Single Arm Phase II Study to Assess the Efficacy of Pembrolizumab Plus Radiotherapy or Ablation in Metastatic Colorectal Cancer Patients

PROTOCOL FACE PAGE FOR
MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

Principal Investigator/Department:	Neil H. Segal, MD, PhD	Medicine
Co-Principal Investigator(s)/Department:	Leonard B. Saltz, MD Josh Yamada, MD Stephen Solomon, MD	Medicine Radiation Oncology Interventional Radiology
Investigator(s)/Department:	Ghassan Abou-Alfa, MD Andrea Cercek, MD Andrew Epstein, MD James Harding, MD David Ilson, MD, PhD Yelena Janjigian, MD David Kelsen, MD Nancy Kemeny, MD Geoffrey Ku, MD Diane Reidy-Lagunes, MD Eileen O‟ Reilly, MD Armin Shahrokni, MD Zsofia K. Stadler, MD Anna Varghese, MD Rona Yaeger, MD Kenneth Yu, MD Nitya Raj, MD Abraham Wu, MD Constantinos Sofocleous, MD Joseph P. Erinjeri, MD Jill Gluskin, MD Vaios Hatzoglou, MD Ellen Hollywood, NP Erica Kaufman, NP Patricia Gabriel, NP Jinru Shia, MD Marinela Capanu, PhD	Medicine Medicine Medicine Medicine Medicine Medicine Medicine Medicine Medicine Medicine Medicine Medicine Medicine Medicine Medicine Medicine Medicine Radiation Oncology Interventional Radiology Interventional Radiology Radiology Radiology Nursing Nursing Nursing Pathology Epidemiology and Biostatistics
Consenting Professional(s)/Department:	Ghassan Abou-Alfa, MD Andrea Cercek, MD Andrew Epstein, MD James Harding, MD David Ilson, MD, PhD Yelena Janjigian, MD David Kelsen, MD Nancy Kemeny, MD Geoffrey Ku, MD	Medicine Medicine Medicine Medicine Medicine Medicine Medicine Medicine Medicine

	Diane Reidy-Lagunes, MD	Medicine
	Eileen O'Reilly, MD	Medicine
	Armin Shahrokni, MD	Medicine
	Leonard B. Saltz, MD	Medicine
	Neil H. Segal, MD, PhD	Medicine
	Zsofia K. Stadler, MD	Medicine
	Anna Varghese, MD	Medicine
	Rona Yaeger, MD	Medicine
	Kenneth Yu, MD	Medicine
	Nitya Raj, MD	Medicine
	Ellen Hollywood, NP	Nursing
	Erica Kaufman, NP	Nursing
	Patricia Gabriel, NP	Nursing

Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

Memorial Sloan-Kettering Cancer Center
1275 York Avenue
New York, New York 10065

Table of Contents

List of Abbreviations

Abbreviation or Specialized Term	Definition
ADA	anti-drug antibody
ADCC	antibody-dependent cell-mediated cytotoxicity
AE	adverse event
ALT	alanine aminotransferase
APC	antigen presenting cells
AST	aspartate aminotransferase
CD	cluster of differentiation
CI	confidence interval
CIS	carcinoma in situ
C_{max}	maximum observed concentration
CNS	central nervous system
CR	complete response
CRC	colorectal cancer
CRF	case report form
CT	computed tomography
CTC	circulating tumor cells
CTCAE	Common Terminology Criteria for Adverse Events
CTLA-4	cytotoxic T-lymphocyte antigen 4
DC	disease control
DCR	disease control rate
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
DR	duration of response
ECG	electrocardiogram
ECOG	Eastern Cooperative Oncology Group
ELISA	enzyme-linked immunosorbent assays
EMA	European Medicines Agency
EOI	end of infusion
EU	European Union
Fc	fragment crystallizable
FFPE	formalin fixed paraffin embedded
FTIH	First-time-in-human

Abbreviation or Specialized Term	Definition
GCP	Good Clinical Practice
GI	gastrointestinal
HCl	hydrochloride
HCV	hepatitis C virus
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
ICF	informed consent form
ICH	International Conference on Harmonisation
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
IFN	interferon
IgG1κ	immunoglobulin G1 kappa
IHC	immunohistochemistry
IL	interleukin
IM	immunogenicity
IRB	Institutional Review Board
irAE	immune-related adverse event
irRC	immune-related response criteria
IV	intravenous(ly)
IVRS	interactive voice response system
IWRS	interactive web response system
MAb	monoclonal antibody
MedDRA	Medical Dictionary for Regulatory Activities
MDSC	Myeloid derived suppressor cell
miRNA	micro ribonucleic acid
MMR	mismatch repair
mRNA	messenger ribonucleic acid
MRI	magnetic resonance imaging
MRSD	maximum recommended starting dose
MTD	maximum tolerated dose
NCI	National Cancer Institute
NOAEL	no-observed adverse-effect-level
NSCLC	non-small cell lung cancer

Abbreviation or Specialized Term	Definition
OBD	optimal biological dose
ORR	objective response rate
OS	overall survival
PBMC	peripheral blood mononuclear cell
PD	progressive disease
PD-1	programmed death 1
PD-L1	programmed death ligand 1
PFS	progression-free survival
PK	pharmacokinetics
PR	partial response
Q2W	every 2 weeks
RCC	renal cell carcinoma
RECIST	Response Evaluation Criteria in Solid Tumors
RFA	Radiofrequency ablation
RT	Radiation therapy
SAE	serious adverse event
SD	stable disease
SID	subject identification
SMC	Safety Monitoring Committee
SPD	sum of products of diameters
SRT	Safety Review Team
SUSAR	suspected unexpected serious adverse reaction
TIL	tumor infiltrating lymphocyte
TNF-α	tumor necrosis factor alpha
TSH	thyroid-stimulating hormone
ULN	upper limit of normal
US FDA	United States Food and Drug Administration
USA	United States of America
WFI	water for injection
WHO	World Health Organization
w/v	weight/volume

1.0 PROTOCOL SUMMARY AND/OR SCHEMA

Name of Investigational drug: Pembrolizumab	
Title of Study: Single Arm Phase II Study to Assess the Efficacy of Pembrolizumab Plus Radiotherapy or Ablation in Metastatic Colorectal Cancer Patients	
Study Centers: MSKCC	
Study Period: 24 months	**Phase of Development:** II
Objectives: The **primary objective** is Overall Response Rate in disease that is not ablated or radiated (Complete Response and Partial Response). The **secondary objectives** are: 1. Safety and tolerability of Pembrolizumab plus radiotherapy (RT) or ablation 2. Overall Survival of pembrolizumab plus RT or ablation 3. Progression free survival rate at 1 year 4. Progression free survival rate at 2 years The **exploratory objectives** are to evaluate biomarkers that may correlate with activity of pembrolizumab plus RT or pembrolizumab plus ablation in colorectal cancer (CRC), or prospectively identify CRC patients likely to respond to treatment.	
Study Design: This will be a Simon two-stage design, phase II study. It will be conducted to determine the efficacy and safety of (1) pembrolizumab plus RT in subjects with metastatic CRC who are undergoing RT as standard therapy; and, (2) pembrolizumab plus ablation in subjects with metast atic CRC who are undergoing ablation as standard therapy. Eligible patients will have metastatic colorectal cancer and received at least two prior standard therapy for metastatic disease. Patients will be stratified according to eligibility for RT (cohort 1) or ablation (cohort 2). Patients in **cohort 1** will have at least one locally advanced or metastatic lesion for which palliative RT is considered appropriate standard therapy, and at least one other measurable index lesion that will not undergo RT. Patients in **cohort 2** will have at least one metastatic lesion for which palliative ablation is considered appropriate standard therapy, and at least one other measurable index lesion that will not undergo ablation. All subjects will undergo ablation or begin RT within 1 week prior to dose 1 of pembrolizumab. Subjects will receive pembrolizumab via IV infusion at 200 mg every three weeks (Q3W), and continue treatment Q3W until progression of disease, initiation of alternative cancer therapy, unacceptable toxicity, or other reasons to discontinue treatment occur, up to 24 months. Patients who had a prior response or stable disease without another reason to discontinue therapy may resume treatment for an additional 12 months upon disease progression. Repeat palliative RT in cohort 1, or ablation in cohort 2, will be permitted in select cases for the treatment of isolated lesions, provided a target lesion remains. Patients will be evaluated by physical exam and routine blood tests every three weeks during the study period. CT or MRI will be performed during screening, and then at 9 week intervals. Tumor measurements and deter mination of tumor responses will be performed according to RECIST 1.1. Subjects may continue to receive pembrolizumab beyond radiographic progression in the absence of clinical deterioration, and after discussion with the Principal Investigator. All subjects will be followed up to 2 years for survival or until the study closes. The primary endpoint of this trial is the response rate in CRC treated with RT plus pembrolizumab (cohort 1) or ablation plus pembrolizumab (cohort 2). A two-stage Simon"s optimal design will be employed to test the null hypothesis that the true response rate is ≤5% versus the alternative hypothesis that the true response rate is at least 25% with type I and II error rates of 10% each. Each cohort will be evaluated separately for this purpose. In the first stage, we will accrue 9 patients in each cohort. If 0 objective tumor responses (PR or CR) are observed among the 9 subjects treated in a cohort, then subject enrollment will be terminated in that cohort. If at least 1 response is observed among the 9 subjects treated in a cohort, then the study will be expanded to enroll a total of 24 treated subjects in that cohort. At the end of the study, if 2 or less objective tumor responses are observed in a cohort, then the study will be considered not worthy of further investigation in that particular cohort. If at the end of the study ≥3 tumor responses per RECIST 1.1 are observed in a cohort, then further investigation of pembrolizumab plus RT and/or pembrolizumab plus ablation will be considered worthwhile. The study will complete when all subjects have either progressed or discontinued from the study for other reasons. This study requires accrual of a minimum of 18 subjects and up to a maximum of 48 subjects if both cohorts are expanded to the second stage. The accrual time is estimated to be 2 years. Tumor biopsies for research purposes will be	

mandatory in all patients. Pre-treatment biopsies will be obtained during fiducial placement for RT, at the time of ablation, or during a separate procedure. On treatment biopsies will be performed,1 week after completing RT or undergoing ablation (of the radiated/ ablated lesion) and then 4 weeks after dose #1 of pembrolizumab (of a non-radiated/ ablated lesion). Tumor immunohistochemistry may be performed for PD-L1 expression and quantification of tumor infiltrating lymphocyte (TIL) subtypes, including CD8+ T-cells, CD4+ T-cells, Regulatory T-cells (Treg), and Myeloid Derived Suppressor Cells (MDSCs). Peripheral blood samples will be obtained in all patients at baseline then weeks 3, 6, and 9 for all patients. Additional blood draws beyond week 9 are permitted based upon interesting clinic/immunological findings. Plasma may be analyzed for change in antibody responses to a broad panel of antigens (seromics). Flow cytometry may be performed for peripheral blood immune cell phenotype and their activation status, including CD8+ T-cells, CD4+ T-cells, Tregs, and MDSCs. The amount of PDL-1 expression, changes in immune cell repertoire and activation status within the tumor and peripheral blood as well as immune responses against specific tumor antigens will be correlated with clinical outcome in an exploratory manner in order to identify predictive makers for response, and to further our understanding of pembrolizumab plus RT, and pembrolizumab plus ablation in CRC.

Diagnosis and Main Criteria for Inclusion in the Study:

Inclusion Criteria

1. Willing and able to provide written informed consent.
2. Histologically- or cytologically- confirmed CRC.
3. Locally advanced or metastatic CRC.
4. Subjects have received at least two standard available therapies.
5. At least one tumor for which palliative RT is considered appropriate standard therapy (cohort 1); or, at least one tumor for which palliative ablation is considered appropriate standard therapy (cohort 2).
6. At least one index lesion that will not undergo RT or ablation, and is measurable based on RECIST 1.1.
7. ≥ 18 years of age on day of signing informed consent.
8. Consent for tumor biopsies and blood draws for research purposes.
9. Consent for use of available archived tissue for research purposes.
10. Performance status of ECOG 0 or 1.
11. Adequate organ function, defined as:
    - Absolute Neutrophil Count ≥ 1,500/mm^3.
    - Platelet count ≥ 100,000/mm^3.
    - Serum creatinine ≤ 1.5 x ULN or CrCl ≥60 mL/min.
    - AST and ALT ≤ 2.5 × ULN or ≤ 5 × ULN for subjects with liver metastases.
    - Bilirubin ≤ 1.5 × ULN or Direct bilirubin ≤ ULN.
    - INR/PT and PTT ≤1.5 X ULN unless on anticoagulant therapy and PT/PTT within therapeutic range.
12. Adequate method of contraception.

Exclusion Criteria

1. Currently participating in/ has participated in an investigational study within 4 weeks.
2. Diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial t reatment.
3. Prior monoclonal antibody within 4 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered > 4 weeks earlier.
4. Prior chemotherapy, targeted small molecule therapy, within 2 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent (exc. Grade 2 neuropathy).
5. If subject received major surgery, they must have recovered adequately from the t oxicity and/or complications from the intervention prior to starting therapy.
6. Known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or in s itu cervical cancer that has undergone potentially curative therapy.
7. Known CNS metastases and/or carcinomatous meningitis. Subjects with previously treated

	brain metastases may participate provided they are stable without evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to trial treatment.
8.	Active autoimmune disease requiring systemic treatment within the past 3 months or a documented history of clinically severe autoimmune disease, or a syndrome that requires systemic steroids or immunosuppressive agents.
9.	Interstitial lung disease or active, non-infectious pneumonitis.
10.	Active infection requiring systemic therapy.
11.	History or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject"s participation for the full duration of the trial, or is not in the best interest of the subject to p articipate, in the opinion of the treating investigator.
12.	Known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
13.	Pregnant 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.
14.	Prior therapy with an anti-P D-1, anti-P D-L1, anti-P D-L2, anti-CD137, or anti-Cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) antibody (including ipilimumab or any other antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways).
15.	Known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).
16.	Known active Hepatitis B or C.
17.	Live vaccine within 30 days prior to the first dose of trial treatment.
Number of Subjects: 18 to 48, including patients in cohort 1 (n= 9 to 24) and cohort 2 (n=9 to 24).	

2.0 OBJECTIVES AND SCIENTIFIC AIMS

- The primary objective is Overall Response Rate in disease that is not ablated or radiated of pembrolizumab plus RT or pembrolizumab plus ablation in subjects locally advanced/metastatic CRC, according to RECIST v1.1.
- The secondary objectives are to determine:
    - Safety and tolerability of pembrolizumab plus RT or ablation
    - Overall Survival of pembrolizumab plus RT or ablation
    - Progression free survival rate at 1 year
    - Progression free survival rate at 2 years
- The exploratory objectives are to evaluate biomarkers that may correlate with activity of pembrolizumab plus RT or ablation in CRC, or prospectively identify CRC patients likely to respond to treatment.

3.0 BACKGROUND AND RATIONALE

3.1 Colorectal cancer

Worldwide, CRC is the third most common form of cancer in men, with 663,000 cases (10% of the total) and second most common in women, with 571,000 cases (9.4% of the total) per year. Each year there are about 608,000 deaths from colon cancer which is approximately 8% of all cancer deaths making colorectal cancer the fourth most common cause of cancer death [1]. In 2012 in the U.S. an estimated 103,170 new cases were diagnosed with 51,690 deaths [2]. Treatment of CRC depends largely on the stage of the disease, which is most commonly rated according to tumor, nodes, and metastasis (TNM) criteria. The initial treatment is surgery. However, post-surgery metastatic disease occurs in 40%-60% of patients and the prognosis for patients who develop advanced metastatic disease is poor. Over the past decade, progress has been made in the role of systemic therapy for the palliation of advanced colorectal cancer. With the introduction of oxaliplatin, irinotecan, anti-VEGF therapies, and anti-EGFR therapies, the median life expectancy of patients has been increased to about 29 months [3]. Despite these therapeutic advances, patients with unresectable, metastatic and/or recurrent CRC, remain incurable. There is a substantial unmet medical need for more effective and less toxic therapies, especially for those patients with advanced disease that have not responded or have become resistant to the existing standard treatments. The development of novel approaches to treatment is greatly needed in order to improve outcomes in such patients.

3.2 Immune Augmentation in Colorectal Cancer

Immune augmentation, including checkpoint-directed therapies, has been studied in patients with CRC, and isolated tumor responses have been observed. Our group performed a study of CTLA-4 blockade with tremelimumab (15 mg/kg every 90 days) in 47 patients with previously treated CRC. Grade 3/4 treatment-related adverse events (AEs) included diarrhea (n = 5; 11%), ulcerative colitis (n = 1; 2%), fatigue (n = 1; 2%), autoimmune thrombocytopenia (n = 1; 2%), and hypokalemia (n = 1; 2%). Of 45 response-evaluable patients, 44 did not reach second dose

(43 progressive disease; one discontinuation). Twenty-one patients (45%) lived ≥ 180 days after enrollment. One patient (2%; 90% CI, < 1% to 10%) had a stable pelvic mass and substantial regression in an adrenal mass (partial response) [4]. Another study was a dose escalation study of PDL-1 blockade with MPDL3280A (0.01-20 mg/kg every three weeks) in 20 patients with solid tumors and lymphoma. Tumor regression was observed in 1 of 4 patients with CRC (partial response). In a first-in-human single-dose, dose-escalation (0.3 to 10 mg/ kg) study of PD-1 blockade with nivolumab in solid tumors, 14 patients with CRC were enrolled. In the phase I multi-dose, dose escalation (1 to 10 mg/kg every 2 weeks) study, 19 patients with CRC were enrolled. Treatment was reasonably well tolerated in both studies. Durable tumor regression (complete response) beyond 3 years was observed in one patient [5-7].

3.3 Rationale for immune therapy plus radiotherapy or ablation in colorectal cancer

RT and ablation, such as cryoablation or radiofrequency ablation, are both standard therapies in patients with metastatic colorectal cancer. Ablation results in tumor destruction by direct heat or cold, and RT results in tumor destruction by ionizing radiation. Either ablation or RT alone is not expected to result in objective systemic benefit alone. However, each modality has been associated with induction of systemic immunity that can be augmented by co-stimulatory blockade with PD-1 leading to potential for tumor shrinkage.

The phenomenon of tumor destruction by RT or ablation leading to shrinking of tumors away from the site of initial treatment has been termed the ***abscopal effect***. Albeit rare, when the abscopal effect occurs, the clinical implications can be profound.

The rationale of the abscopal effect is that *in situ* tumor destruction releases a large amount of tumor antigens. Antigen-presenting cells, such as dendritic cells, then take up these antigens in the periphery and migrate to lymph nodes where they activate CD4+ and CD8+ T-lymphocytes that recognize these tumor-antigens. Immune augmentation via immune co-stimulatory molecules then permits the ensuing immune response to strengthen and destroy cancer.

The combination of tumor cryoablation and immunomodulation has been shown in animal models, by our colleagues, to generate such a systemic anti-tumor response [8]. Specifically, local tumor destruction with cryoablation can lead to exposure of DCs with sufficient quantities of tumor antigens to ultimately lead to DC maturation and activation. Recently, our collaborators (Dr. James Allison‟s group at MSKCC) showed that combination therapy with cryoablation and CTLA4 blockade successfully mediated rejection of metastatic prostate cancer lesions and prevented the growth of secondary tumors in preclinical murine models [8]. Levy et al. from MSKCC showed in a BALB/c mouse model of metastatic colon cancer that cryoablation of a solitary tumor mass plus cyclophosphamide, to selectively depletes regulatory T cells (Tregs), leads to regression of established tumors and protection against tumor rechallenge [9]. Den Brok et al. have also demonstrated this effect *in vivo* using the well-defined murine B16-OVA melanoma cell line using RFA. They demonstrated that RFA alone results in a weak but detectable immune response against OVA, as well as other B16 antigens. Whereas, the combination of co-stimulatory blockade by anti-CTLA-4 together with RFA approximately

doubled the number of mice alive at 70 days post-tumor rechallenge compared with mice that received control antibody plus ablation alone [10].

A similar effect has been shown with RT and blockade of PD-1/PDL-1 interaction. Deng et al. showed that anti-PDL-1 enhanced the effect of RT in a MC38 colorectal tumor model, with substantial delay in tumor growth 34 days after treatment with combination therapy. Tumors measured 27.85 (± 27.85) mm after combination therapy, compared to 278.6 (± 94.20) mm and 457.6 (± 44.24) mm after RT alone, or anti-PDL-1 alone, respectively. A similar benefit was shown in the TUBO breast cancer cell line, with reduced TUBO tumor growth after mice were re-challenged in the opposite flank, implying systemic immunity. This immunological effect was associated with activation of CD8+ T-cells and reduced tumor-infiltrating myeloid-derived suppressor cells (MDSC) [11].

More recently, Dr. Postow from our group reported this abscopal effect in a patient who developed a systemic response to localized RT after having had disease progression while receiving CTLA-4 blockade (ipilimumab). Specifically, a right hilar lymph-node and spleen metastases, which were not the target of RT, showed regression only after the patient received palliative RT after several months of CTLA-4 blockade. A delayed response to the ipilimumab was considered unlikely [12].

The preclinical models and the clinical observation provide compelling rationale to further study tumor ablation and RT in combination with immune augmentation by co-stimulatory blockade. Pembrolizumab is an excellent choice, as this agent has already demonstrated activity in cancer. CRC is an appropriate target for such a study because this disease is (1) frequently associated with Tumor Infiltrating Lymphocytes (TILs), is (2) routinely treated with combination therapies including RT or ablation, and (3) there is substantial unmet need for novel therapies beyond the currently available systemic therapies.

3.3 Pembrolizumab

3.3.1 Pharmaceutical and Therapeutic Background

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decade. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies [13-16]. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors.

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane

glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD -L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues [13]. 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 (MEL). 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.

Pembrolizumab (previously known as MK-3475 or SCH 900475) is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2.

3.3.2 Preclinical and Clinical Trial Data

3.3.2.1 Non-Clinical Toxicology Summary of results

In the 1-month and 6-month toxicology study in cynomolgus monkeys, pembrolizumab administered once a week and once every other week respectively, intravenously up to 200 mg/kg resulted in no adverse treatment related effects. The exposure multiple based on a predicted $AUC_{0\text{-}tau}$ of 4464 µg/day/mL at the maximum anticipated human clinical dose of 10 mg/kg Q2W or Q3W is 15-fold at 200 mg/kg, the NOAEL for the 6-month monkey study. Additionally, in the tissue cross-reactivity study of pembrolizumab with human and monkey tissues demonstrated the expected on-target staining of the membranes of mononuclear leukocytes in both species. Off-target cross-reactivity staining was also noted in both species but was limited to cytoplasm of various cell types/tissues and the stroma (extracellular connective tissue matrix), and was considered related to the

experimental method artifacts, i.e. tissue processing for IHC, that are well recognized limitations of tissue cross-reactivity studies and, thus not considered toxicologically relevant.

No reproductive or developmental toxicity studies are planned with pembrolizumab. Therefore, inclusion of women of childbearing potential in clinical trials should be in accordance with the study protocol and applicable regulatory guidance (e.g., ICH M3(R2): Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals).

3.3.2.2 Clinical Summary of results

As of 18-Oct-2013, 1,000 patients have been treated with pembrolizumab at several dose- schedules, including 10 mg/kg every 2 weeks. Pembrolizumab has been generally well tolerated, as expected based on preclinical findings and other anti-PD-1 monoclonal antibodies. As of 18-Oct-2013 no serious infusions reactions have been reported in PN001, however, since the potential exists in anti-PD-1 monoclonal antibodies, investigators should be vigilant to this possibility. Less than 1% of patients thus far assayed had confirmed positive ADA samples and among these, no or no clear impact on exposure has been observed. There is no contraindication to further clinical investigation with pembrolizumab. Pharmacokinetics were as expected, based on pembrolizumab being an IgG mAb and based on preclinical data, which support dosing once every 2 or 3 weeks. pembrolizumab monotherapy induces an ORR of 25%/27% in patients with ipilimumab- exposed melanoma by central independent RECIST and oncology review/investigator assessed irRC, respectively. Pembrolizumab monotherapy induces an ORR of 39%/43% in patients with ipilimumab-naive melanoma by central independent RECIST and oncology review/investigator assessed irRC, respectively. These responses are remarkably durable. The preliminary 1-year survival rate for patients, many of whom have had multiple therapies, including ipilimumab, who receive pembrolizumab is 81%. Pembrolizumab monotherapy induces an ORR of 21%/24% in patients with previously-treated NSCLC by central independent RECIST/investigator assessed irRC, respectively, with these responses also remarkably durable. Preliminary data suggest higher levels of PD-L1 expression in tumors of NSCLC are associated with increased activity (ORR 67% by investigator assessed irRC/57% by central independent RECIST); additional data are required to define the optimal PD-L1 cut point.

The most commonly reported treatment emergent AEs experienced are fatigue (43.8%), nausea (26.7%), cough (25.3%), pruritus (24.6%), diarrhea (22.3%) and rash (21.5%). Immune-related adverse events were reported in 21.4% of melanoma patients; most of these events (15.8%) were considered drug-related by the investigator. The most commonly reported, immune-related adverse events across the dose-schedules are rash (3.2%), pruritus (2.9%), vitiligo (2.9%), hypothyroidism (2.7%), arthralgia (2.2%), diarrhea (2.2%), and pneumonitis (1.9%). Review of the overall benefit:risk ratio of pembrolizumab favors enrollment of eligible patients into clinical trials of pembrolizumab.

3.3.2.3 Rationale for Dose Selection/Regimen/Modification

An open-label Phase I trial (Protocol 001) was conducted to evaluate the safety and clinical activity of single agent pembrolizumab. The dose escalation portion of that trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors. 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. Recent data from other clinical studies within the pembrolizumab program has shown that a lower dose of pembrolizumab and a less frequent schedule may be sufficient for target engagement and clinical activity.

PK data analysis of pembrolizumab administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life (refer to IB). Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days). This early PK and pharmacodynamic data provides scientific rationale for testing a Q2W and Q3W dosing schedule.

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. 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 norm alized dosing or a fixed dose across all body weights. Pembrolizumab has been found to have a wide therapeutic range based on the melanoma indication.

MK-3475 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 MK-3475 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 NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

The rationale for further exploration of 2 mg/kg and comparable doses of pembrolizumab in solid tumors is based on: 1) similar efficacy and safety of pembrolizumab when dosed at either 2 mg/kg or 10 mg/kg Q3W in melanoma patients, 2) the flat exposure-response relationships of pembrolizumab for both efficacy and safety in the dose ranges of 2 mg/kg Q3W to 10 mg/kg Q3W, 3) the lack of effect of tumor burden or indication on distribution behavior of pembrolizumab (as assessed by the population PK model) and 4) the assumption that the dynamics of pembrolizumab target engagement will not vary meaningfully with tumor type.

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 patient exposures in the exposure range established in melanoma as associated with maximal efficacy response and 3) will maintain individual patients 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.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

This will be a Simon two-stage design, phase II study. It will be conducted to determine the efficacy and safety of (1) pembrolizumab plus RT in subjects with metastatic CRC who are undergoing RT as standard therapy; and, (2) pembrolizumab plus ablation in subjects with metastatic CRC who are undergoing ablation as standard therapy.

4.2 Intervention

Patients will be stratified according to eligibility for RT (cohort 1) or ablation (cohort 2). Patients in **cohort 1** will have at least one locally advanced or metastatic lesion for which palliative RT is considered appropriate standard therapy, and at least one other measurable index lesion that will not undergo RT. Patients in **cohort 2** will have at least one metastatic lesion for which palliative ablation is considered appropriate standard therapy, and at least one other measurable index lesion that will not undergo ablation. Subjects in cohort 2 will undergo ablation of one or more lesions. The ablation method is at the discretion of the interventional radiologist. All subjects will undergo ablation or begin RT within 7 days prior to starting pembrolizumab. Pembrolizumab dosing may continue during RT. Subjects will receive pembrolizumab via IV infusion at 200 mg every three weeks (Q3W), and continue treatment Q3W until progression of disease, initiation of alternative cancer therapy, unacceptable toxicity, or other reasons to discontinue treatment occur, up to 24 months. Patients who had a prior response without another reason to discontinue therapy after 24 months will continue clinic visits, routine blood work (CBC, COMP, CEA) and CT/MRI every 9 weeks, and may resume treatment for an additional 12 months upon disease progression. Repeat palliative RT in cohort 1, or ablation in cohort 2, will be permitted in select cases for the treatment of isolated, non-target lesions. Patients will be evaluated by physical exam and routine blood tests every three weeks during the study period. CT or MRI will be performed during screening, and then at 9 week intervals. Tumor measurements and determination of tumor responses will be performed according to RECIST 1.1.

Subjects may continue to receive pembrolizumab beyond radiographic disease progression in the absence of clinical deterioration, and after discussion with the Principal Investigator.

All subjects will be followed up to 2 years for survival or until the study closes. Exploratory research studies to evaluate the effect of this therapy will be performed in patients using

research blood draws, and tumor biopsy at baseline, 1 week after completing RT or undergoing ablation (from the radiated/ablated lesion), and then 4 weeks after dose #1 of pembrolizumab (from a non-radiated/ablated lesion),for research purposes.

4.3 Estimated Duration of Subject Participation

Subjects may be treated for up to 24 months, with the option to resume treatment for an additional 12 months upon disease progression, unless there is anot her reason to discontinue treatment. All subjects will be followed for survival for up to 2 years unless the Principal Investigator or Merck decides to end the study.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1 Cryoablation

The cryoablation will be performed percutaneously under image guidance as standard therapy in accordance with institutional standard practice.

5.2 Radiofrequency ablation

The radiofrequency ablation (RFA) will be performed percutaneously under image guidance as standard therapy in accordance with institutional standard practice.

5.3 Radiotherapy

Radiotherapy will be performed using external beam ionizing radiation or by brachytherapy as standard therapy in accordance with institutional standard practice.

5.4 Pembrolizumab

Pembrolizumab will be provided by Merck. Pembrolizumab will be prepared and administered as per MSKCC guidelines. Please refer to investigator brochure for additional information

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations. Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

6.1 Subject Inclusion Criteria

1. Be willing and able to provide written informed consent/assent for the trial.
2. Histologically- or cytologically- confirmed CRC.
3. Metastatic or recurrent CRC.

4. Subjects have received two or more standard available therapies known to prolong survival and for which they would be considered eligible. Such therapies should include regimens containing oxaliplatin and irinotecan in combination with a fluoropyrimidine if appropriate (e.g., FOLFOX and FOLFIRI or their variants).

5. At least one tumor for which palliative RT is considered appropriate standard therapy (cohort 1); or, at least one tumor for which palliative ablation is considered appropriate standard therapy (cohort 2).

6. At least one index lesion that will not undergo RT or ablation, and which is measurable based on RECIST 1.1.

7. Be ≥ 18 years of age on day of signing informed consent.

8. Consent for tumor biopsies and blood draws for research purposes.

9. Consent for use of available archived tissue for research purposes.

10. Have a performance status of 0 or 1 on the ECOG Performance Scale.

11. Demonstrate adequate organ function as defined in Table 6.1, all screening labs should be performed within 6 weeks of treatment initiation.

Table 6.1 Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Renal	
Serum creatinine **OR** Measured or calculated[a] 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 **OR** ≤ 5 X ULN for subjects with liver metastases
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) 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 ≤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]Creatinine clearance should be calculated per institutional standard.	

12. Female subject of childbearing potential should have a negative serum pregnancy within 2 weeks prior to starting radiation therapy or undergoing ablation.

13. Female subjects of childbearing potential should be willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication (Reference Section 9.5.2). Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.

14. Male subjects should agree to use an adequate method of contraception starting with the first dose of study therapy through 120 days after the last dose of study therapy.

6.2 Subject Exclusion Criteria

1. Is currently participating in or has participated in a study of an investigational agent or using an investigational device within 4 weeks of the first dose of treatment.

2. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment.

3. Has had a prior monoclonal antibody within 4 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.

4. Has had prior chemotherapy, targeted small molecule therapy within 2 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.

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

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

6. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or in situ cervical cancer that has undergone potentially curative therapy.

7. Has known active central nervous system (CNS) metastases and/or carcinom atous meningitis. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least four weeks prior to the first dose of trial treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to trial treatment.

8. Has an active autoimmune disease requiring systemic treatment within the past 3 months or a documented history of clinically severe autoimmune disease, or a syndrome that requires systemic steroids or immunosuppressive agents. Subjects with vitiligo or resolved childhood asthma/atopy would be an exception to this rule. Subjects that

require intermittent use of bronchodilators or local steroid injections would not be excluded from the study. Subjects with hypothyroidism stable on hormone replacement or Sjorgen‟s syndrome will not be excluded from the study.

9. Has evidence of interstitial lung disease or active, non-infectious pneumonitis.

10. Has an active infection requiring systemic therapy.

11. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject‟s participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

12. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

13. Is pregnant 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.

14. Has received prior therapy with an anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, or anti-Cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) antibody (including ipilimumab or any other antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways).

15. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).

16. Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).

17. Has received a live vaccine within 30 days prior to the first dose of trial treatment.

7.0 RECRUITMENT PLAN

This study will be available to all patients seen at Memorial Hospital, who meet the eligibility criteria outlined in section 6.0.

Memorial Hospital is a referral center for CRC. In addition, the study may be placed on the institutional Website to maximize patient recruitment. Patients will be identified from medical oncology clinics for treatment of their disease.

The investigators take due notice of the NIH policy concerning inclusion of women and minorities in clinical research populations. There will be no limitation to access with regard to race or gender. Patients will be required to read, agree to, and sign an IRB-approved informed consent form prior to registration on this trial. The registration procedure will be conducted as described in section 15.0. Patients will not receive payment for their participation on this study.

8.0 PRETREATMENT EVALUATION

To be completed prior to starting RT or ablation and within 6 weeks of starting pembrolizumab:

- CT scan with contrast (chest, abdomen and pelvis). If patient is unable to receive CT contrast, or the abdominal/pelvic target lesion is indeterminate on CT scan then MRI with contrast (abdomen and pelvis) plus CT chest without contrast may be perfromed. Non-contrast CT CAP may be used if the target lesion(s) do not require contrast for accurate measurements.
- 12-lead Electrocardiogram (EKG).
- Signed informed consent for study participation.
- History and physical examination, including height, weight, vital signs (temperature, pulse rate, respiration rate, blood pressure), and performance status (ECOG).
- Serum pregnancy test for all women of childbearing potential (within two weeks of starting RT or ablation). If the test result is positive related to pregnancy, the patient will not be allowed to participate in this study.
- CBC with differential and platelet count, serum chemistries (Na, Cl, BUN, Creatinine, K, CO2, and glucose), LFTs (AST, ALT, alkaline phosphatase, total bilirubin), calcium, albumin, total protein, and CEA
- Serology for HepBsAg, HepBcAb and hepatitis C antibody (negative test acceptable prior to screening period)
- Blood test for research purposes.
- Perform baseline tumor biopsy for research purposes at the time of fiducial placement or ablation or during a separate procedure.

9.0 TREATMENT/INTERVENTION PLAN

9.1 Dosing Instructions and Schedule

Drug	Dose/ Potency	Dose Frequency	Route of Administration	Regimen/ Treatment Period	Use
Pembrolizumab	200 mg	Q3W	IV infusion	n/a	Experimental

The first day of dosing is considered Day 1. Subjects will receive pembrolizumab as an IV infusion. Refer to „MK-3475 Drug Preparation Instructions" manual.

Trial treatment should be administered on Day 1 of each cycle. Trial treatment may be administered up to 3 days before or after the scheduled Day 1 of each cycle due to administrative reasons. All trial treatments will be administered on an outpatient basis.

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

9.2 Monitoring of Dose Administration

Subjects will be monitored during and after infusion with assessment of vital signs per institutional practice.

In the event of an infusion-related reaction, refer to See appendix C for guidance.

As with any antibody, allergic reactions to dose administration are possible. Appropriate drugs and medical equipment to treat acute anaphylactic reactions must be immediately available, and study personnel must be trained to recognize and treat anaphylaxis. The study site must have immediate access to emergency resuscitation teams and equipment in addition to the ability to admit subjects to an intensive care unit if necessary.

9.3 Concomitant Medications

9.3.1 Permitted Concomitant Medication

Investigators may prescribe concomitant medications or treatments (e.g., acetaminophen, diphenhydramine) deemed necessary to provide adequate prophylactic or supportive care except for those medications identified as “excluded” as listed in Section 9.3.2.

9.3.2 Excluded Concomitant Medications

Subjects must be instructed not to take any medications, including over-the-counter products, without first consulting with the investigator.

The following medications are considered exclusionary during the study. The Principal Investigator must be notified if a subject receives any of these during the study.

- Any investigational anticancer therapy.
- Any concurrent chemotherapy (except as permitted above), immunotherapy, or biologic therapy. Concurrent use of hormones for non-cancer-related conditions (e.g., insulin for diabetes and hormone replacement therapy) is acceptable.
- Immunosuppressive medications including, but not limited to systemic corticosteroids (>10 mg/day prednisone or equivalent), methotrexate, azathioprine, and tumor necrosis factor alpha (TNF-α) blockers. Use of immunosuppressive medications for the management of investigational product-related AEs, in subjects with contrast allergies is acceptable. In addition, use of inhaled and intranasal corticosteroids is permitted.

9.4 Dose Modification

Refer to APPENDIX C for guidance in the event of a drug-related adverse event. Pembrolizumab dosing may continue in the event of an unrelated, grade 1 or tolerable grade 2 adverse event.

Subjects who experience a recurrence of the same severe or life-threatening event at the same grade or greater with re-challenge of pembrolizumab should be discontinued from trial treatment.

9.4.1 Supportive Care Guidelines

- Nausea/vomiting: Nausea and vomiting should be treated aggressively, and consideration should be given in subsequent cycles to the administration of prophylactic antiemetic therapy according to standard institutional practice. Subjects should be strongly encouraged to maintain liberal oral fluid intake.

- Anti-infectives: Subjects with a documented infectious complication should receive oral or IV antibiotics or other anti-infective agents as considered appropriate by the treating investigator for a given infectious condition, according to standard institutional practice.

9.5 Diet/Activity/Other Considerations

9.5.1 Diet

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

9.5.2 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. Non-pregnant, non-breast-feeding women may be enrolled if they are willing to use 2 methods of birth control or are considered highly unlikely to conceive. Highly unlikely to conceive is defined as 1) surgically sterilized, or 2) postmenopausal (a woman who is ≥45 years of age and has not had menses for greater than 1 year will be considered postmenopausal), or 3) not heterosexually active for the duration of the study. The two birth control methods can be either two barrier methods or a barrier method plus a hormonal method to prevent pregnancy. Subjects should start using birth control from study Visit 1 throughout the study period up to 120 days after the last dose of study therapy.

The following are considered adequate barrier methods of contraception: diaphragm, condom (by the partner), copper intrauterine device, sponge, or spermicide. Appropriate hormonal contraceptives will include any registered and marketed contraceptive agent that contains an estrogen and/or a progestational agent (including oral, subcutaneous, intrauterine, or intramuscular agents).

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study they must adhere to the contraception requirement (described above) for the duration of

the study and during the follow-up period defined in section 11.1.2 - Pregnancies. If there is any question that a subject will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

9.5.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab, the subject will immediately be removed from the study. The site 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 will be reported to Merck without delay and within 24 hours if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn). The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to Merck. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to Merck and followed as described above and in Section 11.1.2.

9.5.4 Use in Nursing Women

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

9.6 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator or the Sponsor if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons.

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.
- Confirmed radiographic disease progression

  Note: A subject may be granted an exception to continue on treatment with confirmed radiographic progression if clinically stable or clinically improved.

- Unacceptable adverse experiences
- Intercurrent illness that prevents further administration of treatment
- 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

- Completed 24 months of treatment with pembrolizumab

  Note: 24 months of study medication is calculated from the date of first dose. Subjects who stop pembrolizumab after 24 months may be eligible for up to one year of additional study treatment at the discretion of the PI if they responded during the initial 24 months and then progressed after stopping study treatment.
- Administrative reasons

After the end of treatment, each subject will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for 90 days after the end of treatment). Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent or becoming lost to follow-up, for up to 2 years. After documented disease progression each subject will be followed by telephone (if not otherwise following up at Memorial Hospital) for overall survival until death, withdrawal of consent, the end of the study, or 2 years, whichever occurs first.

9.7 Clinical Criteria for Early Trial Termination

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

1. Quality or quantity of data recording is inaccurate or incomplete

2. Poor adherence to protocol and regulatory requirements

3. Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects

4. Plans to modify or discontinue the development of the study drug

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

9.8 RESEARCH BLOOD AND BIOPSY SPECIMENS

9.8.1 Research blood

For all patients, blood specimens will be obtained for research purposes during screening or on day 1, then at weeks 3, 6 and 9 (± 3 days). Additional blood draws beyond week 9 are permitted based upon interesting clinic/immunological findings.

Specimens should be collected prior to drug administration. Four (4) tubes of blood are to be collected in BD Vacutainer® CPT™ Cell Preparation Tubes with Sodium Heparin. Each tube should contain approximately 10 cc of blood. Peripheral blood mononuclear cells and plasma will then be isolated per institutional practice in the Immune Monitoring Facility (IMF).

9.8.2 Research biopsy

A pre-treatment tumor biopsy will be obtained at the time of ablation or fiducial placement or during a separate procedure. On treatment biopsies will be performed 1 week (±3 days) after completing RT or undergoing ablation (from the radiated/ ablated lesion), and then 4 weeks (±1

week) after dose #1 of pembrolizumab (from a non-radiated/ ablated lesion). For patients that are unable to have biopsy 4 weeks after dose #1 of pembrolizumab, the procedure may be delayed up until Week 9.

Patients will be permitted to continue enrollment and treatment on protocol in the event that insufficient material was obtained from the biopsy. The on treatment biopsy will not be required if this is no longer considered appropriate at the time of the planned procedure, for example, if the tumor is no longer accessible or the procedure is deemed to be unsafe. Tumor lesion planned for on-treatment biopsy may be an index lesion if $\geq$ 2 cm in at least one diameter.

If clinically practical, subjects will undergo up to 5 core biopsies. Three core biopsies will be placed in formalin and processed for FFPE, 2 core biopsies will be immediately frozen in liquid nitrogen and then stored at -80°C (appendix B). All tissue obtained during biopsy procedures will be sent to the IMF facility for analysis under this study as indicated below. If the patient undergoes a routine procedure, tissue may be obtained for future correlative analyses. Up to 20 x 5μm slides (unstained) may be obtained from each routine procedure where tissue was extracted. Tissue from routine procedures may be sent directly to the IMF facility for immediate processing (e.g. isolation of Tumor Infiltrating Lymphocytes).

9.8.3 Correlative studies

Pharmacodynamic changes may be evaluated for associations with clinical activity, and safety (adverse event) data. Tissue may be used for correlative studies such as IHC, tumor mutation analysis, proteomic analysis, and immunodiversity. PDL-1 immunohistochemistry will be done by a Merck designated laboratory. Other assessment will be done at MSKCC.

9.8.3.1 Whole Blood

Flow cytometry will be performed at baseline and during treatment to assess baseline and changes in composition/activation status of lymphocyte subsets present in peripheral blood mononuclear cell preparations (PBMCs). Lymphocyte subsets to be assayed may include, but are not limited to CD8+ and CD4+ T-cell subsets (activated; effector/memory; regulatory) and populations of those cells as defined by the expression of activation, exhaustion, or signaling markers such as ICOS, HLA-DR, PD-1, CTLA-4, and/or intracellular IFNγ. NK cell populations may be monitored in a similar fashion with a focus on characterizing subsets defined by the expression of activation markers (e.g. NKG2D; IL-21R) and/or by markers that are associated with the potential of NK cells to lyse target cells (e.g., CD107a, granzyme, perforin). Additional flow cytometry-based assays will focus on defining and monitoring the abundance of myeloid-derived suppressor cells (MDSCs), a cell type which appears to negatively impact anti-tumor activity and which has been shown to promote immune escape by limiting activated CD8 T-cell infiltration into the tumor microenvironment [17]. Immune cells may be evaluated using HLA-A2-restricted tetramer assays to detect and quantify the presence of T cells directed against specific antigens which are anticipated to be presented to the immune system due to study treatment. Detecting on-treatment increases in these T cell populations may be considered evidence of adaptive immune responses in CRC.

9.8.3.2 Plasma

To understand the prevalence of circulating proteins and the impact they may have on the clinical activity and/or safety of pembrolizumab treatment, the protein concentrations of a panel

of cytokines, chemokines, and other relevant immunomodulatory, soluble factors may be investigated by ELISA and/or other relevant multiplex-based protein assay methods. Examples of analytes to be assessed may include but are not limited to factors induced by IFNγ signaling (e.g., T cell chemoattractants CXCL9; CXCL10) and other factors generally involved in inflammatory processes. Plasma may be used also to assess the presence and/or concentration of anti-tumor antibodies using a mulitplex platform such as Invitrogen‟s Protoarray platform(c). Levels of sPD-L1 in peripheral blood may also be assessed.

9.8.3.3 Tissue Biopsies and/or archived tissue

The presence of TILs within tumors in response to pembrolizumab treatment will be evaluated baseline and on-treatment biopsies and, when feasible, tissue acquired from routine procedures. Archived tissue (up to 20 x 5 μm slides), tissue acquired from routine procedures (up to 20 x 5 μm slides per procedure), and biopsy tissue may be analyzed using immunohistochemistry for PD-L1 expression and other immune-related genes, and gene expression (microarray and/or RT- QPCR) research platforms. Laser Capture Microdissection may be utilized to enrich specific regions of tumor material for use in similar or additional downstream applications, which may include in-situ hybridization, flow cytometry, ELISA, and/or assessment of miRNA. In all cases, the goal may be to determine the abundance of a battery of immunoregulatory genes or proteins associated with cancer cells and/or cancer-interacting lymphocytes derived from biopsied material. Other biomarkers may be evaluated as determined by additional data. Remaining specimens may be stored for future studies related to CRC immunity.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

Study Calendar

Period	Screening	Treatment									End of study visit[15]
Cycle[1]	<6 weeks[2]	1	1	2	2	3	4	5	6	7+	
Week		0	1	3	4	6	9	12	15	18+	
Informed consent	x										
Medical history	x										
EKG	x										
CT/MRI[3]	x						x			x	x
Height[4]	x										
Physical examination	x	x		x		x	x	x	x	x	x
Vital signs/ Performance status[4]	x	x		x		x	x	x	x	x	x
Report medications	x	x		x		x	x	x	x	x	x
Pulse oximetry		x		x		x	x	x	x	x	x
Report side effects				x		x	x	x	x	x	x
CBC[5,6]	x	x		x		x	x	x	x	x	x
Comp[5,7]	x			x		x	x	x	x	x	x
Thyroid function (TSH, fT3,fT4)	x					x		x		odd cycles	
CEA[8]	x	x					x			x	x
Hepatitis B and C[9]	x										
Pregnancy test if female (Serum)[10]	x										
Research blood tests [5,11]	x			x		x	x				
Obtain archived tissue [12]	x										
Research tumor biopsy [13]	x		x		x						
RT (Cohort 1) [14]	x										
Ablation (Cohort 2) [14]	x										
Pembrolizumab		x		x		x	x	x	x	x	

1. Each cycle is approximately 3 weeks in duration, corresponds to 1 completed treatment.
2. Procedures must be performed within 6 weeks prior to dose 1 of Pembrolizumab on Day 1.
3. CT or MRI will be performed during screening, then at 9 week (±1 wk) intervals and at final visit if more than 4 weeks from prior imaging. If patient is unable to receive CT contrast or the abdominal/ pelvic target lesion is indeterminate on CT then MRI with contrast (abdomen and pelvis) plus CT without contrast (chest) may be performed. Imaging may be delayed up to 2 weeks if patient is receiving additional RT or ablation.
4. Vital signs to include heart rate, respiratory rate, blood pressure, and weight.
5. Blood to be collected prior to dosing any study medications.
6. Hematology to include standard complete blood cell (CBC) panel.
7. Comprehensive metabolic panel included sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, total protein, albumin, bilirubin, alkaline phosphatase, AST, ALT, calcium.
8. CEA to be obtained during screening, day 1 and then within two weeks (or on day) of subsequent imaging.
9. Serology for HepBsAg, HepBcAb and hepatitis C antibody (unless previously tested negative).
10. Serum pregnancy test is required within 2 weeks of starting radiation or ablation
11. Blood draws for research purposes performed during screening or day 1, then weeks 3, 6 and 9 for all patients. Additional blood draws beyond week 9 are permitted based upon interesting clinic/immunological findings for all patients.

12. Up to 20 x 5µm slides (unstained) of archived tissue will be requested on all patients for research purposes. For patients undergoing routine procedures during the study, additional tissue (up to 20 x 5µm slides (unstained)) may be obtained from each routine procedure. for research purposes.
13. Research tumor biopsy will be performed during fiducal placement or ablation or during a separate procedure, then 1 week (±3 days) after completing RT or ablation (from the radiated/ablated lesion) and 4 weeks after dose #1 of pembrolizumab (±1 week) from a non-ablated/radiated lesion. For patients that are unable to have biopsy 4 weeks after dose #1 of pembrolizumab, the procedure may be delayed up until Week 9.
14. RT begins or ablation is performed during cycle 1, within one week prior to dose 1 of Pembrolizumab.
15. EOS visit occurs 4(±1 wk) weeks after last dose of pembrolizumab. CT scan or MRI to be done only if last imaging was conducted greater than four weeks prior to the date of the Final Visit.

11.0 TOXICITIES/SIDE EFFECTS

The most commonly reported treatment emergent AEs related to pembrolizumab are fatigue (43.8%), nausea (26.7%), cough (25.3%), pruritus (24.6%), diarrhea (22.3%) and rash (21.5%). Immune-related adverse events were reported in 21.4% of melanoma patients; most of these events (15.8%) were considered drug-related by the investigator. The most commonly reported, immune-related adverse events across the dose-schedules are rash (3.2%), pruritus (2.9%), vitiligo (2.9%), hypothyroidism (2.7%), arthralgia (2.2%), diarrhea (2.2%), and pneumonitis (1.9%).

11.1 SAFETY MONITORING

Subjects will be evaluated for occurrence of AEs at each visit. Events will be characterized and reported as described below. Safety will also be monitored by performing physical exams and routine laboratory procedures.

11.1.1 Adverse Events and Serious Adverse Events

Definitions of AEs, non-serious AE, and serious adverse events (SAEs) are provided in this section. Additionally, provided in the sections below are reporting guidelines for any AE or SAE occurring during this study.

Definition of Adverse Event and Non-Serious Adverse Event

The following definition of AE will be used for the study: “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 (investigational) product, whether or not related to medicinal (investigational) product.”

This definition includes any abnormalities or anomalies that were not seen at baseline or which worsened during the course of the study, if present at baseline.

A “non-serious” adverse event is any event that does not meet the definition of “serious adverse event” as presented, below.

Reporting and Treating Non-Serious Events

It is the responsibility of the investigator to perform regular assessments for AEs. Subjects will be regularly queried about the occurrence of any AEs and will be monitored throughout the

study for reactions to study drug and/or study procedures. The investigator and clinical staff will record all AEs, whether volunteered by or elicited from the subject, at any time during a subject‟s participation in the study. Abnormal laboratory findings (e.g., hematology, comprehensive metabolic panel) or other abnormal assessments (e.g., vital signs) will be recorded as AEs if they are judged as clinically significant by the investigator.

All subjects experiencing an AE will be evaluated by the investigator and monitored until resolution of the events or until the investigator deems the event clinically stable and/or at an acceptable level. Unless the event requires hospitalization (SAE), medical treatment will be provided to the subjects at the unit and treatment medication and/or medical procedures will be provided per the treating-investigator‟s clinical discretion. All clinically significant AEs, including clinically significant laboratory abnormalities, will be followed until resolution. AEs meeting the definition of SAEs require special reporting in addition to documentation in the CRDB as described below.

All AEs, including clinically significant laboratory and assessment abnormalities will be recorded according to “Common Terminology Criteria for Adverse Events” V4.0 (CTCAE) and must be recorded in the CRDB. Events occurring prior to initiation of first dose should be recorded on the Medical History page of the CRDB. Any AE occurring after initiation of first dose of study drug should be recorded on the Adverse Event page of the CRDB. AEs should be recorded in the CRDB using the medical terminology found in the source documentation. Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology. It is the investigator‟s responsibility to provide his/her assessment of the relationship of the event to the study drug and the severity of the event using the following scales:

- Relationship

  - *Unrelated*: The AE is clearly attributable to a concurrent illness, concurrent medication, clinical state, or environmental factor other than the investigative agent.
  - *Unlikely*: The occurrence of the AE does not follow the study in a temporal sequence and/or based upon available subject information, e.g., medical history, disease process, known pharmacology of drug, a relationship between the drug and AE is unlikely.
  - *Possible*: The AE follows a reasonable temporal sequence from the time of study drug administration, but it is possible that other factors; e.g., subject‟s clinical state or concomitant mediations, environmental factors, or the drug‟s pharmacology may have caused the AE.
  - *Probable*: The AE follows a reasonable temporal sequence from the time of study drug administration, follows a known response pattern of the medication class, and cannot be reasonably explained by other factors.

- Severity

The severity of all adverse events should be graded according to the Common Terminology Criteria for Adverse Events (CTCAE) V4.0. For those adverse events not listed in the CTCAE, the following grading system should be employed:

- *Mild* (CTCAE Grade 1): Transient symptoms, awareness of sign/symptom, but easily tolerated and no interference with subject‟s daily activities
- *Moderate* (CTCAE Grade 2): Marked signs/symptoms that interfere with subject‟s usual activities, but still acceptable
- *Severe* (CTCAE Grade 3): Incapacitating signs/symptoms which cause considerable interference with the subject‟s daily activities, unacceptable
- *Life-threatening* (CTCAE Grade 4): Life-threatening of disabling AE
- *Death* (CTCAE Grade 5): Death-related AE. See CTCAE Guidelines for assigning Grade 5.

Definition of Serious Adverse Event

The following definition of SAE applies for the study: “A serious AE means any AE occurring at any dose that results in any of the following outcomes: death, a life-threatening AE, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, a congenital anomaly/birth defect, or overdose of study drug. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered a serious AE when, based upon appropriate medical judgment, they may jeopardize the subject or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. A life-threatening AE is any AE that places the subject or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred (e.g., it does not include a reaction that, had it occurred in a more severe form, might have caused death).” Reporting and Treating Serious Adverse Events as per section 17.2.

Pregnancies

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them), including the pregnancy of a male subject's female partner that occurs during the trial or within 120 days of completing the trial completing the trial, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier. All subjects and female partners of male subjects who become pregnant must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 5 calendar days to the MSKCC Safety Office and within 2 working days to Merck Global Safety (Attn: Worldwide Product Safety; FAX 215 993-1220)

11.1.3 Other Events of Special Interest

Hepatic Function Abnormality

Hepatic function abnormality is defined as any increase in ALT or AST to greater than 3 × ULN and concurrent increase in bilirubin to greater than 2 × ULN. Concurrent findings are those that derive from a single blood draw or from separate blood draws taken within 8 days of each other. Follow-up investigations and inquiries will be initiated promptly by the investigational site to determine whether the findings are reproducible and/or whether there is objective evidence that clearly supports causation by a disease (e.g., cholelithiasis and bile duct obstruction with distended gallbladder) or an agent other than the investigational product.

Events of hepatic function abnormality (as defined above) should be recorded according to the definitions of AE and SAE:

- If an event of hepatic function abnormality is considered to be related to a pre-existing condition and does not represent a worsening of this condition and/or is considered to be within the range of normal physiological fluctuation for the subject, the event does not meet the definition of an AE and does not need to be recorded as such.

- If a definitive diagnosis for an underlying condition unrelated to the investigational product is established for an event of hepatic function abnormality, the diagnosis should be recorded as an AE/SAE.

- If no definitive diagnosis is determined for an event of hepatic function abnormality, the term “hepatic function abnormal” should be used to report the AE/SAE.

Definition of an Overdose for This Protocol and Reporting of Overdose to Merck
For purposes of this trial, an overdose will be defined as any dose exceeding the prescribed dose for pembrolizumab by 20% over the prescribed dose. No specific information is available on the treatment of overdose of pembrolizumab. In the event of overdose, subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated. Pembrolizumab does not need to be discontinued in case of overdose, but it does need to be reported.

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

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

All reports of overdose with and without an adverse event must be reported within 5 calendar days to the MSKCC Safety Office and within 2 working days to Merck Global Safety . (Attn: Worldwide Product Safety; FAX 215 993-1220)

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

For the purposes of this study, patients will be evaluated for response every 3 cycles (approximately 9 weeks), or as clincially indicated if interim toxicity occurs mandating cancer staging re-assessment. RECIST 1.1 criteria will be used.

CT scan with contrast of the chest, abdomen, and pelvis

- CT scans should be performed with contiguous cuts in slice thickness of 5 mm or less. Spiral CT should be performed using a 5-mm contiguous reconstruction algorithm.

MRI scans

- MRI of the abdomen and pelvis is acceptable for measurement of lesions provided that the same anatomical plane is used for serial assessments. If possible, the same imaging device should be used for serial evaluations. In case of MRI, measurements will be preferably performed in the axial (transverse) plane on contrast-enhanced T1-weighted images. However, there are no specific sequence recommendations.

Measurability of Tumor Lesions

Tumor lesions will be categorized as follows:

- **Measurable Lesions -** Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:
  - 10 mm by CT scan (irrespective of scanner type) and MRI (no less than double the slice thickness and a minimum of 10 mm)
  - 10 mm caliper measurement by clinical exam (when superficial)
  - Malignant lymph nodes are considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm).
- **Nonmeasurable Lesions -** Nonmeasurable lesions are defined as all other lesions (or sites of disease), including small lesions (longest diameter < 10 mm or pathological lymph nodes with ≥ 10 to < 15 mm short axis). Lesions considered truly nonmeasurable include the following: leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.
- **Target Lesions -** All lesions up to a maximum of 5 lesions total (and a maximum of 2 lesions per organ) representative of all involved organs should be identified as target lesions. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected.

- **Non-target Lesions -** It is possible to record multiple nontarget lesions involving the same organ as a single item on the case record form (e.g., “multiple enlarged pelvic lymph nodes” or “multiple liver metastases”)

Response Criteria

Evaluation of Target Lesions

- **Complete Response -** Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm (the sum may not be “0” if there are target nodes).
- **Partial Response -** At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.
- **Progressive Disease -** At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression.)
- **Stable Disease -** Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum of diameters while on study.

Evaluation of Non-target Lesions

- **Complete Response -** Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (< 10 mm short axis).
- **Non-complete response/Non-progressive disease -** Persistence of 1 or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.
- **Progressive Disease -** Unequivocal progression of existing non-target lesions will be defined as the overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. In the absence of measurable disease, change in non-measurable disease comparable in magnitude to the increase that would be required to declare PD for measurable disease. Examples include an increase in a pleural effusion from „trace" to „large," an increase in lymphangitic disease from localized to widespread.

Appearance of New Lesions

The appearance of new lesions is considered PD according to RECIST v 1.1 guidelines. Considering the unique response kinetics that have been observed with immunotherapy, new lesions may not represent true disease progression. In the absence of rapid clinic al deterioration, subjects may continue to receive treatment with pembrolizumab.

Evaluation of Overall Response

Table 12 provides overall responses for all possible combinations of tumor responses in target and non-target lesions with or without the appearance of new lesions.

Table 12 Evaluation of Overall Response

Target Lesions	Non-target Lesions	New Lesions	Overall Response
Complete response	Complete response	No	Complete response
Complete response	Not evaluable	No	Partial response
Complete response	Non-complete response / non-progressive disease	No	Partial response
Partial response	Non-progressive disease and not evaluable [a]	No	Partial response
Stable disease	Non-progressive disease and not evaluable [a]	No	Stable disease
Not all evaluated	Non-progressive disease	No	Not evaluable
Progressive disease	Any	Yes/No	Progressive disease
Any	Progressive disease	Yes/No	Progressive disease
Any	Any	Yes	Progressive disease

[a] Not evaluable is defined as either when no or only a subset of lesion measurements are made at an assessment.

13.0 CRITERIA FOR REMOVAL FROM STUDY

In the absence of serious toxicity or complications, all patients will continue treatment for up to 2 years. In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

- Disease progression (unless the patient continues treatment beyond progression).
- Development of an intercurrent medical condition or need for concomitant treatment that precludes further participation in the trial.
- Unacceptable toxicity or any adverse event that precludes further participation in the trial.
- The investigator removes the patient from the trial in the best interests of the patient.
- Patient death.
- Study completion or discontinuation for any reason.
- Patient withdraws consent to continued participation in the trial or is lost to follow up.

Subjects who are permanently discontinued from receiving investigational product will return for end of study visit, unless consent is withdrawn, the subject is lost to follow-up or begins another treatment. All subjects will be followed for survival every 3 months for up to 2 years. If not otherwise following up at Memorial Hospital, patient‟s will be contacted by phone for survival follow up.

If consent is withdrawn, the subject will not receive any further investigational product or further study observation.

14.0 BIOSTATISTICS

The primary endpoint of this trial is the response rate in CRC treated with RT plus pembrolizumab (cohort 1) or ablation plus pembrolizumab (cohort 2). No response is expected in a non-radiated or non-ablated lesion with these current therapies and we consider a response rate ≤5% as unacceptable. A two-stage Simon‟s optimal design will be employed to test the null hypothesis that the true response rate is ≤5% versus the alternative hypothesis that the true response rate is at least 25% with type I and II error rates of 10% each. Each cohort will be evaluated separately for this purpose. In the first stage, we will accrue 9 patients in each cohort. If 0 objective tumor responses (PR or CR) are observed among the 9 subjects treated in a cohort, then subject enrollment will be terminated in that cohort. If at least 1 response is observed among the 9 subjects treated in a cohort, then the study will be expanded to enroll a total of 24 treated subjects in that cohort. At the end of the study, if 2 or less objective tumor responses are observed in a cohort, then the study will be considered not worthy of further investigation in that particular cohort. If at the end of the study ≥3 tumor responses per RECIST 1.1 are observed in a cohort, then further investigation of pembrolizumab plus RT and/or pembrolizumab plus ablation will be considered worthwhile.

Secondary outcomes, including toxicity, the additional measures of efficacy and exploratory objectives will be summarized by cohort.

The study will complete when all subjects have either progressed or discontinued from the study for other reasons. This study requires accrual of a minimum of 18 subjects and up to a maximum of 48 subjects if both cohorts are expanded to the second stage. The accrual time is estimated to be 9 months to 2 years for both cohorts.

Antitumor Activity

Assessments of antitumor activity will be based on the ORR, PFS, and OS. Response Evaluation Criteria in Solid Tumors guidelines (v1.1) [18] with modifications to account for the unique response patterns observed with immunotherapy will be used to determine tumor response.

The ORR is defined as the proportion of subjects CR or PR based on RECIST criteria. The exact 95% CI of ORR will be estimated using the binomial distribution. Progression-free survival will be measured from the start of treatment with pembrolizumab until the documentation of disease progression or death due to any cause, whichever occurs first. Progression-free survival will be censored on the date of last tumor assessment documenting absence of tumor progression for subjects who are still alive prior to data cutoff, dropout, or the initiation of alternate anticancer treatment. Subjects having no tumor assessments after the start of treatment with pembrolizumab will have PFS censored on the first date of treatment with pembrolizumab. Progression-free survival will be evaluated using the Kaplan-Meier method (Kaplan and Meier, 1958). Overall survival will be determined as the time from the start of

treatment with pembrolizumab until death. For subjects who are alive at the end of study or lost to follow-up, OS will be censored on the last date when subjects are known to be alive. The OS will be evaluated using the Kaplan-Meier method.

Safety and Tolerability Analyses

All recorded adverse events will be listed and tabulated by system organ class, preferred term and treatment. Any significant vital signs and clinical laboratory test results will be listed and summarized. Any significant physical examination findings, and clinical laboratory results will be listed.

Biomarker Analysis

Exploratory research studies will be done to evaluate the effect of this therapy will be performed using research blood draws and tumor biopsies at baseline, 1 week after completing RT or undergoing ablation (from the radiated/ablated lesion), and then 4 weeks after dose #1 of pembrolizumab (from a non-radiated/ablated lesion).

The pharmacodynamic effect of pembrolizumab and RT or ablation on Tumor Infiltrating Lymphocytes (TILs), such as CD4+ and CD8+ T-cells, and expression of tumor markers, such as PD-L1, will be assessed by summary statistics, and investigated graphically to explore patterns of change from pre-treatment to post-treatment specimens.

The pharmacodynamic effect of pembrolizumab and RT or ablation on markers in peripheral blood, such as ICOS, HLA-DR, PD-1, CTLA-4; and, serum proteins, such as CXCL9; CXCL10, will be assessed by summary statistics, and investigated graphically to explore patterns of change over time, i.e.: pretreatment, then week 3, and week 9.

In addition, the relationship of TIL changes and tumor marker expression with measures of peripheral blood markers will be summarized descriptively.

Associations between the markers and response by RECIST will be explored.

Fisher"s exact test will be employed to assess associations between categorical variables while Spearman's rank correlation will be used for continuous variables. Wilcoxon signed rank test will be used to test for differences in continuous expression tumor markers between pre- and post-treatment specimens while McNemar"s test will be used to assess these relationships for binary markers. Fisher"s exact test will be employed to assess associations between categorical variables.

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. Registrations must be submitted via the PPR Electronic Registration System (http://ppr/). The completed signature page of the written consent/RA or verbal script/RA, a completed Eligibility Checklist and other relevant documents must be uploaded via the PPR Electronic Registration System.

15.2 Randomization

n/a

16.0 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team.

The data collected for this study will be entered into a secured database (Clinical Research Database, CRDB) at Memorial Sloan-Kettering Cancer Center. Source documentation will be available to support the computerized patient record.

16.1 Quality Assurance

Weekly registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action. Random-sample data quality and protocol compliance audits may be conducted by the study team, at a minimum of two times per year, more frequently if indicated.

16.2 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled “Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials”, which can be found at http://cancertrials.nci.nih.gov/researchers/dsm/index.html. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC DSM Plans can be found on the MSKCC Intranet at http://mskweb5.mskcc.org/intranet/_assets/_tables/content/359689/Data_safety%20Monitoring07.pdf

17.0 PROTECTION OF HUMAN SUBJECTS

Participation in this trial is voluntary. All patients will be required to sign a statement of informed consent, which must conform to IRB guidelines.

Inclusion of Women and Minorities: Memorial Sloan-Kettering Cancer Center has filed forms HHS 441 (civil rights), HHS (handicapped individual), 639-A (sex discrimination), and 680 (age discrimination); we also take due notice of the NIH policy concerning inclusion of women and minorities in clinical research populations. Patients of all races, both male and female, will be accepted into the protocol. The proposed study population is as described in section 7.0.

Exclusion of Lactating or Pregnant Women: Children have been excluded from this study. Colorectal adenocarcinoma is an adult cancer. Thus, the relevance of these drug to the pediatric population has not been established. Lactating and pregnant women are also excluded because of potential anti-proliferative effects of chemotherapy that may be harmful to the developing fetus or nursing infant.

Benefits: It is possible that this treatment will result in shrinkage of colorectal cancer or in a stabilization of an otherwise progressing disease. It is not known, of course, whether these or any other favorable events will occur. It is not known whether this treatment will affect the overall survival of the patients.

Costs: The patient will be responsible for the costs of standard medical care, including, CT scans, all drug administration fees and all hospitalizations, even for complications of treatment. Pembrolizumab will be supplied to patients by Merck at no cost. Patients will not be responsible for the costs of blood procurement obtained for research purposes , the cost of special testing of any tissue for research purposes, or the cost for obtaining the tumor biopsy for research purposes.

Incentives: No incentives will be offered to patients/subjects for participation in the study.

Alternatives: Patients may be eligible for other investigational studies, or focus on palliative care options.

Confidentiality: Every effort will be made to maintain patient confidentiality. Research and hospital records are confidential. Patient‟s name or any other personally identifying information will not be used in reports or publications resulting from this study. The Food and Drug Administration or other authorized agencies (e.g., qualified monitors) may review patients records and pathology slides, as required.

17.1 Privacy

MSKCC‟s Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

17.2 Serious Adverse Event (SAE) Reporting

Any SAE must be reported to the IRB/PB as soon as possible but no later than 5 calendar days. The IRB/PB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at sae@mskcc.org. The report should contain the following information:

Fields populated from CRDB:

- Subject‟s name (generate the report with only initials if it will be sent outside of MSKCC)
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
    - A explanation of how the AE was handled
    - A description of the subject‟s condition
    - Indication if the subject remains on the study
    - If an amendment will need to be made to the protocol and/or consent form.

The PI‟s signature and the date it was signed are required on the completed report.

For IND/IDE protocols: The CRDB AE report should be completed as above and the FDA assigned IND/IDE number written at the top of the report. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND Office.

17.2.1 Any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any subject from the time the consent is signed through 90 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to Merck product, must be reported within 5 calendar days to the MSKCC Safety Office and within 2 working days to Merck Global Safety unless specified in the ECI reporting guidance (APPENDIX C).

Non-serious Events of Clinical Interest will be forwarded to Merck Global Safety and will be handled in the same manner as SAEs.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to Merck product that is brought to the attention of the investigator at any time outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor and to Merck.

SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety facsimile number: +1-215-993-1220

A copy of all 15 Day Reports and Annual Progress Reports is submitted as required by FDA, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local regulators. Investigators will cross reference this submission according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally investigators will submit a copy of these reports to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215 993-1220) at the time of submission to FDA.

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

Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) (see APPENDIX C) and must be recorded as such on the Adverse Event case report forms/worksheets and reported within 5 calendar days to the MSKCC Safety Office and to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220) according to the ECI reporting guidance (APPENDIX C).

Events of clinical interest for this trial include:

1. an overdose of Merck product, as defined in Section 11.1.3 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.

2. an elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.

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

3. In the event a subject develops any of the following AEs, a detailed narrative of the event should be reported as an ECI to the Sponsor within 5 calendar days to the

MSKCC Safety Office and to Merck Global Safety according to the ECI reporting guidance (APPENDIX C):

a. Grade ≥ 3 diarrhea
b. Grade ≥ 3 colitis
c. Grade ≥ 2 pneumonitis
d. Grade ≥ 3 hypo- or hyperthyroidism

A separate guidance document has been provided entitled “event of Clinical Interest and Immune-Related Adverse Event Guidance Document.” This document provides guidance regarding identification, evaluation and management of ECIs and irAEs. Additional ECIs are identified in this guidance document and also need to be reported to the Sponsor within 5 calendar days to the MSKCC Safety Office and to Merck Global Safety according to the ECI reporting guidance (APPENDIX C).

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

ECIs that occur in any subject from the date of first dose through 90 days following cessation of treatment, or the initiation of a new anticancer therapy, whichever is earlier, whether or not related to the Merck"s product, must be reported within 5 calendar days to the MSKCC Safety Office and to Merck Global Safety according to the ECI reporting guidance (APPENDIX C).

18.0 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.

5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

19.0 REFERENCES

1. Ferlay, J., et al., *Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008.* Int J Cancer, 2010. **127**(12): p. 2893-917.
2. *American Cancer Society. Cancer Facts and Figures 2011*.
3. Meyerhardt, J.A. and R.J. Mayer, *Systemic therapy for colorectal cancer.* N Engl J Med, 2005. **352**(5): p. 476-87.
4. Chung, K.Y., et al., *Phase II study of the anti-cytotoxic T-lymphocyte-associated antigen 4 monoclonal antibody, tremelimumab, in patients with refractory metastatic colorectal cancer.* J Clin Oncol, 2010. **28**(21): p. 3485-90.
5. Lipson, E.J., et al., *Durable Cancer Regression Off-Treatment and Effective Reinduction Therapy with an Anti-PD-1 Antibody.* Clin Cancer Res, 2013. **19**(2): p. 462-8.
6. Brahmer, J.R., et al., *Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates.* J Clin Oncol, 2010. **28**(19): p. 3167-75.
7. Topalian, S.L., et al., *Safety, activity, and immune correlates of anti-PD-1 antibody in cancer.* N Engl J Med, 2012. **366**(26): p. 2443-54.
8. Waitz, R., M. Fasso, and J.P. Allison, *CTLA-4 blockade synergizes with cryoablation to mediate tumor rejection.* Oncoimmunology, 2012. **1**(4): p. 544-546.
9. Levy, M.Y., et al., *Cyclophosphamide unmasks an antimetastatic effect of local tumor cryoablation.* J Pharmacol Exp Ther, 2009. **330**(2): p. 596-601.
10. den Brok, M.H., et al., *In situ tumor ablation creates an antigen source for the generation of antitumor immunity.* Cancer Res, 2004. **64**(11): p. 4024-9.
11. Deng, L., et al., *Irradiation and anti-PD-L1 treatment synergistically promote antitumor immunity in mice.* J Clin Invest, 2014. **124**(2): p. 687-95.
12. Postow, M.A., et al., *Immunologic correlates of the abscopal effect in a patient with melanoma.* N Engl J Med, 2012. **366**(10): p. 925-31.
13. Sharpe, A.H. and G.J. Freeman, *The B7-CD28 superfamily.* Nat Rev Immunol, 2002. **2**(2): p. 116-26.
14. Dong, H., et al., *Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion.* Nat Med, 2002. **8**(8): p. 793-800.
15. Brown, J.A., et al., *Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production.* J Immunol, 2003. **170**(3): p. 1257-66.
16. Francisco, L.M., P.T. Sage, and A.H. Sharpe, *The PD-1 pathway in tolerance and autoimmunity.* Immunol Rev, 2010. **236**: p. 219-42.
17. Lesokhin, A.M., et al., *Monocytic CCR2(+) myeloid-derived suppressor cells promote immune escape by limiting activated CD8 T-cell infiltration into the tumor microenvironment.* Cancer Res, 2012. **72**(4): p. 876-86.

18. Eisenhauer, E.A., et al., *New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1).* Eur J Cancer, 2009. **45**(2): p. 228-47.

20.0 APPENDICES

APPENDIX A: Requisition For Blood Specimens
APPENDIX B: Requisition For Biopsy Specimens
APPENDIX C: PEMBROLIZUMAB PROGRAM (MK-3475) EVENT OF CLINICAL INTEREST GUIDANCE DOCUMENT, Version 5.0

APPENDIX A: REQUISITION FOR BLOOD SPECIMENS

Single Arm Phase II Study to Assess the Efficacy of Pembrolizumab Plus Radiotherapy or Ablation in Metastatic Colorectal Cancer Patients

A) Section A: Patient information (To be completed by RSA) Patient initials: _____ Patient study ID#: _____ Site: _____	**B) Section B: Sample information** (To be checked off by RSA) **4 CPT tubes (10 cc) will be collected at the following time points** **[ ]**Baseline **[ ]**Week 3 **[ ]**Week 6 **[ ]**Week 9 **[ ]**Week ____
C) Section C: Sample Collection information (To be completed by phlebotomy) Drawn By:_____ Date/ Time:_____	Sample Collection Instructions: 1. Gently invert all tubes 8-10 times at room temperature immediately after collection 2. Write patient initials, date, and time of collection on each tube 3. Place all collected tubes in biohazard ziplock bag 4. Send all specimens at room temperature via Stat Messengers to _____
D) Section D: Sample Processing information (To be completed by laboratory personnel) Lab ID#: _____ Received by: _____ Date/Time: _____	
E) **Section E: Sample shipping information** (To be completed by laboratory personnel) Sent by: _____ Date/Time: _____ Received by: _____ Date/Time: _____	Sample shipping Instructions Ship samples on dry ice to: Dr. Jianda Yuan Memorial Sloan-Kettering Cancer Center Zuckerman Research Building 425 East 66th street, Z1545 New York, NY 10065

APPENDIX B: REQUISITION FOR BIOPSY SPECIMENS OR LEFT OVER TISSUE OBTAINED DURING A ROUTINE PROCEDURE

Single Arm Phase II Study to Assess the Efficacy of Pembrolizumab Plus Radiotherapy or Ablation in Metastatic Colorectal Cancer Patients

A) Section A: Patient information (To be completed by RSA) Patient initials: _____ Patient study ID#: _____ Site: _____	**B) Section B: Sample information** (To be checked off by RSA) **[ ]** Baseline: **5 Core Biopsies** **[ ]** Week 1: **5 Core Biopsies** **[ ]** Week 4: **5 Core Biopsies** **[ ]** Routine Procedure: **5 Cores or divided tumor**
C) Section C: Sample Collection information (To be completed by physician or designee) Obtained By:_____ Procedure: _____ Date/ Time:_____	Sample Collection Instructions: 1. Cores # 1, 2, 5: Place into formalin (3-5 cc) 2. Cores # 3, 4: Place in sterile Nunc tube and snap freeze in liquid nitrogen *3. Core #__: Place in RPMI (5 cc)* *[If tissue remains, collect/ process as for core #5]*
D) Section D: Storage/Processing information (To be completed by laboratory personnel) Lab ID#: _____ Received by: _____ Date/Time: _____	Sample Storage/ Processing Instructions 1. Cores # 1, 2, 5: Store at 4-8°C 2. Cores # 3, 4: Store at -70 to -80°C *3. Core #___: Process immediately for isolation of tumor infiltrating lymphocytes.*
E) **Section E: Sample shipping information** (To be completed by laboratory personnel) Sent by: _____ Date/Time: _____ Received by: _____ Date/Time: _____	Sample shipping Instructions Ship samples 1, 2, and 5 at room temperature; samples 3 and 4 on dry ice, attention: Dr. Jianda Yuan Memorial Sloan-Kettering Cancer Center Zuckerman Research Building 425 East 66th street, Z1545 New York, NY 10065