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A Pilot Study of Pembrolizumab and Neoadjuvant Radiation for Large, High-Risk, Soft Tissue Sarcomas

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PRINCIPAL INVESTIGATOR SIGNATURE PAGE

Signature of Sponsor-Investigator	dd/mm/yyyy
Lee Cranmer, MD, PhD	
Printed Name of Sponsor-Investigator	
Institution Name: _____	

By my signature, I agree to personally supervise the conduct of this study at my study site and to ensure its conduct is in compliance with the protocol, informed consent, Institutional Review Board (IRB)/Ethics Committee (EC) procedures, instructions from Merck representatives, the Declaration of Helsinki, ICH Good Clinical Practices Guidelines, and local regulations governing the conduct of clinical studies.

Table of Content

List of Tables

List of Figures

1.0 TRIAL SUMMARY

Abbreviated Title	Neoadjuvant Pembro And Rads for Sarcoma
Trial Phase	Pilot Study
Clinical Indication	Localized soft tissue sarcomas
Trial Type	Single-arm, open label
Type of control	Historical
Route of administration	IV
Trial Blinding	None
Treatment Groups	All subjects receive standard of care radiation therapy + pembrolizumab
Number of trial subjects	26
Estimated enrollment period	12 months
Estimated duration of trial	3 years
Duration of Participation	Treatment in 3 months (subjects will be followed up to for 5 years)
Estimated average length of treatment per subject	3 months

2.0 COMMONLY USED ABBREVIATIONS:

AE	Adverse Event
APC	Antigen Presenting Cell
APP	Advanced Practice Provider (Nurse practitioners or physician assistants)
BMP	Basic Metabolic Panel
CBC	Complete Blood Count
CMP	Complete Metabolic Panel
CR	Complete Response
CRF	Case Report Forms
CTCAE	Common Terminology Criteria for Adverse Events
CTL	Cytotoxic T Lymphocyte
DLT	Dose Limiting Toxicity
DSMB	Data Safety Monitoring Board
Gy	Gray
EBRT	External Beam Radiation Therapy
ECI	Events of Clinical Interest
ECOG	Eastern Cooperative Oncology Group
IB	Investigator Brochure
IMRT	Intensity Modulated Radiation Therapy
IO	Immuno-oncology
IV	Intravenous
mAb	Monoclonal Antibody
MHC	Major Histocompatibility Complex

OS	Overall Survival
OTC	Over the Counter
PD-1	Programed Death Receptor 1
PD-L1 & 2	Program Death Receptor Ligands 1 & 2
PFS	Progression-free Survival
RECIST	Response Evaluation Criteria in Solid Tumors
RR	Response Rate
SAE	Serious Adverse Event
SD	Stable Disease
SBRT	Stereotactic Body Radiation Therapy
STS	Soft Tissue Sarcoma
TSH	Thyroid Stimulating Hormone
Treg	Regulatory T cell

3.0 TRIAL DESIGN

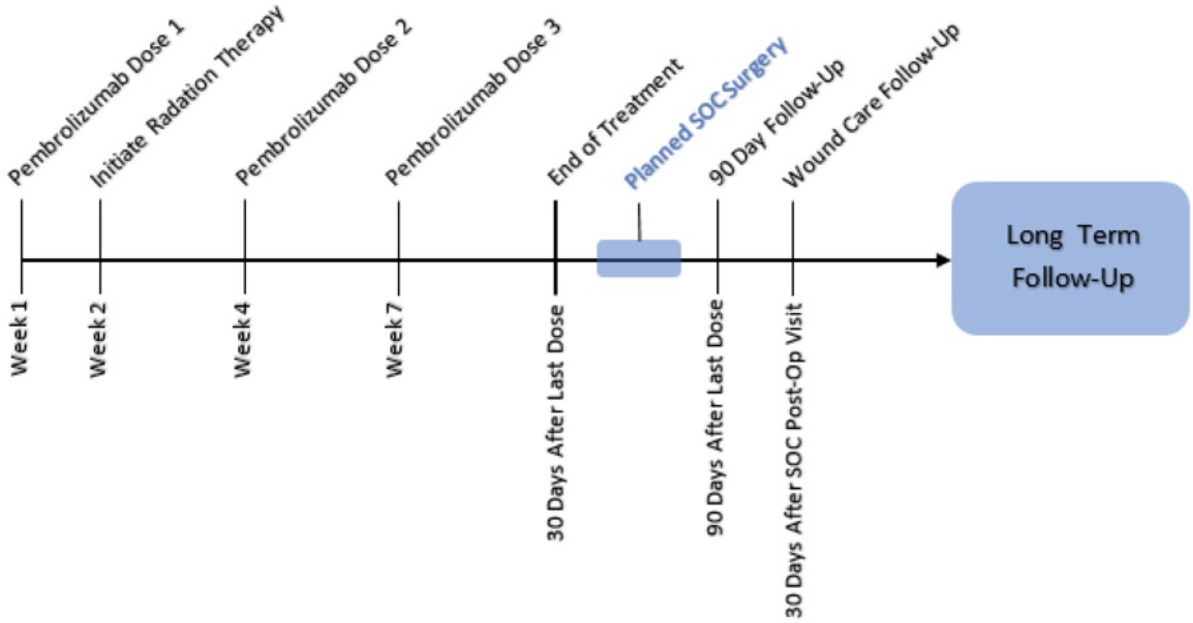
3.1 Introduction

Although localized sarcoma is potentially curable, approximately 50% of subjects with large or high-grade tumors will ultimately develop incurable disease[1,2]. This is a pilot study to examine the addition of the anti-PD-1 targeted, monoclonal-antibody pembrolizumab to standard, neoadjuvant radiation for subjects with high-risk soft tissue sarcomas planning for surgery with curative intent. Pembrolizumab has been combined in the past using hypofractionated radiation, but has never been combined with conventionally fractioned radiation with curative intent in soft tissue sarcoma subjects before. This trial will confirm the safety of this novel combination. A large randomized trial would be required to definitively demonstrate a relapse-free-survival benefit in this population. This study will instead focus on characterizing T-cell responses to this neoadjuvant combination, with a particular focus on the repertoire of tumor infiltrating lymphocytes (TIL). Tumor necrosis and radiographic response by RECIST criteria will be used as surrogates for efficacy, as has been done in other pilot trials combining radiation with novel therapies.

3.2 Treatment Plan

This is an open-label, single-arm, pilot study. Subjects will have localized, intermediate or high grade, large STS tumors.

Figure 1 - Study Schema



Subjects will receive neo-adjuvant radiation per institutional standards, generally 50 Gy given over 5-6 weeks. Subjects will receive their first pembrolizumab dose 1 week prior to

starting radiation. Pembrolizumab will then be administered every 3 weeks, for a maximum of 3 infusions. The standard approved dosing schedule for pembrolizumab will be used in this study, which is a flat 200 mg dose intravenously every 3 weeks. Surgery will be performed as per standard of care, typically 4-6 weeks after the completion of neoadjuvant radiation.

3.3 Safety Monitoring on Study and Post-Treatment Follow-Up

Enrollment will begin with a single cohort of three subject slots. No additional enrollment will be permitted until the first three subjects have completed a full treatment schedule (defined as C1D1 of pembrolizumab through the End of Treatment visit). This temporary pause and review of data is to ensure thorough safety monitoring of the first three subjects enrolled. The data will be reviewed to ensure that pembrolizumab in combination with radiation therapy does not increase the toxicity profile of either treatment in a manner that is unexpected or compromises subject safety.

During this initial 3-subject enrollment, if one or more of the three subjects experiences a serious unexpected adverse event at least possibly related to pembrolizumab or radiation treatment, the temporary enrollment hold will be extended as the Sponsor-Investigator evaluates the potential risk and safety. During this enrollment hold, the Sponsor-Investigator will review all currently collected safety data and make an evaluation as to whether to permit further accrual.

If none of the first three subjects experience a serious unexpected adverse event related to pembrolizumab or radiation treatment, enrollment may continue without restriction. If at any point in time there is greater than 30% of subjects who have unexpected, probably related SAEs grade III or higher that do not resolve to grade 2 within 2 weeks, the trial will pause enrollment for safety review by the Sponsor-Investigator.

All subjects will have an End of Treatment visit 30 days (± 14 days) after the completion of pembrolizumab therapy, which may coincide with the pre-operative visit. Subjects will have a Wound Care Follow-Up visit 30 days (± 14 days) after their first standard of care post-operative visit to determine how the wound healing is proceeding. If there are wound healing complications or any other ongoing toxicities related to treatment at that time, the subject will continue to be monitored until the toxicity/complication resolves, improves to maximal anticipated improvement, or the study closes.

Subjects will be followed for as many as 5 years after completing treatment for survival determination. This may be done via publicly available records, medical record review, or by phone.

3.4 Biopsy and Analysis of Tumor

All subjects must have pre-treatment tissue available to be considered eligible. This may be

a biopsy collection performed for this trial or recent archival tissue. For subjects who are having a research biopsy performed only for the study, this must be completed within four weeks prior to the first pembrolizumab treatment (Week 1, Day 1). Archival tissue from a recent biopsy may be used in place of a fresh tissue biopsy so long as the tissue was collected within 90 days prior to the first pembrolizumab treatment (Week 1, Day 1). If archival tissue is used, an entire block or split of the standard of care block is required. If this is not feasible, archival tissue will not be accepted and the subject will need to undergo a fresh research biopsy.

Biopsies for this study will be core needle biopsies, unless a different type of biopsy is clinically indicated. If collected for clinical purposes, both clinical and research tissue samples will be obtained. Archival tissue from prior surgeries related to this diagnosis may be requested for research relating to exploratory endpoints at PI discretion.

Clinical tumor samples analyzed by the University of Washington Pathology Department as part of standard of care will be examined by a qualified pathologist (as determined by the Sponsor-Investigator) to determine the extent of tumor necrosis.

3.5 Radiation

Neoadjuvant radiation will be given as per standard of care at a subject's treating institution. Typically this will be 50 Gy, though a minimum of 45 Gy is required. Radiation will be delivered with conventional fraction sizes of 1.8-2.0 Gy daily into given over 5-6 weeks in daily fractions. However, a minimum of 25 fractions is required. Radiation protocols will be tailored to the specific subject's needs and may include a variety of techniques including 3-dimensional conformal external beam radiation therapy (3D CRT) or Intensity Modulated Radiation Therapy (IMRT).

While not typical, occasionally oral or topical steroids are used by some providers to minimize radiation-related toxicity as part of normal standard of care. This is allowed, however, subjects participating in this trial should not use these agents prophylactically. Topical steroids (1% hydrocortisone cream) may be used for grade 2 or higher radiation toxicity. Oral and intravenous steroids should only be used as described in Section 7.2. Other standard supportive care medications, including Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), may be used at the discretion of the treating physician for radiation-related toxicity.

4.0 OBJECTIVE(S) & HYPOTHESIS(ES)

4.1 Primary Objective(s)

1. To determine the rate of complete histopathologic necrosis following the combination of pembrolizumab and neoadjuvant radiation.

4.2 Secondary Objective(s)

1. To determine response based on RECIST criteria following the combination of radiation and pembrolizumab
2. To confirm the tolerability and safety of the combination of neoadjuvant pembrolizumab and radiation in a population with localized STS based on CTCAE v5.0.

4.3 Exploratory Objectives

1. To make preliminary estimates of event-free survival and overall survival at one year.
2. To analyze changes occurring in tumor immune infiltrates following pembrolizumab and standard of care, neoadjuvant radiation, including but not limited to:
   - Number of CD4 and CD8 T cells
   - Phenotype of infiltrating T cells
   - Number of FoxP3 Regulatory T cells
   - Number of macrophages
   - Phenotype of Macropahges
   - Gene expression in immune infiltrates
   - For tumors with known immunogenic targets, the number and phenotype of antigen specific T cells
3. To determine volumetric size response and perform quantitative radiomic analysis.
4. To measure the time of wound healing following surgery and the rate of wound healing complications following definitive surgery.

5.0 ENDPOINTS

5.1 Primary Endpoint

1. Rate of necrosis following treatment with neoadjuvant radiation and pembrolizumab treatment.

5.2 Secondary Endpoints

1. Response rate (complete, partial, overall) of combination treatment with pembrolizumab and radiation therapy as defined by RECIST v1.1 criteria
2. Adverse event profile (based on National Cancer Institute Common Terminology Criteria for Adverse Events [CTCAE] v5.0) of all subjects

5.3 Exploratory Endpoints

1. Progression-free survival rate and median overall survival rate (OS) at 12 months, based on RECIST v1.1
2. Changes in tumor immune infiltrates as described in Section 4.3
3. Identify any potential predictors of treatment response and relapse-free survival, when comparing pre-treatment imaging with imaging prior to surgery (after completion of radiotherapy and concurrent pembrolizumab) utilizing volumetric size response and quantitative radiomic analyses.
4. Rate of wound healing and incidence rate of any wound healing complications following definitive surgery

6.0 BACKGROUND & RATIONALE

6.1 Background

Refer to the approved labeling for detailed background information on MK-3475 (Pembrolizumab).

6.1.1 Pharmaceutical and Therapeutic Background

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

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune

responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer (NK) cells. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma. This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

6.1.2 Preclinical and Clinical Trial Data

Refer to the FDA Package Insert for Pembrolizumab for Preclinical and Clinical data.

6.2 Rationale

6.2.1 Rationale for the Trial and Selected Subject Population

While a randomzied trial would be required to definitively prove benefit of pembrolizumab in the neoadjuvant setting, this would require over 200 subjects and would likely take a year to accrue at 3-5 centers and would require years of follow-up for conclusive results. This

trial is proposed to estimate the potential benefit in the highest risk subjects. It is in such subjects that a prospective randomized trial could be conducted to detect benefit. Furthermore, sarcomas are superficial, allowing for easily accessible pre-treatment biopies and are often very large (frequently >10 cm), allowing large tumor volumes to be available for correlative studies. This clinical situation offers a uniques opportunity to study changes in tumor biology that may be applicable to all cancer types.

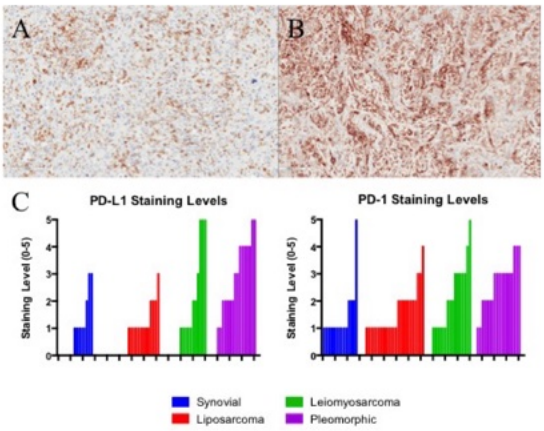


Figure 2 - A) High PD-1 staining in a high grade, metastatic sarcoma. B) Profuse membranous tumor cell staining for PD-L1 in a leiomyosarcoma tumor. C) Levels of staining were scored by a pathologist experienced at interpreting PD-1 and PD-L1 staining (1 = focal, 2 >5%, 3 >25%, 4 >50%, 5 > 75%). Graph showing intensity of staining for individual sarcoma subtypes.

6.2.1.1 Neo-adjuvant Radiation for Soft Tissue Sarcomas

For subjects with small, low grade localized soft tissue sarcomas (STS), the combination of neo-adjuvant radiation and surgery can lead to excellent long-term disease free survival (DFS) rates of >80%. [3] However, over one-half of subjects with a new diagnosis of localized STS will have larger, higher-grade tumors, approximately 50% of who will develop fatal metastatic disease.[1,2] Adjuvant and/or neo-adjuvant chemotherapy has been studied extensively, yet its role remains controversial. Practice patterns vary widely, to a large degree because the benefit is unclear and the agents used (doxorubicin and ifosfamide) are relatively toxic.[4] Radiation for high-risk STS improves local control; at most sarcoma specialty centers, it is given preoperatively/neoadjuvantly. [3] An agent with the potential to synergize with radiation and decrease the chance of metastatic spread would be highly desirable.

6.2.1.2 PD-1 and PD-L1 Expression in Soft Tissue Sarcomas (STS)

We have seen T-cell tumor infiltration in a variety of sarcoma subtypes and the amount of infiltrating T cells has been associated with shorter disease specific survival, supporting the idea that check point inhibition might be an effective strategy.[5] One recent study found PD-1^{+} lymphocytes in 65% of STS tumors and PD-L1 expression by IHC in 58% of STS tested. [6]

To better define the pattern of PD-L1 expression in specific STS subtypes, the Pollack Lab entered into a collaboration with Merck to analyze 81 formalin-fixed, paraffin-embedded FFPE) sarcoma samples from the University of Washington Tissue Bank for PD-L1 and PD-1 expression by IHC. Ninety percent of tumors tested positive for PD-1 staining and 59% for PD-L1. Significantly higher expression levels were seen in higher-grade tumors and pleomorphic undifferentiated sarcoma. Detectable PD-L1 was seen in a subset of each sarcoma subtype tested, including n=11 (58%) of leiomyosarcoma (LMS) and n=7 (47%) synovial sarcoma (SS). PD-1 expressing lymphocytes were also see in a majority of tumors

including n=16 (84%) LMS and n=15 (100%) SS. High (4+) or very high (5+) PD-L1 expression was seen in 10 sarcoma including 3 LMS (Figure 2). Seven tumors had high or very high PD-1 expression including one SS, and two LMS. In contrast, even though 100% of the SS tumors had some PD-1 expression, none of the SS tumors had either 4+ or 5+ PD-1 expression. Only n=4 (27%) of SS tumors had >2+ expression for PD-L1. Higher-grade tumors were associated with higher levels of both PD-L1 ($p = 0.03$) and PD-1 expression ($p = 0.05$).

These data are particularly important. They indicate that therapy targeting the PD-1 pathway may be broadly active among STS. Given the great heterogeneity of this class of tumors, the finding of a biologically common pathway that may be important in the pathogenesis of the condition may be particularly actionable. In addition, the relatively high degree of expression of PD-L1 in high-grade tumors may be especially useful, as it is these tumors that are most prone to developing distant metastasis. Thus, our preliminary data suggest that the PD-1 pathway may be very useful as a target in the treatment of STS.

6.2.1.3 Rationale for the combination of Pembrolizumab and Neoadjuvant Radiation

The rationale for combining radiation and immunotherapy has been written about extensively. [7-10] However, these studies have focused on hypofractionated radiation in the metastatic setting. None of these published studies have examined the use of pembrolizumab with the neoadjuvant radiation schedules that are typically used in the curative setting for many malignancies (using high doses but in low-dose daily fractions). To more closely examine the expression of PD-1 and its ligand in the context of neoadjuvant treatment, we performed a retrospective study of sarcoma subjects who received neoadjuvant radiation where pre- and post-treatment tumor was available. While PD-1 and PD-L1 were similar following treatment, PD-L2 increased significantly ($p < 0.05$), suggesting that blockade of PD-1 and PD-L2 might stimulate anti-tumor immunity leading to tumor

destruction and potentially the elimination of minimal residual disease.

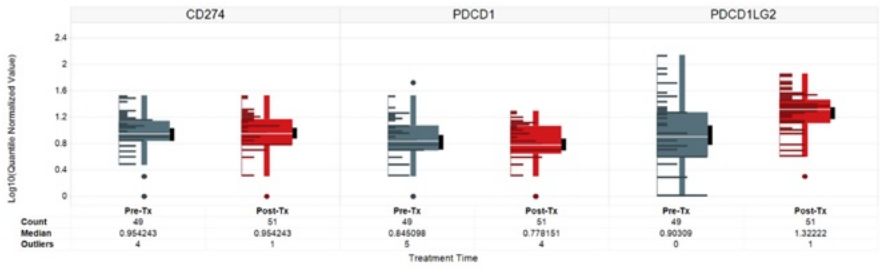


Figure 3 - Gene expression based on a nanostring evaluation of sarcoma tumors before and after neoadjuvant radiation. PD1 (left) and PD-L1 (middle) were relatively unchanged following neoadjuvant radiation. However, PD-L2 (right) increased significantly p <0.05)

6.2.2 Rationale for Dose Selection/Regimen/Modification

The dose of pembrolizumab will be a fixed dose of 200 mg IV every 3 weeks. The safety of pembrolizumab has been studied extensively as monotherapy. An open-label Phase I trial was conducted to evaluate the safety and clinical activity of single agent pembrolizumab. The dose escalation portion of this trial evaluated three dose levels in subjects with advanced solid tumors: 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W). All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first-in-human study of pembrolizumab showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No MTD was identified to date.

Pharmacokinetic (PK) data analysis of pembrolizumab administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life. Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days).

A population PK analysis has been performed using serum concentration time data from 476 subjects. Within the resulting population PK model, clearance and volume parameters of pembrolizumab were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. Pembrolizumab has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg Q3W body weight based regimen are anticipated to remain well within the established exposure margins of 0.5 – 5.0 for pembrolizumab in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W vs. the proposed dose regimen of 2 mg/kg Q3W (i.e. 5-fold higher dose and exposure). The population PK evaluation

revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the non-small cell lung cancer (NSCLC) and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

The choice of the 200 mg Q3W as an appropriate dose for the switch to fixed dosing is based on simulations performed using the population PK model of pembrolizumab showing that the fixed dose of 200 mg every 3 weeks will provide exposures that 1) are optimally consistent with those obtained with the 2 mg/kg dose every 3 weeks, 2) will maintain individual subject exposures in the exposure range established in melanoma as associated with maximal efficacy response and 3) will maintain individual subjects exposure in the exposure range established in melanoma that are well tolerated and safe.

A fixed dose regimen will simplify the dosing regimen to be more convenient for physicians and to reduce potential for dosing errors. A fixed dosing scheme will also reduce complexity in the logistical chain at treatment facilities and reduce wastage. Multiple studies have looked at the safety of checkpoint inhibitors generally, and pembrolizumab specifically with radiation in a variety of malignancies. Anti-PD-1 therapy is now combined with radiation both in the context of clinical trials but also in the context of routine standard of care when subjects on PD-1 targeted treatments require radiation and can be performed without any significantly increased toxicity.[11] Radiation can be added to rescue lung cancer subjects presenting on nivolumab.[12] Indeed, in sarcoma the combination has been reported to be well tolerated and successful in treating subject's cancer following off-label use.[13] Indeed, if toxicity were to be seen it would be most likely be found in the treatment of brain metastasis as the brain is such a sensitive area. However, even in the brain, the combination appears to be safe without apparent increased autoimmunity or increased radiation toxicity with either PD-1 blockade alone or in combination with anti-CTLA4.[11,14,15]

6.2.3 Rationale for Endpoints

6.2.3.1 Primary Endpoint

Combining neoadjuvant radiation with pembrolizumab will likely have a higher rate of complete necrosis compared with historical controls. Complete or near-complete (>95%) necrosis after administration of neoadjuvant therapy is strongly associated with relapse-free survival prolonged in trials of chemotherapy combined with radiation of soft-tissue sarcoma.[16,17] Rates of complete necrosis in trials with chemoradiation for soft tissue sarcoma are consistently <15%.[18,19] Other small pilot trials of agents aimed at radiosensitization have also used complete necrosis as a surrogate for clinical activity.[20-22] A significant improvement in the rate of complete necrosis would be a strong rationale to pursue a randomized trial of pembrolizumab combined with neoadjuvant radiation in the curative setting.

6.2.3.2 Secondary Endpoints

6.2.3.2.1 Response Rate

It is hypothesized that subjects treated with pembrolizumab in combination with neoadjuvant radiation will have a higher rate of partial response compared with historical controls. Traditionally, neoadjuvant radiation does not lead to dramatic reduction in tumor size prior to surgery. While an improvement in the partial response rate is not necessary for the combination of pembrolizumab and radiation to lead to improved outcomes, if an improved response rate was observed, this would strongly suggest clinical activity.

6.2.3.2.2 Safety of Pembrolizumab and Radiation

Multiple studies have looked at the safety of checkpoint inhibitors generally, and pembrolizumab specifically with radiation in a variety of malignancies. Anti-PD-1 therapy is now combined with radiation both in the context of clinical trials but also in the context of routine standard of care when subjects on PD-1 targeted treatments require radiation and can be performed without any significantly increased toxicity.[11] Radiation can be added to rescue lung cancer subjects presenting on nivolimab.[12] Indeed, in sarcoma the combination has been reported to be well tolerated and successful in treating subject's cancer following off-label use.[13] If toxicity were to be seen it would most likely be found in the treatment of brain metastasis as the brain is such a sensitive area. However, even in the brain the combination appears to be safe without apparent increased autoimmunity or increased radiation toxicity with either PD-1 blockade alone or in combination with anti-CTLA4.[11,14,15]

7.0 METHODOLOGY

7.1 Entry Criteria

Most subjects with newly-diagnosed, large and high-grade soft-tissue sarcomas planning for neoadjuvant radiation and surgery will be eligible. All subjects meeting the eligibility criteria will be considered for enrollment. No exceptions to eligibility will be granted.

7.1.1 Subject Inclusion Criteria

In order to be eligible for participation in this trial, the subject must:

1. Be willing and able to provide written informed consent for the trial.
2. Be ≥ 18 years of age on day of signing any informed consent documents.
3. Have measurable disease based on RECIST 1.1 criteria.
4. Have newly diagnosed disease or localized recurrent or oligometastatic lesions that are candidates for radiation.

a. **NOTE:** Subjects may not have any prior systemic therapy or radiation for this sarcoma. They may have received systemic therapy and/or radiation for a different cancer.

b. **NOTE:** Oligometastatic disease will be defined as 3 or fewer detectable lesions with plans to radiate all detectable disease with conventionally fractionated radiation prior to resection.

5. Have an intermediate or high-grade soft tissue sarcoma at the discretion of the reviewing Sarcoma pathologist.

6. The tumor must be at least 3 cm in maximum dimension for intermediate-grade tumors, or 1.5 cm in maximum dimension for high-grade tumors.

7. Have plans to undergo neo-adjuvant radiation and surgery with curative intent. A minimum of 45 Gy is necessary, planned to be administered over a minimum of 25 fractions.

8. Be willing to provide tissue from a newly obtained core, incisional or excisional biopsy of a tumor lesion. Archival tissue from a recent clinical or research biopsy (within 90 days prior to Week 1 treatment) may be used in place of a fresh tissue biopsy.

9. Have a performance status of 0 or 1 on the ECOG Performance Scale or >70% on the Karnofsky Scale. Evaluation of performance status is to be performed within 7 days prior to the date of enrollment.

10. Demonstrate adequate organ function as defined in Table 1. All screening laboratory tests should be performed within 28 days of enrollment.

Table 1 - Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L[a]
Renal	
Serum creatinine **OR** Measured or calculated[b] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN

Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN
Albumin	≥2.5 mg/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a]Criteria must be met without erythropoeiten dependency and without packed red blood cell (pRBC) transfusion within last two weeks. [b] Creatinine clearance should be calculated per institutional standard.	

11. Female subjects of childbearing potential should have a negative serum pregnancy within 72 hours prior to receiving the first dose of study medication. If negative serum pregnancy is confirmed during screening window greater than 72 hours prior to first dose, that will be sufficient for eligibility confirmation, however, a confirmatory test must be completed and resulted within 72 hours prior to receiving the first dose of study medication on Week 1, Day 1.

12. All individuals of child-bearing potential must be willing to use an adequate method of contraception as outlined in **Appendix B: Women of Childbearing Potential Definitions and Methods of Contraception**, from the first dose of the study medication through 120 days after the last dose of study medication.

7.1.2 Subject Exclusion Criteria

The subject must be excluded from participating in the trial if the subject:

1. Has had prior radiation to affected area.

2. Has one of the following sarcoma subtypes where neoadjuvant chemotherapy is established as practice at our institution: extra-skeletal Ewing's sarcoma, Embryonal Rhabdomyosarcoma, Alveolar Rhabdomyosarcoma

a. **NOTE:** Pleomorphic rhabdomyosarcoma is allowed. Bone sarcomas including Osteosarcoma, Ewing's sarcoma and Chondrosarcoma are not allowed. Extra-skeletal Osteosarcoma is considered a soft tissue sarcoma and is allowed.

3. Has a diagnosis of immunodeficiency or has an active autoimmune disease that has required systemic treatment in the past 2 years except replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid).

4. Is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment.

5. Has a known history of active TB (Bacillus Tuberculosis).

6. Hypersensitivity (≥ Grade 3) to pembrolizumab and/or any of its excipients.

7. Has a history of a second malignancy, unless potentially curative treatment has been completed with no evidence of malignancy for 2 years.

    a. **NOTE:** The time requirement does not apply to participants who underwent successful definitive resection of basal cell carcinoma of the skin, squamous cell carcinoma of the skin, superficial bladder cancer, in situ cervical cancer, or other in-situ cancers.

8. Has current or a history of any distant metastatic disease (including brain).

    a. **NOTE:** An isolated or oligo-metastatic regional recurrence may be allowed if all other criteria are met, curative attempt is being pursued.

9. Has known history of (non-infectious) pneumonitis or interstitial lung disease that required steroids, or has current evidence of pneumonitis or interstitial lung disease.

10. Has an active infection requiring systemic therapy.

11. Has known psychiatric or substance abuse disorders that would interfere with adherence to the requirements of the trial.

12. Is pregnant (positive urine pregnancy test within 72 hours prior to enrollment) or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment. If a urine pregnancy test is positive or cannot be confirmed negative, a serum pregnancy test will be required.

13. Has received prior therapy with an anti-PD-1, anti-PD-L1, anti-CTLA4 or anti-PD-L2 agent or with an agent directed to another stimulatory or co-inhibitory T-cell receptor (eg, CTLA-4, OX 40, CD137).

14. Has a known history of Human Immunodeficiency Virus (HIV) infection.

15. Has known history of Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected) infection.

16. Has received a live vaccine or live-attenuated vaccine within 30 days of planned start of study therapy. Administration of killed vaccines is allowed. *Note: Any licensed COVID-19 vaccine (including for Emergency Use) is allowed in the study as long as they are mRNA vaccines, adenoviral vaccines, or inactivated vaccines. These vaccines will be treated just as any other concomitant therapy. Investigational vaccines (i.e., those not licensed or approved for Emergency Use) are not allowed.*

17. Has received an investigational agent or has used an investigational device within 4 weeks prior to study intervention administration.

18. Has a history or current evidence of any condition, therapy, or laboratory abnormality or other circumstance that might confound the results of the study, interfere with the subject's participation for the full duration of the study, such that it is not in the best interest of the subject to participate, in the opinion of the treating investigator or has not adequately recovered from any major surgery or has ongoing surgical complications.

19. Has had an allogenic tissue/solid organ transplant.

7.2 Trial Treatments

The treatment to be used in this trial is outlined below in Table 2.

Table 2 - Trial Treatment

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
Pembrolizumab	200 mg	Q3W	IV infusion	Day 1 of each 3 week cycle x 3	Experimental
Radiation	≥45 Gy	Daily	N/A	5-6 weeks	Standard
Surgery	N/A	Once	N/A	N/A	Standard

7.2.1 Pembrolizumab

Pembrolizumab will be administered per institutional standard at the Fred Hutchinson Cancer Center as an outpatient therapy. Subjects will receive a maximum of three doses of pembolizumab on study.

Pembrolizumab dose calculation, reconstitution, preparation of the infusion fluid, and administration will be completed per institutional standard, referencing the package insert.

7.2.1.1 Dose Selection/Modification

7.2.1.1.1 Dose Selection

Subjects will receive a flat dose of 200 mg of pembrolizumab intravenously every 3 weeks, up to a maximum of three doses. Please refer to Table 2 - Trial Treatment for additional information.

7.2.1.1.2 Dose Modification and Toxicity Management for Immune-Related AEs Associated with Pembrolizumab and Immuno-oncology (IO) combination partners

AEs associated with pembrolizumab or IO/IO combination exposure, , may represent an immunologic etiology. These immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of pembrolizumab or combination treatment and may affect more than one body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of pembrolizumab or combination treatment, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Based on the severity of irAEs, withhold or permanently discontinue pembrolizumab or combination treatment and administer corticosteroids. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab are provided in Table 3 and Table 6.

Note: The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab. If after the evaluation the event is determined not to be related to pembrolizumab, the investigator does not need to follow the treatment guidance.

Table 3 - Dose modification and toxicity management guidelines for immune-related AEs associated with pembrolizumab and IO combination partners

General instructions:

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

Immune-related AEs	Toxicity grade or conditions (CTCAE v5.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper • Add prophylactic antibiotics for opportunistic	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment
	Recurrent Grade 2, Grade 3 or 4	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus).

	Grade 4	Permanently discontinue		• Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.
AST / ALT elevation or Increased bilirubin	Grade 2[A]	Withhold	• Administer corticosteroids (initial dose of 0.5- 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable)
	Grade 3[B] or 4[C]	Permanently discontinue	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold[D]	• Initiate insulin replacement therapy for participants with T1DM • Administer anti-hyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated.	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue[D]		
Hyperthyroidism	Grade 2	Continue	• Treat with nonselective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders.
	Grade 3 or 4	Withhold or permanently discontinue[D]		

Hypothyroidism	Grade 2-4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyroinine) per standard of care	• Monitor for signs and symptoms of thyroid disorders.
Nephritis: grading according to increased creatinine or acute kidney injury	Grade 2	Withhold	• Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper.	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Neurological Toxicities	Grade 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology or exclude other causes
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Asymptomatic cardiac enzyme elevation with clinical suspicion of myocarditis (previously CTCAE v4.0 Grade 1)	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 2, 3 or 4	Permanently discontinue		
Exfoliative Dermatologic Conditions	Suspected SJS, TEN, or DRESS	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology or exclude other causes
	Confirmed SJS, TEN, or DRESS	Permanently discontinue		
All other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	• Based on type and severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event.[E]		

	Recurrent Grade 3 or Grade 4	Permanently discontinue		

AE(s)=adverse event(s); ALT= alanine aminotransferase; AST=aspartate aminotransferase; CTCAE=Common Terminology Criteria for Adverse Events; DRESS=Drug Rash with Eosinophilia and Systemic Symptom; GI=gastrointestinal; IO=immuno-oncology; ir=immune related; IV=intravenous; SJS=Stevens-Johnson Syndrome; T1DM=type 1 diabetes mellitus; TEN=Toxic Epidermal Necrolysis; ULN=upper limit of normal.

Note: Non-irAE will be managed as appropriate, following clinical practice recommendations.

[A] AST/ALT: >3.0 to5.0 x ULN if baseline normal; >3.0 to 5.0 x baseline, if baseline abnormal; bilirubin:>1.5 to 3.0 x ULN if baseline normal; >1.5 to 3.0 x baseline if baseline abnormal

[B] AST/ALT: >5.0 to 20.0 x ULN, if baseline normal; >5.0 to 20.0 x baseline, if baseline abnormal; bilirubin:>3.0 to 10.0 x ULN if baseline normal; >3.0 to 10.0 x baseline if baseline abnormal

[C] AST/ALT: >20.0 x ULN, if baseline normal; >20.0 x baseline, if baseline abnormal; bilirubin: >10.0 x ULN if baseline normal; >10.0 x baseline if baseline abnormal

[D] The decision to withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician. If control achieved or ≤ Grade 2, pembrolizumab may be resumed.

[E] Events that require discontinuation include, but are not limited to: encephalitis and other clinically important irAEs.

7.2.1.1.3 Dose Modification and Toxicity Management of Infusion-Reactions Related to Pembrolizumab

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

Table 4 - **Pembrolizumab Infusion-Reaction Dose modification and Treatment Guidelines**

NCI CTCAE Grade	Treatment	Premedication at Subsequent Dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires therapy or infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids, Antihistamines, NSAIDs, Acetaminophen, Narcotics Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. If symptoms resolve within 1 hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g. from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the participant should be premedicated for the next scheduled dose. **Participants who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further study drug treatment**	Participant may be premedicated 1.5h (± 30 minutes) prior to infusion of _____ with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of analgesic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: Epinephrine**, IV fluids, Antihistamines, NSAIDs, Acetaminophen, Narcotics, Oxygen, Pressors, Corticosteroids Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately. **Participant is permanently discontinued from further study drug treatment.**	No subsequent dosing
Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v5.0 (CTCAE) at http://ctep.cancer.gov		

7.2.1.1.4 Other Allowed Dose Interruption for Pembrolizumab

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

7.2.2 Radiation Therapy

Radiation will be given according to institutional standard/standard of care, and is expected to be administered over an average of 5-6 weeks duration. Radiation therapy may be completed at a local institution, however if radiation therapy is to be planned and administered outside of the research site, the outside Radiation Oncologist will be required to consult with a Consortium-affiliated Radiation Oncologist working on this trial prior to determining eligibility and prior to initiating protocol therapy. Please refer to Table 2 - Trial Treatment for additional information.

7.2.3 Surgery

Surgical resection is to be completed per institutional standard/standard of care, after completion of protocol pembrolizumab and radiation therapy. Surgery should be completed no less than 4 weeks from last dose of radiation or last dose of pembrolizumab therapy, whichever is later, and must occur after completion of the End of Treatment visit. Surgery must occur no later than 8 weeks after the last dose of radiation or pembrolizumab therapy, whichever is later. Approval of delays greater than 8 weeks between last dose of study therapy and surgical date may be approved by the Sponsor-Investigator on a case-by-case basis. Subjects who do not proceed to surgery for any reason will be replaced.

7.2.4 Timing of Dose Administration and Dose Delays

Trial treatment should be administered on Day 1 of each cycle after all procedures and assessments have been completed as detailed on the Trial Flow Chart (Section 8.0). Pembrolizumab may be administered up to 3 days before or after the scheduled Day 1 of each cycle for administrative reasons. Participants should be placed back on pembrolizumab therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor-Investigator. Dose delays in pembrolizumab administration will not affect timing or administration of radiation therapy. Dose delays in radiation therapy will be managed per institutional guidelines/standard of care and will not affect timing or administration of pembrolizumab therapy.

7.2.5 Trial Blinding/Masking

This is an open-label trial. The Sponsor-Investigator, treating physicians, subject and clinic/study staff will all be aware of the treatment being administered.

7.2.6 Acceptable Concomitant Medications

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

All concomitant medications received within 28 days before the first dose of trial treatment and through the Wound Care Follow-Up visit should be recorded and evaluated for SAEs and ECIs as defined in Section 9.4.

7.2.7 Prohibited Concomitant Medications

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

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Live or attenuated vaccines within 30 days before the first dose of trial intervention and while participating in the trial.
    - Note: Any licensed COVID-19 vaccine (including for Emergency Use) in a particular country is allowed in the study as long as they are mRNA vaccines, replication-incompetent adenoviral vaccines, or inactivated vaccines. These vaccines will be treated just as any other concomitant therapy. Investigational vaccines (i.e. those not licensed or approved for Emergency Use) are not allowed**.**
    - Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids except when used for the following purposes:
    - To modulate symptoms of an AE that is suspected to have an immunologic etiology
    - For the prevention of emesis
    - To premedicate for IV contrast allergies

  - To treat asthma or COPD exacerbations (only short-term oral or IV use in doses >10 mg/day prednisone equivalent)
  - For chronic systemic replacement not to exceed 10 mg/day prednisone equivalent
- Other glucocorticoid use except when used for the following purposes:
  - For topical use or ocular use
  - Intraarticular joint use
  - For inhalation in the management of asthma or chronic obstructive pulmonary disease

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

The Exclusion Criteria describes other medications which are prohibited in this trial in Section 7.1.2. Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for a specifically prohibited medication during the trial, discontinuation from trial therapy may be required. The Sponsor-Investigator will discuss any questions regarding this with the Merck Clinical team.

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

7.3 Rescue Medications & Supportive Care

7.3.1 Supportive Care Guidelines

Subjects should receive appropriate supportive care measures per institutional standard as deemed necessary by the treating investigator. Supportive care guidelines for the management of adverse events with potential immunologic etiology are outlined along with the dose modification guidelines in Section 7.2.1.1.2, [**Table 3**].

7.3.2 Radiation Related Toxicities

The expected toxicities related to radiation therapy may vary in relation to the site of irradiation. The most commonly observed toxicities include fatigue (Grade 3), and skin toxicities (Grade 3) such as rash, color changes, burning, blistering, or desquamation. Skin toxicities should be managed by radiation-oncology staff utilizing standard of care protocols.

The radiation oncologist may list expected toxicities for a given radiation treatment prior to administration of radiation treatment in the subject's medical record. Strict prospective

quality assurance of all radiation plans will be performed prior to initiation of radiotherapy, per institutional standards, to minimize risk of serious toxicity.

Toxicities of radiation should be treated according to standard institutional practices, including the prescription of steroids, if appropriate. Subjects will continue treatment if steroids are used to treat radiation-associated toxicity.

7.3.3 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm.

For this trial, male subjects will be considered to be of non-reproductive potential if they have azospermia (whether due to having had a vasectomy or due to an underlying medical condition).

Please refer to Appendix B: Women of Childbearing Potential Definitions and Methods of Contraception for further details regarding definitions of childbearing potential for women and appropriate methods of contraception while on study.

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study subjects of childbearing potential must adhere to the contraception requirement described in Appendix B throughout the study period up to 120 days after the last dose of pembrolizumab. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

7.3.4 Use in Pregnancy

If a subject or subject's partner inadvertently becomes pregnant while the subject receiving study treatment with pembrolizumab, the Sponsor-Investigator and Merck will immediately be made aware (within 24 hours for Sponsor-Investigator and within 2 business days for Merck), and the subject will immediately be removed from the study. If the subject or partner of subject becomes pregnant, there will be an additional consent form required (pregnancy or pregnant partner consent) to follow the pregnancy to outcome. The study team will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy and/or any related serious adverse events (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn) will be reported within 24 hours to the Sponsor-Investigator and within 2 working days to Merck.

The Sponsor-Investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to Merck.

7.3.5 Use in Nursing Women

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

7.4 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be removed from the trial at the discretion of the Sponsor-Investigator for any reason. In addition, a subject may be withdrawn by the Sub-Investigator or the Sponsor-Investigator if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. When a subject discontinues/withdraws prior to 90 Day Follow-Up Visit, all applicable activities scheduled for the 90 Day Follow-Up visit should be performed at the time of discontinuation.

A subject will be considered withdrawn and no longer followed if they enroll onto hospice care.

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

- The subject or legal representative (such as a parent or legal guardian) withdraws consent.
- Unacceptable adverse experiences as described in Section 7.2.1.1
- Intercurrent illness that prevents further administration of treatment
- Sponsor-Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up
- Administrative reasons
- Subject progresses (clinically and/or radiologically) and is no longer a surgical candidate.

7.4.1 Discontinuation of Study Therapy after CR

All subjects will complete the three scheduled pembrolizumab injections prior to the first radiographic assessment of response unless one of the criteria is met in Section 7.4.

7.5 Subject Replacement Strategy

Should any subjects fail to complete at least 2 doses of pembrolizumab and surgery, they will be replaced.

7.6 Clinical Criteria for Early Trial Termination

Early trial termination will be the result of the criteria specified below:

- Quality or quantity of data recording is inaccurate or incomplete
- Poor adherence to protocol and regulatory requirements
- Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects
- Plans to modify or discontinue the development of the study drug

In the event of Merck's decision to no longer supply study drug, ample notification will be provided so that appropriate adjustments to subject treatment can be made.

8.0 TRIAL FLOW CHART

8.1 Study Flow Chart

Table 5 - Schedule of Assessments

Evaluation		Treatment Period							
Scheduling Window: (± 3 days unless otherwise specified)	Screening (28-day Window)	Week 1	Week 2	Week 4	Week 7	End of Treatment[1]	90-Day Follow-up[2]	Wound Care Follow-Up[3]	Survival Follow-up
Informed Consent	X								
Inclusion/Exclusion Criteria	X								
Demographics and Medical History	X								
Prior and Concomitant Medication Review	X	X		X	X	X	X		
Pembrolizumab Administration[4]		X		X	X				
Radiation Therapy[5]			X	X	X				
Post-study anticancer therapy status							X	X	X
Survival Status[6]							X	X	X
Review Adverse Events		X		X	X	X	X	X	
Physical Examination (Including ECOG)[10]	X	X		X	X	X	X	X	
Vital Signs and Weight	X	X		X	X		X		
Pregnancy Test[7]	X	X		X	X	X			
PT/INR and aPTT	X					X			
CBC with Differential, Blood Chemistry	X	X		X	X	X	X		
Urinalysis	X	X							
T3, FT4 and TSH	X	X			X	X	X		
Tumor Imaging	X					X[8]			
Tumor tissue (biopsy)[9]	X					X[9]			
Correlative Studies Blood Collection		X		X	X	X	X		

[1] This visit is to be completed 30 days (± 14 days) after the last dose of pembrolizumab. This visit may be scheduled to coincide with the standard of care outpatient pre-operative surgical appointment. This visit must occur prior to any scheduled surgery.
[2] This visit is to be completed 90 days (± 14 days) after the last dose of pembrolizumab. This visit may be scheduled to coincide with the standard of care outpatient post-operative surgical appointment. This visit must be completed after any scheduled surgery.
[3] This will be conducted 30 days (±14 days) after the standard of care post-operative visit has been completed. If the 90-Day Follow-Up visit occurred within the ± 14 day window for this visit, this visit may be omitted.
[4] Pembrolizumab may be administered with a window of ±3 days from Day 1 of each scheduled week for a maximum of three doses.
[5] Given according to standard of care, beginning on Day 1 of Week 2 (± 3 days). Exact duration of dosing dependent on standard of care radiation plan.
[6] After the Wound Care Follow-Up Visit (or 90 Day Follow-Up Visit if the Wound Care Follow-Up visit is omitted), subjects will be contacted by telephone or medical record review every 12 weeks (± 2 weeks) to assess for disease status, recurrence, and survival status until 1 year after initiation of pembrolizumab, death, withdrawal of consent, or the end of the study, whichever occurs first. After the first year, then subjects will be contacted via telephone or medical record review every 6 months (± 2 months) to assess for disease status, recurrence, and survival status until 5 years after initiation of pembrolizumab, until death, withdrawal of consent, or the end of the study, whichever comes first.
[7] Female subjects of child-bearing potential must have a negative serum pregnancy test at screening. Subsequent cycles may be either serum or urine tests. If more than 72 hours elapses between screening negative serum pregnancy assessment and C1D1, the negative serum pregnancy should be reconfirmed prior to administration of C1D1 treatment.
[8] End of Treatment disease assessment via imaging must be completed within 3 weeks prior to planned surgery.
[9] Archival tissue may be used instead of fresh biopsy collection. If archival tissue is utilized at baseline, tissue must have been collected within 90 days prior to planned Week 1 Day 1. End of treatment tissue will be collected from surgical resection. If no surgical resection occurs, this tissue will not be collected.
[10] ECOG performance status must be confirmed within 7 days prior to the date of C1D1. If more than 7 days elapse between ECOG assessment and C1D1, the ECOG performance status should be reconfirmed prior to administration of C1D1 treatment.

9.0 TRIAL PROCEDURES

9.1 Trial Procedures

The Trial Flow Chart - Section 8.1 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 treating investigator.

Furthermore, additional evaluations/testing may be deemed necessary by the Sponsor-Investigator 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.

9.2 Screening Evaluations

Each subject who enters into the screening period for the study receives a unique subject identification number before any study-related procedures are performed. The subject identification number will be assigned. This number will be used to identify the subject throughout the clinical study and must be used on all study documentation related to that subject.

The subject identification number must remain constant throughout the entire clinical study; it must not be changed after initial assignment, including if a subject is rescreened.

A signed and dated Institutional Review Board (IRB) approved informed consent form (latest approved version) must be obtained from each subject prior to performing any study-specific procedures. All subjects must personally sign and date the consent form before commencement of study-specific procedures. Adverse events are to be collected for a subject once they have signed the informed consent.

Screening evaluations will be performed for all subjects to determine study eligibility. These evaluations must be obtained ≤28 days prior to enrollment, unless otherwise indicated. Baseline imaging should be obtained as close to initiation of protocol therapy as possible.

The following procedures are to be completed during the 28-day screening period, after signed informed consent has been obtained, designated in the Study Flow Chart.

- Demographics (if allowed by local regulations, date of birth, sex, race, and ethnicity)
- Physical examination (including physical exam, medical/cancer history, ECOG performance status assessment)
- Prior/concomitant medications and procedures evaluation: all medications taken, and procedures completed within ≤28 days prior to signing of informed consent
- Vital signs (height [screening only], weight, temperature, sys/dias blood pressure, respiration rate, and pulse)

- Local Laboratory Assessments: blood chemistry, complete blood count (CBC) + differential, complete urinalysis with microscopic evaluation, serum pregnancy test (women of child-bearing potential), thyroid function, PT/INR and aPTT
- CT or MRI must be done within the 28-day screening period, but preferably should be done as close to enrollment as possible. CT/MRI may be used from prior to consent, as long as no more than 28 elapse days between imaging date and enrollment date.
- Tumor tissue biopsy or archival tissue (within 90 days of pembrolizumab treatment initiation)

A subject is considered enrolled when the treating investigator or Sponsor-Investigator decides that the subject has met all eligibility criteria. The investigator is to document this decision and date, in the subject's medical record and/or in/on the electronic case report form (eCRF). Subjects should initiate treatment within 7 days of being enrolled to the study (28-day screening window from date of consent to enrollment date, plus up to an additional 7 days from enrollment date to C1D1 [total elapsed time from initiating screening to initiating therapy may not exceed 35 days]).

9.3 Treatment Period

A subject is considered on treatment when pembrolizumab is first administered. This is considered Week 1. Subjects should initiate treatment within 7 days of being enrolled to the study. Pembrolizumab is to be administered after all other protocol-specified pre-dose assessments have been performed during each visit that it is required. Subjects will continue therapy until completion of planned course of treatment, disease progression, or unacceptable AEs. Subjects should be instructed to immediately inform the Sponsor-Investigator (PI) of any AEs.

9.3.1 Day 1 Assessment

Subjects must be evaluated by the Sponsor-Investigator or delegated Sub-Investigator prior to each infusion. In general, these visits will be the day of the infusion but could be up to 2 days prior for all visits after Week 1.

The following assessments will be performed on Day 1 of Week 1:

- Physical examination
- Concomitant medication and procedures evaluation
- Vital signs (weight, temperature, sys/dias blood pressure, respiration rate, and pulse)
- ECOG performance status
- CBC with differential
- Thyroid function tests
- Clinical chemistry panel
- Urine or serum pregnancy test (women of child-bearing potential only)
- Urinalysis

- Adverse Event assessment
- Correlative studies blood collection

All Day 1 evaluations for Week 1 may be omitted if screening evaluations are performed within 72 hours of Week 1 Day 1.

The following assessments will be performed on Day 1 of Week 2 only (± 3 days):

- Initiation of radiation therapy

All Day 1 Week 2 assessments may be completed at a local site, if the subject is receiving radiation therapy locally. These assessments are considered routine care and can be obtained via medical record review.

The following assessments will be performed on Day 1 of Week 4 and Week 7 (± 3 days) unless otherwise indicated:

- Physical examination
- Concomitant medication and procedures evaluation
- Vital signs (weight, temperature, sys/dias blood pressure, respiration rate, and pulse)
- ECOG performance status
- CBC with differential
- Clinical chemistry panel
- Thyroid function tests (Week 7 only)
- Urine or serum pregnancy test (women of child-bearing potential only)
- Adverse Event assessment
- Correlative studies blood collection

9.3.2 Disease Assessment

Disease status will be assessed by CT or MRI scan. At baseline, imaging must include chest in addition to any other areas of known disease (i.e., if a subject's tumor is located in an extremity, the extremity must also be imaged). Image preparation and evaluation will follow the specifications provided in the RECIST version 1.1. The same modality (CT or MRI) must be used at screening and throughout the study.

CT/MRI scans to be performed at the following frequency:

- ≤28 days prior to enrollment
- End of Treatment Visit (± 14 days). Imaging must occur within 3 weeks prior to scheduled surgery.

An unscheduled scan for suspected disease recurrence/progression may be performed at any time at investigator discretion.

9.3.3 End of Treatment Visit

The End of Treatment visit should be conducted approximately 30 days (± 14 days) after the last dose of pembrolizumab. This visit may be scheduled to coincide with the standard of care outpaptient pre-operative surgical appointment. This visit must occur prior to any scheduled surgery.

The following assessments will be performed at the End of Treatment visit:

- Physical examination
- Concomitant medication and procedures evaluation
- ECOG performance status
- Urine or serum pregnancy test (women of child-bearing potential only)
- CBC with differential
- Clinical chemistry panel, including PT/INR and aPTT and thyroid function tests
- Adverse Event assessment
- Correlative studies blood collection
- Disease assessment via imaging studies
- Tumor tissue collection (to be obtained from planned surgical resection – may be omitted if surgical resection does not occur)

9.3.4 90 Day Follow-Up Visit

The 90 Day Follow-Up visit should be conducted approximately 90 days (± 14 days) after the last dose of pembrolizumab. This visit may be scheduled to coincide with the standard of care outpatient post-operative surgical appointment. This visit must be completed after any scheduled surgery.

The following assessments will be performed at the 90 Day Follow-Up visit:

- Physical examination
- Vital signs (weight, temperature, sys/dias blood pressure, respiration rate, and pulse)
- Concomitant medication and procedures evaluation
- ECOG performance status
- CBC with differential
- Clinical chemistry panel, including thyroid function tests
- Adverse Event assessment
- Correlative studies blood collection
- Post-study anticancer therapy and survival status

9.3.5 Wound Care Follow-Up Visit

The following assessments will be performed 30 days (± 14 days) after the standard of care post-operative visit:

- Physical examination
- ECOG performance status
- Post-study anticancer therapy and survival status
- Adverse event assessment, only to determine progression of surgical wound healing
-

Please review footnote 3 of Section 8.1 for further details regarding scheduling of this visit.

9.3.6 Survival Follow-up

Disease status and any subsequent anticancer therapy information status will continue to be monitored. Subjects will be contacted by telephone or medical record review every 12 weeks (± 2 weeks) after the 30 Day Follow-Up Visit to assess for disease status, recurrence, and survival status until 1 year after initiation of pembrolizumab therapy, death, withdrawal of consent, or the end of the study, whichever occurs first. After the first year, then subjects will be contacted either by telephone or medical record review every 6 months (± 1 month) to assess for disease status, recurrence, and survival status until 5 years after initiation of pembrolizumab, death, withdrawal of consent, or the end of the study, whichever comes first.

9.4 Clinical Procedures/Assessments

9.4.1 Assessing and Recording Adverse Events

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Study 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 5.0. Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

An adverse event is defined as any untoward medical occurrence in a subject 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), 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 pembrolizumab and/or radiation, is also an adverse event.

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

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

All adverse events that occur after the consent form is signed but before treatment initiation must be reported to the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

All adverse events must be recorded from the day informed consent is signed through the Wound Care Follow-Up visit. Such events will be recorded at each examination on the Adverse Event electronic case report forms and subject-specific worksheets.

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 last dose of pembrolizumab or 30 days following cessation of pembrolizumab if the participant initiates a new anticancer therapy – whichever is earlier should also be followed and recorded.

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

9.4.2.1 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 an other important medical event

Note: In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, are reportable to Merck in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by Merck for collection purposes.

- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose. For purposes of this study, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater. No specific information is available on the treatment of overdose of pembrolizumab. In the event of overdose, the participant should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

For the time period beginning when the consent form is signed until treatment is started, any serious adverse event, including death due to any cause must be reported within 24 hours to the Sponsor-Investigator and within 2 working days to Merck Global Safety only if

it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment initiation through 90 days following cessation of pembrolizumab or 30 days post following cessation of pembrolizumab if patient initiates a new anticancer therapy (whichever is earlier), any serious adverse event, including death due to any cause whether or not related to the Merck product, must be reported within 24 hours to the Sponsor-Investigator and within 2 working days to Merck Global Safety. Additionally, any SAE brought to the attention of an investigator at any time outside of the time period specified above must be reported immediately to Merck if the event is considered drug related.

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

SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety facsimile number: +1-215-661-6229

9.4.2.2 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported within 2 working days to Merck Global Safety (Attn: Worldwide Product Safety; FAX 215 661 6229).

For the time period beginning when the consent form is signed until treatment initiation, any ECI, that occurs to any subject must be reported within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment initiation through 120 days following cessation of treatment, any ECI, whether or not related to Merck product, must be reported 2 working days to Merck Global Safety.

Events of clinical interest for this trial include:

1. 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.
    a. 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.

An investigator who is a qualified physician will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 5.0. 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 6 - Evaluating Adverse Events

V5.0 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 for an elective procedure to treat a pre-existing condition that has not worsened is not a serious adverse event. A pre-existing condition is a clinical condition that is diagnosed prior to the use of a Merck product and is documented in the subject's medical history.); 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) **(**although not serious per ICH definition, is reportable to the Sponsor within 24 hours and to Merck within 2 working days to meet certain local requirements); **or**	

	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event for collection purposes. An overdose that is not associated with an adverse event must be reported within 24 hours to the Sponsor and to Merck within 2 working days.	
	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 Merck product to be discontinued?	
Relationship to Merck Product	Did Merck product cause the adverse event? The determination of the likelihood that 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 Merck product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely Merck product caused the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to 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 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

Relationship to Merck Product (continued)	**The following components are to be used to assess the relationship between the test drug and the AE: (continued)**	
	Dechallenge	Was Merck product discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the Sponsor's product; or (3) the trial is a single-dose drug trial); or (4) Sponsor's product(s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to Merck product in this study? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial); or (3) Sponsor's product(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 MERCK PRODUCT, OR IF REEXPOSURE TO MERCK PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE SPONSOR 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 Merck 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 Merck product relationship).**	

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

9.4.2.3 Sponsor Responsibility for Reporting Adverse Events

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

9.4.3 Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor-Investigator and to Merck

For purposes of this trial, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater. 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 the overdose of pembrolizumab, the adverse event(s) will be reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of pembrolizumab meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose will be reported 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-Investigator and within 2 working days to Merck Global Safety (Attn: Worldwide Product Safety; FAX 215-661-6229).

9.4.4 Reporting of Pregnancy and Lactation to the Sponsor-Investigator 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) that occurs during the trial.

Such events must be reported within 24 hours to the Sponsor-Investigator and within 2 working days to Merck Global Safety (Attn: Worldwide Product Safety; FAX 215 661-6229). Please refer to Section 7.3.4 for additional information on pregnancy and pregnancy reporting.

Exception: A positive pregnancy test at the time of initial screening is not a reportable event unless the participant has received study intervention.

9.4.5 Tumor Tissue Collection and Correlative Studies Blood Sampling

At time points specified in Study Flow Chart, up to 40 mL PBMC and 10 mL serum will be frozen to be analyzed for flow cytometry, functional analysis, cytokine analysis and other assays.

For research pretreatment biopsies, an estimated five to six core samples will be collected from pre-treatment biopsies: 2-3 cores will be formalin-fixed for immune-histochemistry; and 2-3 cores should be flash-frozen in RNALater. An additional 1-2 cores may be placed in RPMI and processed for flow cytometry, at investigator discretion. The cores will be used for future studies requiring RNA; lymphocyte expansion and tissue slice culture; and flow cytometry. If at least five cores are unable to be retrieved during the biopsy, the Sponsor-Investigator will make the determination on what testing will be done on each core that is procured. Allocation of cores into media may be adjusted at investigator discretion. Pre- and post-radiation samples will be tested for PD-L1 and PD-1 IHC staining.

If pre-treatment archival tissue (obtained within 90 days of Week 1) is used instead of fresh biopsy, the archival block should be split into a research block. If this is not feasible, archival tissue will not be accepted and the subject should undergo a research pre-treatment biopsy instead.

All subjects should have tissue available post-treatment. Because sarcomas are large, additional laboratory studies should be able to be performed on post-treatment tissue. If possible, 5-6 large aliquots of the fresh tumor resection will be collected. One to two aliquots should be placed in RNALater, one to two aliquots in RPMI (at Investigator's discretion), and the rest in formalin for creation of FFPE blocks. If excess tissue is not used, this will be preserved as part of the sarcoma tumor repository, if subjects consent to this. If tissue is not used and subjects do not indicate that they would like it stored, the tissue will be destroyed within two years from the end of the study.

Flow cytometry or multiplex IHC may be performed on both pre and post treatment samples in order to quantify T cell infiltrates and characterize T cell phenotype. Expression of inhibitory markers (PD-1, CTLA-4, LAG-3) will be tested on infiltrating T cells. Additionally, stromal immune cells will be characterized with respect to tumor associated macrophage phenotype (CD163, CD115, CD206). Expanded lymphocytes will be tested to determine tumor specificity against a single cell suspension made from the resection specimen. PBMC from pre and post treatment samples will be tested to determine if changes in T cell phenotype observed in the tumor are observed systemically. Additional or alternative analysis of tumor or blood specimens may be completed at investigator discretion.

9.4.6 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below in Table 7.

Table 7 - Laboratory Tests

Hematology	Chemistry	Urinalysis	Other
Hematocrit	Albumin	Blood	Serum β-human chorionic gonadotropin†
Hemoglobin	Alkaline phosphatase	Glucose	PT (INR)
Platelet count	Alanine aminotransferase (ALT)	Protein	aPTT
WBC (total and differential)	Aspartate aminotransferase (AST)	Specific gravity	Total thriiodothyronine (T3)
Red Blood Cell Count	Lactate dehydrogenase (LDH)	Microscopic exam *(If abnormal)*	Free tyroxine (T4)
Absolute Neutrophil Count	Carbon Dioxide	Urine pregnancy test †	Thyroid stimulating hormone (TSH)
Absolute Lymphocyte Count	Uric Acid		
	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		
	Creatinine		

† Perform on women of childbearing potential only. If urine pregnancy results cannot be confirmed as negative, a serum pregnancy test will be required.

10.0 STATISTICAL ANALYSIS PLAN

10.1 Statistical Analysis Plan Summary

The rationale for combining radiation and immunotherapy has been written about extensively [7-10]. However, these studies have focused on hypofractionated radiation in the metastatic setting and none of these published studies have examined the use of Pembrolizumab with the neoadjuvant radiation schedules that are typically used in the curative setting for many malignancies. Safety will be evaluated by CTCAE 5.0 and tabulated for qualitative description.

With regards to efficacy analysis, RECIST evaluations will be performed and tabulated in both qualitative and quantitative formats. This is a pilot trial that is not powered to give definitive determination of efficacy. As RECIST partial responses are not typically seen following neoadjuvant radiation alone, any objective responses would be highly interesting and encouraging of further study either for soft-tissue sarcomas generally or for a specific subtype where a response was observed.

The rate of complete necrosis will also be analyzed. It is assumed that the rate of complete necrosis in this subject population is approximately 15% after chemoradiation [18,19]. A design with 26 subjects has 94.4% power to detect an improvement in the complete necrosis rate from 15% to 40%. The observation of at least 7 subjects with complete necrosis would be considered evidence to rule out a 15% complete necrosis rate. With 26 subjects, binary proportions can be estimated to within 20%. Any toxicity with at least 10% prevalence has at least a 94% chance of being observed.

Event free survival and overall survival at 1 and 5 years will be determined in order to estimate the potential benefit in the highest risk subjects. We will generate Kaplan Meier curves to illustrate these overall survival rates and event-free survival rates. Using data already being analyzed under IRB approved protocols, we will compare these to historical controls at our institution using Cox's proportional hazard model. Subjects' demographic varialbes (such as age and gender) will be incorporated in the analysis.

Biologic specimens will be compared using gene expression, flow cytometry and mIHC between pre and post treatment samples. This data will be utilized to quantify cellular infiltrates and create a detailed profile of T cell and macrophage phenotypes. For most samples, it is expected to obtain sufficient cell counts to flow sort live single cell suspension samples, CD45 neg (tumor) populations, and CD4+, CD8+ and CD11B+ populations for RNA extractions so that the RNAseq results on individual cellular populations may be compared.

The study center is experienced in expanding tumor infiltrating lymphocytes (TIL), and in most cases is able to expand TIL to numbers sufficient for functional assays. All tumor samples will be subject to TIL expansion. If tumors demonstrate defined immunogenic antigens, cytokine release assays will be performed to interrogate changes in T cell function

between pre and post treatment samples. If relevant, either expanded TIL or single cell suspension samples may be sent for TCR sequencing to determine how therapy impacts the TCR repertoire.

A historical series has been collected of such samples treated with radiation alone (protocol #9013) and the change in these two data sets will be compared using two-sample T-test. Pathway analysis tools (such as Gene Set Enrichment Analysis) may also be used to evaluate if relevant biological pathways (such as immun-response pathways) show any alerations with respect to the therapy. Kaplan Meier curves depicting genes of interest, mIHC results and clinical outcomes will be reported. Cluster analysis based on gene expressions may be performed, and followed by the conduct of subtype analysis, where appropriate. Gene expression's impact on the survival time will be evaluated, if appropriate, and its AUC (area under the curve) will be reported if such biomarkers show up in anlaysis.

11.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

11.1 Investigational Product

The Sponsor-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 8.

Table 8 - Product Descriptions

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

11.2 Packaging and Labeling Information

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

11.3 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.

11.4 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.

11.5 Returns and Reconciliation

The Sponsor-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 Sponsor-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.

12.0 DATA AND SAFETY MONITORING PLAN

Institutional support of trial monitoring will be in accordance with the FHCC/University of Washington Cancer Consortium Institutional Data and Safety Monitoring Plan. Under the provisions of this plan, FHCC Clinical Research Support (CRS) coordinates data and compliance monitoring conducted by consultants, contract research organizations, or FHCC employees unaffiliated with the conduct of the study. Independent monitoring visits occur at specified intervals determined by the assessed risk level of the study and the findings of previous visits per the institutional DSMP.

In addition, protocols are reviewed at least annually and as needed by the Consortium Data and Safety Monitoring Committee (DSMC), FHCC Scientific Review Committee (SRC) and the FHCC/University of Washington Cancer Consortium Institutional Review Board (IRB). The review committees evaluate accrual, adverse events, stopping rules, and adherence to the applicable data and safety monitoring plan for studies actively enrolling or treating subjects. The IRB reviews the study progress and safety information to assess continued acceptability of the risk-benefit ratio for human subjects. Approval of committees as applicable is necessary to continue the study.

The trial will comply with the standard guidelines set forth by these regulatory committees and other institutional, state and federal guidelines.

13.0 ADMINISTRATIVE AND REGULATORY DETAILS

13.1 Institutional Review Board

In accordance with federal regulations (21 CFR 312.66), an Institutional Review Board (IRB) that complies with regulations in 21 CFR 56 must review and approve this protocol and the informed consent form prior to initiation of the study.

13.2 Consent

The Sponsor-Investigator or his associate must explain verbally and in writing the nature, duration, and purpose of the study and possible consequences of treatment. Subjects must also be informed that they may withdraw from the study at any time and for any reason without jeopardizing their future treatment. In accordance with federal regulations (21 CFR 312), all subjects must sign the IRB-approved consent form.

13.3 Termination of Study

The Sponsor-Investigator reserve the right to terminate this study at any time. The FDA may also terminate the study.

13.4 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 requirements for 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.

14.0 REFERENCES

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15.0 APPENDICES

15.1 Appendix A: Performance Status

15.1.1 ECOG Perfomance Status

Table 9 - 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.	

15.1.2 Karnofsky Performance Status

Table 10 - Karnofsky Performance Status

Karnofsky Status	Karnofsky Grade
Normal, no complaints	100
Able to carry on normal activities. Minor signs or symptoms of disease	90
Normal activity with effort	80
Care for self. Unable to carry on normal activity or to do active work	70
Requires occasional assistance, but able to care for most of his needs	60
Requires considerable assistance and frequent medical care	50
Disabled. Requires special care and assistance	40
Severely disabled. Hospitalization indicated though death no imminent	30
Very sick. Hospitalization necessary. Active supportive treatment necessary	20
Moribund	10
Dead	0

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15.2 Appendix B: Women of Childbearing Potential Definitions and Methods of Contraception

15.2.1 Definitions

15.2.1.1 Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming postmenopausal unless permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy, and bilateral oophorectomy.

Women in the following categories are not considered WOCBP

- Premenarchal
- Premenopausal female with 1 of the following:
    - Documented hysterectomy
    - Documented bilateral salpingectomy
    - Documented bilateral oophorectomy
      Note: Documentation can come from the site personnel's review of the participant's medical records, medical examination, or medical history interview.
- Postmenopausal female
    - A postmenopausal state is defined as 12 months of amenorrhea in a woman over age 45 years in the absence of other biological or physiological causes.

15.2.2 Contraception Guidance for Women of Childbearing Potential

One of the highly effective methods of contraception listed below is required during study duration and until the end of relevant systemic exposure, defined as 120 days after last treatment. Local laws and regulations may require use of alternative and/or additional contraception methods.

Highly Effective Contraceptive Methods That Are User Dependent

Failure rate of < 1% per year when used consistently and correctly.[a]

- Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation
    - oral
    - intravaginal
    - transdermal
- Progestogen-only hormonal contraception associated with inhibition of ovulation
    - oral
    - injectable

Highly Effective Methods That are User Independent

- Implantable progestogen-only hormonal contraception associated with inhibition of Ovulation
- Intrauterine device (IUD)
- Intrauterine hormone-releasing system (IUS)
- Bilateral tubal occlusion
- Vasectomized partner
- Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in related to the duration of the study and the preferred and usual lifestyle of the participant.
    - It is not necessary to use any other method of contraception when complete abstinence is elected.
- WOCBP participants who choose complete abstinence must continue to have pregnancy tests as specified in study calendar.
- Acceptable alternate methods of highly effective contraception must be discussed in the
  event that the WOCBP participants chooses to forego complete abstinence.

[a] *Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for participants participating in clinical studies.*

Less Than Highly Effective Contraceptive Methods That are User Dependent

Failure rate of > 1% per year when used consistently and correctly.

- Male or female condom with or without spermicide. Male and female condoms cannot be used simultaneously.
- Diaphragm with spermicide
- Cervical cap with spermicide
- Vaginal sponge with spermicide
- Progestogen-only oral hormonal contraception, where inhibition of ovulation is not the
  primary mechanism of action

Unacceptable Methods of Contraception

- Periodic abstinence (calendar, symptothermal, post-ovulation methods)
- Withdrawal (coitus interruptus)
- Spermicide only
- Lactation amenorrhea method (LAM)

15.2.3 Contraception Guidance for Males of Childbearing Potential

Male participants with female partners of childbearing potential are eligible to participate if they agree to the following during the treatment and until the end of relevant systemic exposure.

- Inform any and all partner(s) of their participation in a clinical drug study and the need to comply with contraception instructions as directed by the investigator.
- Male participants are required to use a condom for study duration and until the end of relevant systemic exposure defined as 120 days after last systemic dose.
- Female partners of males participating in the study to consider use of effective methods of contraception until the end of relevant systemic exposure, defined as 120 days after last systemic dose.
- Male participants with a pregnant or breastfeeding partner must agree to remain abstinent from penile vaginal intercourse or use a male condom during each episode of penile penetration during the treatment and until 120 days after last systemic dose.
- Refrain from donating sperm for the duration of the study treatment and for 120 days after last systemic dose.