause is found, then it is assumed to be immune-related.

7.2.4 Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 4.0. Any adverse event which changes CTCAE grade over the course of a given episode will have each change of grade recorded on the adverse event case report forms/worksheets.

All adverse events regardless of CTCAE grade must also be evaluated for seriousness.

Table 13 Evaluating Adverse Events

An investigator who is a qualified physician, will evaluate all adverse events as to:

V4.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.
	Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; **disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of Merck product that	
	†Results in death; or	
	†Is life threatening; or places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization [including hospitalization for an elective procedure] for a preexisting condition which has not worsened does not constitute a serious adverse event.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	Is a new cancer; (that is not a condition of the study) or	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours.	
	Other important medical events that 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 subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause the [redacted]roduct to be discontinued?	
Relationship to test drug	Did the Merck product cause the adverse event? The determination of the likelihood that the Merck product caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between the [redacted]roduct and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely the Merck pr[redacted]sed the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to the Merck product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of the Merck product? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

[redacted]
IRB
IRB ID:
Approval Date:
Expiration Date:

Relationship to [redacted] product (continued)	**The following components are to be used to assess the relationship between the test drug and the AE: (continued)**	
	Dechallenge	Was the [redacted] AE resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the Merck product; or (3) the trial is a single-dose drug trial); or (4) [redacted] (s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to the [redacted] in this study? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial); or (3) [redacted] oduct(s) is/are used only one time). NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY THE [redacted] PRODUCT, OR IF REEXPOSURE TO THE M[redacted] PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE U.S. CLINICAL MONITOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding the M[redacted] product or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following	**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of a [redacted] product relationship).**	
Yes, there is a reasonable possibility of [redacted] relationship.	There is evidence of exposure to the [redacted] explained by the Merck product than by another cause.	
No, there is not a reasonable possibility [redacted] k product relationship	Subject did not receive the [redacted] product OR temporal sequence of the AE onset relative to administration of the [redacted] k product is not reasonable OR there is another obvious cause of the AE. (Also entered for a subject with overdose without an associated AE.)	

[redacted] IRB
IRB ID:
Approval Date:
Expiration Date:

7.2.5 Sponsor Responsibility for Reporting Adverse Events

All Adverse Events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations.

8.0 STATISTICAL ANALYSIS PLAN

8.1 Statistical Analysis Plan Summary

A schema to evaluate the safety of administering pembrolizumab with chemoradiation (CRT) is provided below. Successive regimens have progressively longer concurrent treatment times going from post radiation, to 2 weeks overlap, to 6 week overlap if tolerated or increase MK-3475 from 1 MGQ 3 WEEKS to 2 mg Q 3 WEEKS. The safety is evaluated through dose limiting toxicity (DLT) consisting of grade 4 pneumonitis. The 3+3 design with dose de-escalation and starting at Regimen 1 will be used to find the maximum tolerated dose (MTD) regimen. After MTD is found, six more patients will be treated at MTD dose regimen for a better evaluation of the safety of the dosing schedule for further study. The details of the 3+3 design with dose de-escalation are shown below.

Day 0 is the start day for chemoradiation (CRT)

Day 42 is the presumed last day of chemoradiation

Table 4: Trial Design

START of Pembrolizumab	Day of starting Pembrolizumab	Pembrolizumab Dose	Regimen
2-6 WEEKS AFTER CRT	Day 56-84	100 mg Q3 WEEKS	-1
2-6 WEEKS AFTER CRT	Day 56-84	200 mg Q3WEEKS	1
2 WEEKS BEFORE END OF CRT	Day 28	100 mg Q3WEEKS	2
2 WEEKS BEFORE END OF CRT	Day 28	200 mg Q3WEEKS	3
AT START OF CRT	Day 0	100 mg Q3WEEKS	4
At start of CRT	Day 0	200 mg Q3WEEKS	5

With starting dose regimen 1, groups of 3 patients will be entered at a dose level:

- If all 3 patients treated at the dose regimen do not have a dose limiting toxicity (DLT), then the dose will be escalated to the next dose regimen.
- If 1/3 patients have DLT, then 3 more patients will be treated at this dose level. If none of these additional patients has DLT, then the dose will be escalated, otherwise three more patients are treated at the prior dose regimen (if at most 3 patients were previously treated at that prior dose regimen).
- If at least 2/3 patients have DLT, then three more patients are treated at the prior dose regimen (if at most 3 patients were previously treated at that prior dose regimen).

The MTD is the dose regimen that 0/6 (or 0/3 if at dose level –1) or 1/6 patient experience DLT; and at least 2/3 or 2/6 patients treated with the next higher dose regiment will have had DLT.

Note: If the escalation occurs at the last dose regimen (Regimen 5), then the MTD is at or above the last dose regimen. If the de-escalation occurs at dose regimen -1 (at least 2 out of 3 patients or at least 2 out of 6 patients have DLT at dose regimen -1), then the MTD is below the dose regimen -1. In either case, the MTD is not determined from the trial. But for the former, the last regimen (Regimen 5) is safe and still can be used safely for further studies.

8.2 Statistical Analysis Plan

The study design is shown in Study Design/Clinical Plan Section. Table 1 gives three different scenarios for the probabilities of toxicity at the dose regimens. In the first scenario, we assume dose-toxicity rates such that the rate is 5% at dose level –1 and increases gradually to 50% at dose regimen 5. In the second scenario, we assume dose-toxicity rates such that the rate is 12% at dose regimen -1 and 50% at dose regimen 5. In the third scenario, the dose-toxicity rate is 3% at dose regimen -1 and increases slowly to 50% at dose regimen 5.

Table 14. Three scenarios of probabilities of toxicity at dose levels

Dose Regimen	First Scenario of Probability of Toxicity	Second Scenario of Probability of Toxicity	Third Scenario of Probability of Toxicity
-1	0.05	0.12	0.03
1*	0.08	0.15	0.04
2	0.15	0.21	0.06
3	0.24	0.28	0.12
4	0.36	0.38	0.26
5	0.50	0.50	0.50

*: Starting dose level

For the secondary objective, Kaplan Meier estimation method is used to estimate the rates of local and distant metastasis-free survival, progression-free survival, and overall survival. The comparisons to the historical controls are performed using one-sample log-rank test. Local and distant metastasis-free survival is defined as the event without recurrence within the radiation field (i.e. local - planning target volume) and outside of the radiation field (i.e. distance - in other parts of the lung) or recurrence in other organs. Progression-free survival is the event without sign of any progression either locally or distantly, measured from the initiation of chemoradiation. Overall survival is the overall longevity of the patient from time of initiation of chemoradiation to time of death.

From Table 1, the probability that a dose regimen will be declared as the MTD is calculated in Table 2. Also included in Table 2 is the target toxicity level (i.e., the probability of toxicity at the MTD, if MTD is determined in the given dose regimen.)

Table 2 shows that most likely the regimen 2 will be declared as MTD under the first toxicity model scenario. For Scenarios 2, Regimens 1 and 2 are likely to be the MTD. For Scenario 3, Regimen 3 is likely to be the MTD. There is less than a 10% chance that the MTD cannot be determined from the trial in all the scenarios (3.3%, 4.4% and 8.2% for the 1st, 2nd and 3rd scenarios, respectively).

Table 15: Three scenarios of P(DLT) at each dose level and probability of MTD for each dose level

Dose level (i)	-1	1*	2	3	4	5
P(DLT at di)						
8.3 Scenario 1	0.05	0.08	0.15	0.24	0.36	0.50
Scenario 2	0.12	0.15	0.21	0.28	0.38	0.50
Scenario 3	0.03	0.04	0.06	0.12	0.26	0.50
P(declare MTD = Regimen i)						
8.4 Scenario 1	0.06	0.19	0.31	0.29	0.12	0.00
Scenario 2	0.17	0.27	0.27	0.19	0.07	0.00
Scenario 3	0.02	0.04	0.13	0.38	0.35	0.00
P(MTD< Regimen 1) =0.002, P(MTD≥ Regimen 5) =0.031; TTL = 18.3% for 1st scenario						
P(MTD< Regimen 1) =0.026, P(MTD≥ Regimen 5) =0.018; TTL = 20.2% for 2nd scenario						
P(MTD< Regimen 1) =0.0002, P(MTD≥ Regimen 5) =0.082; TTL = 15.9 % for 3rd scenario						

Table 3 shows that, on average, we expect to treat 14-19 patients and observe 3-4 incidences of DLT (≈16.7-22.7%) under the three toxicity model scenarios. The maximum number of patients in the study is 42 (=6*6+6) if 6 patients are used in all the dose regiments.

Table 16: Expected number of patients and DLT incidences at each dose

Dose level (i)	-1	1*	2	3	4	5	Total (%)
Scenario 1							

8.5 P(DLT at di)	0.05	0.08	0.15	0.24	0.36	0.50	
E(# of patients)	0.2	4.1	4.5	4.0	2.4	0.7	15.8
E(DLT incidences)	0.01	0.33	0.67	0.94	0.85	0.37	3.17 (20.1)
Scenario 2							
8.6 P(DLT at di)	0.12	0.15	0.21	0.28	0.38	0.50	
E(# of patients)	0.7	4.6	4.0	2.9	1.5	0.4	14.1
E(DLT incidences)	0.08	0.69	0.85	0.80	0.56	0.21	3.19 (22.7)
Scenario 3							
8.7 P(DLT at di)	0.03	0.04	0.06	0.12	0.26	0.50	
E(# of patients)	0.1	3.4	3.8	4.6	4.4	2.0	18.1
E(DLT incidences)	0.00	0.14	0.23	0.55	1.13	0.98	3.03 (16.7)

9.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

9.1 Investigational Product

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.

Clinical Supplies will be provided by Merck as summarized in Table 7.

Table 17 Product Descriptions

Product Name & Potency	Dosage Form
Pembrolizumab 50 mg	Lyophilized Powder for Injection
Pembrolizumab 100 mg/ 4mL	Solution for Injection

9.2 Packaging and Labeling Information

Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

9.3 Clinical Supplies Disclosure

This trial is open-label; therefore, the subject, 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.

9.4 Storage and Handling Requirements

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label.

Receipt and dispensing of trial medication must 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.

9.5 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 subjects 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.

10.0 ADMINISTRATIVE AND REGULATORY DETAILS

10.1 Confidentiality

The Principal Investigator will ensure that subject privacy and confidentiality of the subject's data will be maintained.

To protect privacy, every reasonable effort will be made to prevent undue access to subjects during the course of the study. All research related interactions with the participant will be

conducted by qualified research staff who are directly involved in the conduct of the research study.

To protect confidentiality, subject files in paper format will be stored in secure cabinets under lock and key accessible only by the research staff. Electronic records of subject data will be maintained using a dedicated web-access secure database, which is housed in an encrypted and password protected server behind the [redacted] firewall. Access to electronic databases will be limited to delegated personnel. The security and viability of the IT infrastructure will be managed by the Office of Human Research Services Bioinformatics team and [redacted] IT department.

Upon completion of the study, research records will be archived and handled per institutional policy.

Subject names or identifiers will not be used in reports, presentations at scientific meetings, or publications in scientific journals.

10.2 Compliance with Financial Disclosure Requirements

The Principal Investigator and Sub-Investigators must comply with applicable federal, state, and local regulations regarding reporting and disclosure of conflict of interest. Conflicts of interest may arise from situations in which financial or other personal considerations have the potential to compromise or bias professional judgment and objectivity. Conflicts of interest include but are not limited to royalty or consulting fees, speaking honoraria, advisory board appointments, publicly-traded or privately-held equities, stock options, intellectual property, and gifts.

The [redacted] reviews and manages research-related conflicts of interest. The Principal Investigator and Sub-Investigators must report conflicts of interest annually and within 30 days of a change in status, and when applicable, must have a documented management plan that is developed in conjunction with the [redacted] approved by the IRB/IEC.

10.3 Compliance with Law, Audit and Debarment

By signing this protocol, the investigator agrees to conduct the trial in an efficient and diligent manner and in conformance with this protocol; generally accepted standards of Good Clinical Practice (e.g., International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Good Clinical Practice: Consolidated Guideline and other generally accepted standards of good clinical practice); and all applicable federal, state and local laws, rules and regulations relating to the conduct of the clinical trial.

All revisions to the protocol must be discussed with, and be prepared by, [redacted]. The investigator should not implement any deviation or change to the protocol without prior review and documented approval/favorable opinion from the IRB of an amendment, except where necessary to eliminate an immediate hazard(s) to study subjects.

If a deviation or change to a protocol is implemented to eliminate an immediate hazard(s) prior to obtaining IRB approval/favorable opinion, as soon as possible the deviation or change will be submitted to:

- IRB for review and approval/favorable opinion
- [redacted]
- Regulatory Authority(ies), if required by local regulations

Documentation of approval signed by the chairperson or designee of the IRB(s)/IEC(s) must be sent to [redacted] of Human Research Services.

If an amendment substantially alters the study design or increases the potential risk to the subject: (1) the consent form must be revised and submitted to the IRB for review and approval/favorable opinion: (2) the revised form must be used to obtain consent from subjects currently enrolled in the study if they are affected by the amendment; and (3) the new form must be used to obtain consent from new subjects prior to enrollment.

If the revision is done via an administrative letter, investigators must inform their IRB.

The investigator also agrees to allow monitoring, audits, IRB/ERC review and regulatory authority inspection of trial-related documents and procedures and provide for direct access to all trial-related source data and documents.

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

The investigator shall prepare and maintain complete and accurate trial documentation in compliance with Good Clinical Practice standards and applicable federal, state and local laws, rules and regulations; and, for each subject participating in the trial, provide all data, and, upon completion or termination of the clinical trial, submit any other reports to the Sponsor as required by this protocol or as otherwise required pursuant to any agreement with the Sponsor.

Trial documentation will be promptly and fully disclosed to the Sponsor by the investigator upon request and also shall be made available at the trial site upon request for inspection, copying, review and audit at reasonable times by representatives of the Sponsor or any regulatory authorities. The investigator agrees to promptly take any reasonable steps that are

requested by the Sponsor as a result of an audit to cure deficiencies in the trial documentation and worksheets/case report forms. The investigator must maintain copies of all documentation and records relating to the conduct of the trial in compliance with all applicable legal and regulatory requirements. This documentation includes, but is not limited to, the protocol, worksheets/case report forms, advertising for subject participation, adverse event reports, subject source data, correspondence with regulatory authorities and IRBs/ERCs, consent forms, investigator's curricula vitae, monitor visit logs, laboratory reference ranges, laboratory certification or quality control procedures and laboratory director curriculum vitae. By signing this protocol, the investigator agrees that documentation shall be retained until at least 2 years after the last approval of a marketing application in an ICH region or until there are no pending contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. Because the clinical development and marketing application process is variable, it is anticipated that the retention period can be up to 15 years or longer after protocol database lock. The Sponsor will determine the minimum retention period and notify the investigator when documents may be destroyed. The Sponsor will determine the minimum retention period and upon request, will provide guidance to the investigator when documents no longer need to be retained. The sponsor also recognizes that documents may need to be retained for a longer period if required by local regulatory requirements. All trial documents shall be made available if required by relevant regulatory authorities. The investigator must consult with and obtain written approval by the Sponsor prior to destroying trial and/or subject files.

ICH Good Clinical Practice guidelines recommend that the investigator inform the subject's primary physician about the subject's participation in the trial if the subject has a primary physician and if the subject agrees to the primary physician being informed.

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

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

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

10.4 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 subject to the [redacted]

submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. Information posted will allow subjects 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.

10.5 Quality Management System

10.5.1 Data Safety Committee

The Rutgers Cancer Institute Human Research Oversight Committee is responsible for annual data and safety monitoring of DUHS sponsor-investigator phase I and II, therapeutic interventional studies that do not have an independent Data Safety Monitoring Board (DSMB). The primary focus of the HROC is review of safety data, toxicities and new information that may affect subject safety or efficacy. Annual safety reviews includes but may not be limited to review of safety data, enrollment status, stopping rules if applicable, accrual, toxicities, reference literature, and interim analyses as provided by the sponsor-investigator. The HROC in concert with the Quality Assurance Monitoring Team oversees the conduct of Rutgers CINJ cancer-related, sponsor-investigator therapeutic intervention and prevention intervention studies that do not have an external monitoring plan, ensuring subject safety and that the protocol is conducted, recorded and reported in accordance with the protocol, standing operating procedures (SOPs), Good Clinical Practice (GCP), and applicable regulatory requirements.

10.5.2 Monitoring

The [redacted] Quality Assurance Monitoring Team will conduct monitoring visits to ensure subject safety and to ensure that the protocol is conducted, recorded, and reported in accordance with the protocol, standard operating procedures, good clinical practice, and applicable regulatory requirements. As specified in the Rutgers CINJ Data and Safety Monitoring Plan, the Quality Assurance Monitoring Team will conduct routine monitoring after the third subject is enrolled, followed by annual monitoring of 1 – 3 subjects until the study is closed to enrollment and subjects are no longer receiving study interventions that are more than minimal risk.

The [redacted] will perform annual reviews on findings from the quality Assurance Monitoring Team visit and additional safety and toxicity data submitted by the Principal Investigator.

Additional monitoring may be prompted by findings from monitoring visits, unexpected frequency of serious and/or unexpected toxicities, or other concerns and may be initiated upon request of [redacted], the Human Research Oversight Committee (HROC), the sponsor, the Principal Investigator, or the IRB. All study documents must be made available upon request to the DCI Monitoring Team and other authorized regulatory authorities, including but not limited to the Nationa[redacted]

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Health, National Cancer Institute, and the FDA. Every reasonable effort will be made to maintain confidentiality during study monitoring.

10.6 Data Management

The policies and procedures of [redacted] department (see: Investigator's Handbook) will govern publication of the trial. It is expected that the results of this trial will be submitted for publication in a timely manner following the conclusion. The Cancer Institute of New Jersey PI, and all co-authors prior to submission or use, must review any abstract or manuscript.

10.6.1 Study Documentation

Study documentation includes but is not limited to source documents, case report forms, monitoring logs, appointment schedules, study team correspondence with Sponsor or regulatory bodies/committees, and regulatory documents that can be found in the DCI-mandated "Regulatory Binder", which includes but is not limited to signed protocol and amendments, approved and signed informed consent forms, FDA Form 1572, CAP and CLIA laboratory certifications, and clinical supplies receipts and distribution records.

Source documents are original records that contain source data, which is all information in original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source documents include but are not limited to hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial. When possible, the original record should be retained as the source document. However, a photocopy is acceptable provided that it is a clear, legible, and an exact duplication of the original document.

10.6.2 Case Report Forms (CRFs)

The electronic CRF stored in [redacted] will be the primary data collection document for the study. The CRFs will be upda[redacted]ely manner following acquisition of new source data. Only the key personnel delegated on the delegation of authority log are permitted to make entries, changes, or corrections in the CRF.

An audit trial will be maintained automatically by the electronic CRF management system. All users of this system will complete user training, as required or appropriate per regulations.

10.6.3 Data Management Procedures and Data Verification

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Users of the electronic CRF will have access based on their specific roles in the protocol.

Completeness of entered data will be checked automatically by the eCRF system, and users will be alerted to the presence of data inconsistencies. Additionally, the data manager and project manager will cross-reference the data to verify accuracy. Missing or implausible data will be highlighted for the PI requiring appropriate responses (i.e. confirmation of data, correction of data, completion or confirmation that data is not available, etc.).

The database will be reviewed and discussed prior to database closure, and will be closed only after resolution of all remaining queries. An audit trail will be kept of all subsequent changes to the data.

10.6.4 Study Closure

Following completion of the studies, the PI will be responsible for ensuring the following activities:
- Data clarification and/or resolution
- Accounting, reconciliation, and destruction/return of used and unused study drugs
- Review of site study records for completeness
- Shipment of all remaining laboratory samples to the designated laboratories

11.0 APPENDICES

11.1 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 (e.g., 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.

11.2 Common Terminology Criteria for Adverse Events V4.0 (CTCAE)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for adverse event reporting. (http://ctep.cancer.gov/reporting/ctc.html)

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11.3 Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 Criteria 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.

In addition, volumetric analysis will be explored by central review for response assessment.

11.4 Events of Clinical Interest Guidance Document

Please refer to separate document.

11.5 Processing and Shipping of Tumor Tissue and Correlative Science Blood Samples

*NOTE: Participating Centers will not be processing specimens locally. All samples will be processed at [redacted] Blood samples should be shipped at room temperature the same day as obtained and must be overnighted to [redacted] Tumor tissue should be collected, without sectioning or processing, into the HTS-[redacted] solution and shipped at 4°C the same day as obtained and must be overnighted to [redacted] When tumor and blood are collected on the same day, they must be shipped together at 4°C. Please refer to instruction sheet with specimen kits.

11.5.1 Tissue Collection

11.5.1.1 Collection of peripheral blood

a. Peripheral blood will be collected by venipuncture into four venous blood collection tubes (four Green Top tubes with sodium heparin: BD vacutainers catalog# 366480).
b. The sample will be transported at room temperature (18°C to 25°C) in a double container from the collection site to the sample processing laboratory.
c. Green Top tubes for peripheral blood mononuclear cell (PBMC) processing should be kept on a rocker at room temperature until processed (to avoid clotting) and processed as soon as possible (within 4 h maximum) after blood collection.
d. The collection tubes will be labeled with the patient ID, date, and time of venous blood draw.
e. Samples will be handled one patient at a time to avoid mix-ups.

11.5.1.2 Collection of tumor tissue and handling of archival tumor tissue

a. Fresh tumor tissue will be collected by biopsy and immersed (completely) in sterile cold (2-7°C) HBSS in a sterile specimen cup (for example, VWR catalog# 15704-088) on ice (care must be taken to not immerse the cup/tissue in ice to avoid freezing the tissue).
b. The specimen cup will be labeled with the patient ID, date, and time of tumor tissue collection.
c. The sample will be transported on ice from the collection site to the sample processing laboratory.
d. Tissue processing should be done as soon as possible after biopsy (and within 3 h maximum) after tissue collection.
e. Samples will be handled one patient at a time to avoid mix-ups.
f. Archival tumor tissue (for example, in paraffin blocks or pathology slides) should be kept at room temperature until shipping.

11.5.2 Tissue Processing

11.5.2.1 Processing of peripheral blood

a. Green Top tubes will be processed as follows:
    i. Green Top tubes will be mixed by inverting the tube gently 6 to 8 times.
    ii. Up to 10 ml of peripheral blood will be added to 10 ml of PBS in a 50 ml Falcon tube and mixed by inverting the tube gently 6 to 8 times.
    iii. The 20 ml peripheral blood/PBS mixture will be overlayed by slow careful pipetting onto a 20 ml layer of Ficoll in a 50 ml tube.
    iv. The peripheral blood/PBS/Ficoll tube will be centrifugated for 20 min at 1750 rpm at room temperature without the break.
    v. Using a transfer pipette the clear top plasma layer will be removed and discarded as biological waste.
    vi. Using a new transfer pipette, the cloudy PBMC layer will be transferred to a 50 ml conical tube.
    vii. Care must be taken not to disrupt the erythrocyte layer during the transfer by using gentle pipetting above the Ficoll layer and keeping the tube stationary.
    viii. The cells will be counted (using a standard hemocytometer) and the total cell count recorded.
    ix. PBS will be added to the PBMC tube up to the 50 ml mark and the tube will be centrifugated for 5 min at 1750 rpm at room temperature with the break.
    x. The supernatant will be discarded and the pellet dissolved in Cryopreserve solution (1 ml Cryopreserve solution per 1 ml of blood) and transferred to cryovials (1 ml per cryovial). The cell concentration and solution volume will be recorded.
    xi. All aliquots will be placed upright in a in a Mr. Frosty (Thermo Scientific catalog #5100-0001) at 4°C for up to 24 hours and then in [redacted]

freezer in a specimen box. All specimens should remain at -80°C or colder prior to shipping. The samples should not be thawed prior to shipping.

11.5.2.2 Processing of tumor tissue

Reference QUALTEK laboratory manual for instruction on processing and shipping of tumor tissue.

Shipping Table

Tissue (processing endpoint)	Storage (before shipping)	Storage during shipping	Time allowed between processing and shipping
Blood (PBMCs from Green Top tubes)	Room Temperature	Room Temperature	Shipped same day as obtained (max <24h)
Tumor (HTS-FRS preserved from fresh tissue)	2-8°C	Ice packs	Shipped same day as obtained (max <24h)
Tumor (formalin fixed from fresh tissue)	Room temperature	Room temperature	Samples can be batched for up to 3 months
Tumor (Archival tissue: blocks or slides)	Room temperature	Room temperature	Samples can be batched for up to 3 months

11.5.3 Shipping

a. Prior to shipping a sample, an e-mail must be sent to [redacted] and [redacted] to advise the recipient ([redacted]) the scheduled shipping time. "Specimens Ready for Shipment" must be included in the subject line. A confirmation e-mail will be returned stating that personnel will be available on the expected delivery date and time. Personnel will generally be available to receive shipments Tuesday through Friday, excluding government holidays. If needed, [redacted] can be contacted directly at [redacted]

b. All weekly processing samples are recommended to ship out via FedEx on the following Monday afternoon for delivery by 10 AM Tuesday (FedEx First Overnight).

c. FedEx return shipment [redacted] Rutgers C[redacted]

d. To request return shipment labels, an e-mail can be sent to [redacted]

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be provided must be listed as well as the number of shipment labels requested. Shipment labels will be provided within 5 business days.

e. Samples and a shipping list containing pertinent sample information should be sent to: [redacted] and specimen records must be made so that one copy can be sent to [redacted] the samples and one can be maintained at the collection site for internal records.

g. Samples from each patient that are shipped under that same shipping condition should be consolidated in a single storage container (such as an 81-place freezer box). Samples from different patients should not be mixed in the same storage container but can be shipping in the same shipping container (as long as they are separated in different storage containers).

h. Please refer to the Post-processing and Shipping Table for post-processing and handling details.

i. Just prior to shipment, storage containers with samples from individual patients should be placed in the shipping container/s and the contents of the package should be matched to the shipping manifest. Both copies of the shipping manifest should be signed and dated and one copy of the shipping manifest and record should be placed in the box.

j. The box should be sealed and a shipping label attached onto the outside of the shipping container. The container should be labeled as containing biohazardous specimens.

k. The shipping date, time, tracking number, and shipping information should be recorded.

l. An e-mail must be sent to [redacted]

m. Once specimens arrive at the Tumor Immunology Laboratory at [redacted] they will be immediately placed at 4°C (for formalin-fixed tumor tissue) or -80°C (or lower for all other tissues and PBMCs) for pending correlative science work or for storage.

n. Upon receipt of the samples, an entry will be made and sample number issued for the expected sample in the Tumor Immunology Laboratory biospecimen repository database.

11.5.4 Safety Precautions

Universal precautions (*i.e.*, a method of infection control in which all human blood and body fluids are treated as if they are infectious for Hepatitis Viruses, Human Imm[redacted]

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virus, and other known and unknown infectious agents) will be utilized when handling all unfixed cells and tissues.

a. Hepatitis B and Hepatitis C viruses may be transmitted through blood and other body fluids, and are associated with acute hepatitis, chronic liver disease, and hepatocellular carcinoma in humans. The probability of seroconversion after needlestick exposure is estimated at 7%. Untreated virus can persist for up to one week at room temperature. All staff who work with human tissue must provide evidence of Hepatitis B vaccination.
b. Human Immunodeficiency virus (HIV) is a retrovirus that causes severe immunodeficiency. Infection increases the risk of developing malignancies, infection by opportunistic organisms, and death. The probability of seroconversion after needlestick exposure is estimated at 0.5%. Infectivity of untreated virus persists for up to one week at room temperature.
c. Other potentially infectious agents, both known and unknown, pose hazards to those working with human tissue. Included are tuberculosis, HTLV1, Coccidiomycosis, Creutzfeldt-Jacob disease, amongst others.
d. Individual institutional and OSHA guidelines must be followed when handling human cells and tissues, and referred to for additional information on bloodborne pathogens, laboratory safety, chemical safety, and biohazardous waste disposal. Briefly:
    i. Personal protective equipment (PPE) must be used at all times while working with human tissue. These include disposable latex or nitrile gloves, face shield, protective splash-resistant laboratory coat (disposable preferred), and covered protective shoes.
    ii. Gloves should be immediately removed and replaced in the event that they become torn or perforated. Gloves must be removed prior to leaving the work area, and disposed of in an appropriate waste disposal container. Hands must be washed in a "clean" sink after removal of gloves.
    iii. Face shields, goggles and masks should be worn whenever a potential for exposure to splashes, spray, splatter, droplets, aerosols of blood or tissue fluid, or other potentially infectious materials may be generated, and if there is a potential for eye, nose or mouth contamination. They should be worn at all times while handling tissue in the for processing.
    iv. Protective lab coats, preferably disposable types, must be donned while working with tissue. Contaminated clothing must be removed prior to leaving the work area, and appropriately laundered or discarded, as per individual institutional guidelines.
    v. All waste must be disposed of prior to leaving the work area. Biohazardous sharps must be properly disposed of in an approved "sharps" container. All other non-sharp waste must be disposed of in an approved orange or red biohazardous waste disposal bag.
    vi. After completion of work with human tissue, all work surfaces must be disinfected with a product that has been demonstrated to be effective against

bacteria, viruses, pseudomonas, tuberculosis and fungi. Product literature should be referred to for appropriate use.

e. Any injuries or exposure to human tissue or potentially infectious biologic agents must be reported promptly as specified in individual institutional safety guidelines.

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