Protocol Title

A Multi-Center, Single Arm, Phase II study of Pembrolizumab (MK-3475) in Combination with Chemotherapy for Patients with Advanced Colorectal Cancer: HCRN GI14-186

Sponsor-Investigator
Kathy D Miller, M.D.
Indiana University School of Medicine

Statistician
Ziyue Liu, PhD
Indiana University, Schools of Public Health and Medicine

Trial Management Provided by
Hoosier Cancer Research Network, Inc.

Funding Support and Pembrolizumab Provided By
Merck Sharp & Dohme Corp.

Investigational New Drug (IND) Application
Exempt per FDA 24FEB2015

Initial Protocol Version Date: 23FEB2015
Amended Protocol Version Date:
06JUL2015
30SEP2015
26FEB2016
02AUG2016
10NOV2016
29APR2019

PROTOCOL SIGNATURE PAGE

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Hoosier Cancer Research Network GI14-186

VERSION DATE: 29APR2019

I confirm I have read this protocol, I understand it, and I will work according to this protocol and to the ethical principles stated in the latest version of the Declaration of Helsinki, the applicable guidelines for good clinical practices, or the applicable laws and regulations of the country of the study site for which I am responsible, whichever provides the greater protection of the individual. I will accept the monitor's overseeing of the study. I will promptly submit the protocol to applicable ethical review board(s).

Instructions to the Investigator: Please **SIGN** and **DATE** this signature page. **PRINT** your name and title, the name and location of the facility in which the study will be conducted, and the expected IRB approval date. Scan and email the completed form to HCRN and keep a record for your files.

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Signature of Investigator Date

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Investigator Name (printed)

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Investigator Title

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Name of Facility

_____

Location of Facility (City and State)

_____ ❑ Not Submitting to IRB

Expected IRB Approval Date

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SYNOPSIS

TITLE	A Multi-Center, Single Arm, Phase II study of Pembrolizumab (MK-3475) in Combination with Chemotherapy for Patients with Advanced Colorectal Cancer: HCRN GI14-186
SHORT TITLE	Phase II study of Pembrolizumab (MK-3475) in combination with Chemotherapy for patients with advanced colorectal cancer
PHASE	II
OBJECTIVES	**Primary Objective** Estimate median progression free survival (mPFS) and compare to historical standard **Primary Endpoint** Median progression free survival as measured from the time of registration to the time of progression per RECIST v1.1 or subject death **Secondary Objectives** 1- Objective response rate (ORR) 2- Disease control rate (DCR) 3- Delayed response 4- Overall Survival (OS) 5- Toxicity of the combination of Pembrolizumab plus mFOLFOX6 6- Response Criteria per established Immune Related Response Criteria **Secondary Endpoints** 1- ORR will be calculated with combining the number of subjects who achieve complete response and partial response per irRC criteria 2- Disease control rate; defined as the sum of subjects with complete response, partial response and stable disease. 3- Delayed response will be evaluated in subjects who achieve stable disease on first tumor assessment but they exhibit an objective response on subsequent tumor evaluations per irRC. 4- Overall Survival will be calculated from the time of registration till the time of subject death 5- Toxicity of therapy will be assessed per CTCAE v4 6- Response rate per Immune Related Response Criteria (irRC) **Exploratory Objectives** 1. Assess the association between TLR4, MyD88, NF-kB expression by IHC and response rate 2. Assess Microsatellite status by IHC

	3. Assess the association between HMGB1 blood level and response rate 4. Assess the association between PD-L1 expression and response rate 5. Blood samples will be stored in the HCRN Biorepository for future correlatives. 6. Tissue samples will be stored in the HCRN Biorepository for future correlatives.
STUDY DESIGN	Multi-institutional, single arm, open-label, phase II study, including a safety run-in cohort. No randomization or blinding involved.
ESTIMATED NUMBER OF SUBJECTS	N = 30
ELIGIBILITY CRITERIA	**Inclusion Criteria** 1. Be willing and able to provide written informed consent for the trial and HIPAA authorization for release of personal health information. **NOTE:** HIPAA authorization may be included in the informed consent or obtained separately. 2. Be ≥ 18 years of age on day of signing informed consent. 3. Have a performance status of 0 or 1 on the ECOG Performance Scale within 14 days prior to registration. 4. Have histological or cytological evidence of colorectal adenocarcinoma with confirmation of metastatic disease either by pathologic or radiologic findings. 5. Have identified tissue from an archival tissue sample (preferably from a metastasis, but sample from primary tumor allowable) or newly obtained core or excisional biopsy of a tumor lesion. 6. Have had no prior systemic therapy for advanced or metastatic disease. Prior adjuvant therapy should have been completed at least 9 months from documentation of metastatic disease. 7. Have measurable disease according to RECIST v1.1 obtained by imaging within 28 days prior to registration. 8. Hemoglobin ≥ 9 g/dL (transfusions are acceptable) 9. Absolute neutrophil count (ANC) ≥ 1.5×10^9/L 10. Platelets ≥ 100×10^9/L 11. Serum creatinine ≤ 1.5 × upper limit of normal (ULN), or measured or calculated creatinine clearance (estimated by Cockcroft-Gault formula below or measured) ≥ 50 mL/min 12. Serum total bilirubin ≤ 1.5 × ULN 13. Aspartate aminotransferase (AST, SGOT) and alanine aminotransferase (ALT, SGPT) ≤ 3 × ULN, unless evidence of liver metastases, then AST/ALT ≤ 5 x ULN

14. International Normalized Ratio (INR) **or** Prothrombin Time (PT) ≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
15. 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
16. Female subject of childbearing potential should have a negative urine or serum pregnancy within 14 days prior to study registration. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required. Subjects of childbearing potential (regardless of sexual orientation, having undergone a tubal ligation, or remaining celibate by choice) are those who meet the following criteria:
    - Has not undergone a hysterectomy or bilateral oophorectomy; or
    - Has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 12 consecutive months).
17. 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.

Exclusion Criteria

1. Has clearly resectable colon cancer liver metastases (CCLM), for example oligometastatic disease involving only one lobe of the liver. Subjects with suspected resectable CCLM should undergo evaluation by a liver surgeon prior to enrollment to document the incurable nature of their disease.
2. Is currently participating in or has participated in a study of an investigational agent or using an investigational device within 4 weeks of registration. Subjects are not permitted to participate in another investigational drug study while being treated on this protocol.
3. Is unable to receive a port or peripherally inserted central catheter (PICC).
4. Has a diagnosis of immunodeficiency or is receiving chronic steroid therapy of prednisone ≥ 10 mg daily or any equivalent dose of corticosteroids.
5. Has previously undergone organ or bone marrow transplantation and is on immunosuppressive therapy
6. Has had major surgery or significant traumatic injury within 4 weeks of study registration. Subject must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy. A diagnostic or research

	biopsy does not exclude subjects from enrollment. Placement of a vascular access device such as a Port-A-Cath is not considered major surgery 7. Has baseline peripheral neuropathy/paresthesia grade ≥ 1. 8. Has a known additional malignancy within the past 3 years. Exceptions include treated localized basal cell or squamous cell carcinoma of the skin, in situ cervical or vulvar carcinoma that has undergone potentially curative therapy, superficial bladder tumors (Ta, Tis & T1), ductal carcinoma in situ (DCIS) of the breast and low grade prostate cancer (Gleason sore 6). Any cancer curatively treated > 3 years prior to registration with no clinical evidence of recurrence is permitted 9. Has known active central nervous system (CNS) metastases and/or carcinomatous 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 trial registration 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 registration. 10. 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. Exceptions to the rule: • Subjects with vitiligo • Subjects with resolved childhood asthma/atopy. • Subjects that require intermittent use of bronchodilators or local steroid injections. • Subjects with hypothyroidism stable on hormone replacement or • Sjögren's syndrome 11. Has a history of pneumonitis that required steroids or current pneumonitis. 12. Has known history of active tuberculosis. 13. Has an active infection requiring systemic therapy (≥ grade 2) for more than 3 days within 1 week of enrollment. 14. 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 Pembrolizumab. 15. Has known hypersensitivity to fluorouracil (5FU), oxaliplatin, or other platinum agents. 16. Known hypersensitivity to pembrolizumab or any of its excipients. 17. Has known dihydropyrimidine dehydrogenase deficiency (DPD) deficiency (testing not required) 18. 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). 19. Has known active Hepatitis B unless patient subject has been on antiviral agents for at least 2 months (baseline testing not required) 20. Has a known history of Human Immunodeficiency Virus (HIV) or Hepatitis C (baseline testing is not required). 21. Has received a live vaccine within 30 days prior to trial registration. 22. 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 site investigator. 23. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial. 24. Has any other psychological, familial, sociological or geographical condition potentially hampering compliance with the study protocol and follow-up schedule. Those conditions should be discussed with the subject before registration in the trial.
STATISTICAL CONSIDERATIONS	This is a multi-institutional, single arm, open-label, phase II study, including a safety run-in cohort, of the combination of pembrolizumab plus FOFLOX in subjects with untreated locally advanced or metastatic colorectal cancer. After a safety run-in cohort of 6 subjects at the tolerated dose level, 24 additional subjects will be enrolled. These 30 subjects will be analyzed as a single cohort for subsequent efficacy and safety analyses. PFS and OS will be estimated by Kaplan-Meier method. Median PFS (mPFS) and OS (mOS) will be estimated with 80% confidence intervals (CIs). The response rates will be estimated with their Agresti-Coull 95% CIs. Duration of response will be summarized by descriptive statistics. Frequencies and proportions will be used to describe toxicities. Common Terminology Criteria for Adverse Events (CTCAE) v4 will be used for assessment of acute and late toxicities during the follow-up period. Logistic regressions will be used for the exploratory objectives studying potential predictors of responses. Historically, first line FOLFOX achieves an mPFS of 9-months. If there are moderate evidences that adding Pembrolizumab will increase mPFS from 9- to around 14-months, a full scale randomized Phase II study will be considered to formally test whether the improvement is meaningful and statistically significant. The sample size N=30 is determined based on budget/recruitment considerations and precision of estimation. We expect that these 30

	subjects will be uniformly accrued within 12-months with an additional 24-months follow up. Kaplan-Meier method will be used to estimate PFS. mPFS and its two-sided 80% CI will be calculated. Consequently, the half-width of the CI from the lower bound to the point estimate of mPFS will be around 4.5-months, which is smaller than the difference between 14- and 9-months. We consider this precision level will allow a decision to be made whether to pursue further studies.
ESTIMATED ENROLLMENT PERIOD	Estimated months: 12 months
ESTIMATED STUDY DURATION	Estimated months: 24 months

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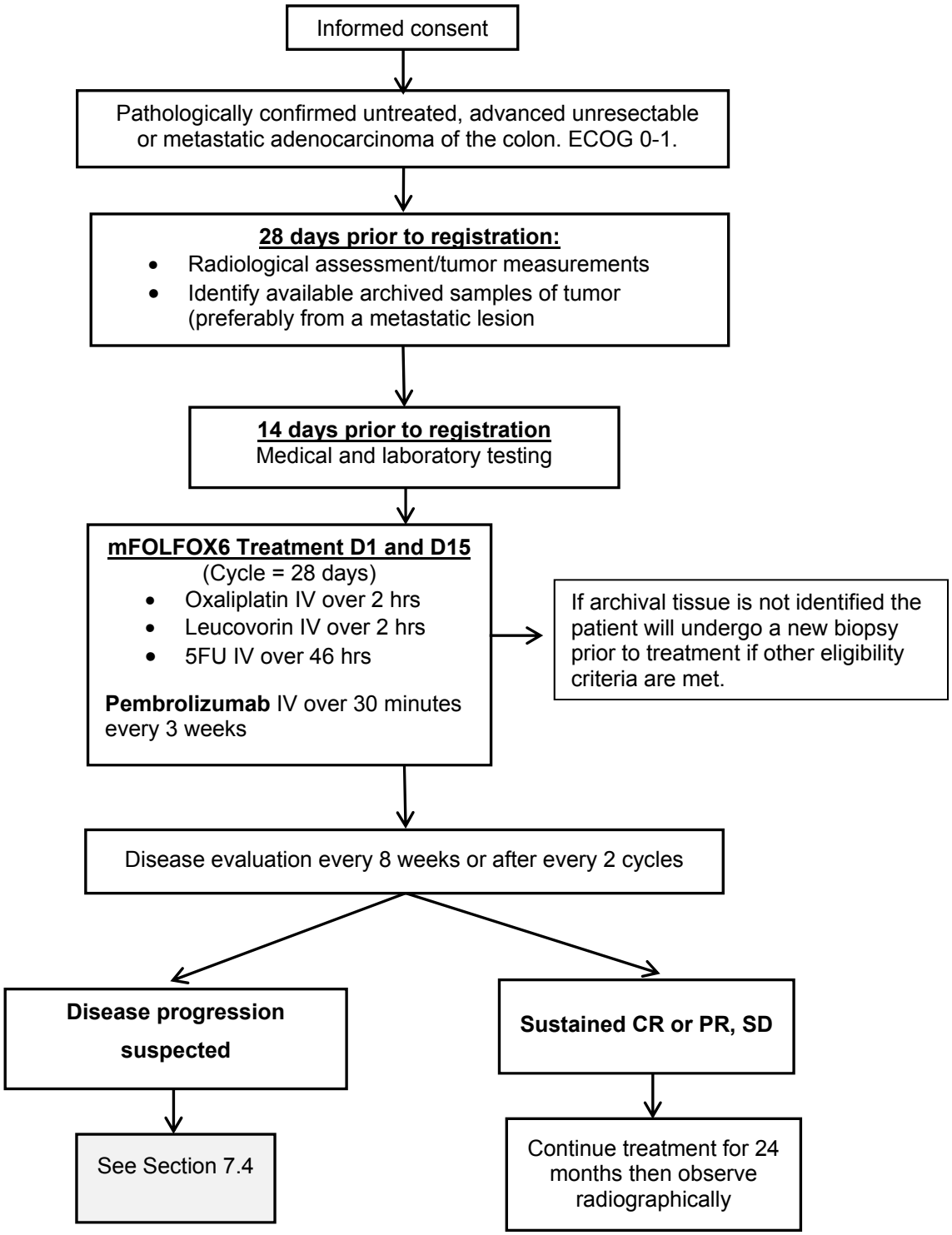
SCHEMA


Informed consent
Pathologically confirmed untreated, advanced unresectable or metastatic adenocarcinoma of the colon. ECOG 0-1.
28 days prior to registration:
Radiological assessment/tumor measurements
Identify available archived samples of tumor (preferably from a metastatic lesion
14 days prior to registration
Medical and laboratory testing
mFOLFOX6 Treatment D1 and D15
(Cycle = 28 days)
Oxaliplatin IV over 2 hrs
Leucovorin IV over 2 hrs
5FU IV over 46 hrs
Pembrolizumab IV over 30 minutes every 3 weeks
If archival tissue is not identified the patient will undergo a new biopsy prior to treatment if other eligibility criteria are met.
Disease evaluation every 8 weeks or after every 2 cycles
Disease progression suspected
See Section 7.4
Sustained CR or PR, SD
Continue treatment for 24 months then observe radiographically

1. BACKGROUND AND RATIONALE

1.1. Background

Human cancers harbor numerous genetic and epigenetic alterations, generating neo-antigens that are potentially recognizable by the immune system. Cancer evolves when intrinsic and extrinsic aberrations occur. Intrinsic genetic errors lead to oncogenic activation and evasion of apoptosis, and an extrinsic aberration involves complex microenvironment interactions, and escaping immune surveillance [1] and immune mediated death [2]. Although an endogenous immune response to cancer is observed in preclinical models and patients, this response is commonly ineffective to eliminate the tumor [3]. This is based on the hypothesis that cancer develops multiple resistance mechanisms, including local immune suppression, induction of tolerance, and systemic dysfunction in T-cell signaling. Additionally, there is a collective evidence that the innate immune system can play a role as an extrinsic tumor suppressor, and in some cases, promote tumor growth [4]. This complex function of innate and adaptive immune system is commonly referred to as immunoediting [5]. The presence of tumor infiltrating lymphocytes (TIL) in the tumor microenvironment is associated with improved outcome in patients with several malignancies including colon cancer [6-10]. Additionally, the concentration of TILs in colorectal liver metastases is associated with improvement in progression free survival, and better response to chemotherapy [11]. The immunomodulatory enzyme indoleamine 2,3-dioxygenase (IDO) that is present in the tumor microenvironment is associated with decreased CD3 tumor infiltrating cells, and higher risk of developing liver metastases from colon cancer tumors[12]. Programmed death 1 (PD-1) is a key immune checkpoint receptor expressed by activated T cells, and it mediates immunosuppression [13]. PD-1, in addition to several inhibitory and stimulatory key immune modulators, regulates the activity and function of T cells. When PD-1 is unbound, the T cells on which it is expressed can engage and kill target cells, but when bound to its ligands PD-L1 and PD-L2, it acts as a brake that inhibits T cell activities particularly during their cytotoxic attack in the tumor itself. Its ligands are overexpressed directly on cancer cells, and their interaction with PD-1 prevents tumor-infiltrating T cells from recognizing their quarry [14].

1.2. Immunotherapy and Cancer

The concept of reprogramming the immune system to recognize cancer as a “foreign entity” has been explored for decades. In fact, BCG was one of the first local immunotherapies used to treat early stage superficial bladder cancer [15]. Since then a tremendous effort has been made to explore several ways of stimulating the immune system and reprogram it to fight cancer. Early studies with interleukin 2 in melanoma and renal cell carcinoma exhibited low response rate, but was associated with first long durable responses in patients with metastatic disease [16]. Sipuleucel-T consists of autologous peripheral blood mononuclear cells, including APCs, that have been activated during a defined period in cell culture with recombinant human PAP–GM-CSF decreased risk of death for men with castrate resistant prostate cancer [17]. While some tumors appear to be more immunogenic than others, recent data supported the potential promise of vaccine therapy in patients with gastrointestinal malignancies such as pancreatic adenocarcinoma [18, 19].

The human immune system is under a complex regulatory structure. Several key factors work as inhibitory checkpoints and others are immune stimulatory [20]. Perhaps the antibody targeting the negative immune checkpoint inhibitors are the most advanced in clinical trials amongst other immune modulatory agents. CTLA4, PD-1, PD-L1 and PD-L2 antagonists have already been evaluated in advanced melanoma with promising results [21-23]. Ipilimumab (CTLA4 antagonist) is the first immune checkpoint inhibitor to be granted FDA approval for the treatment of advanced melanoma. Combination checkpoint inhibitors have demonstrated a promising new era of cancer immune therapy [21]. Proposals to evaluate whether combining a checkpoint inhibitor with an immune stimulatory compound will be safe and effective are underway.

1.3. Pembrolizumab

Pembrolizumab is a highly selective, humanized monoclonal IgG4–kappa isotype antibody against PD-1 that is designed to block the negative immune regulatory signaling of the PD-1 receptor expressed by T cells. The IgG4 immunoglobulin subtype does not engage Fc receptors or activate complement, thus avoiding cytotoxic effects of the antibody when it binds to the T cells that it is intended to activate [24]. In a phase I dose escalation study, 9 patients were enrolled at three different dose levels: 1mg/kg, 3 mg/kg and 10 mg/kg with no observed dose limiting toxicity of the compound with evidence of response in a patient with melanoma [23]. As a result, 135 patients with advanced melanoma were treated with pembrolizumab and experienced a high rate of sustained tumor regression, even in patients who received prior CTLA4 inhibitor [24].

Toxicity associated with pembrolizumab were mainly grade 1 and 2 per the CTCAE, however 13% of patients experienced grade 3 and 4. 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%).

1.3.1 Pembrolizumab Dose

Available PK results in subjects with melanoma, NSCLC, and other solid tumor types support a lack of meaningful difference in PK exposures obtained at a given dose among tumor types. An open-label Phase 1 trial (PN001) in melanoma subjects is being conducted to evaluate the safety and clinical activity of single agent pembrolizumab. The dose escalation portion of this 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 maximum tolerated dose (MTD) has been identified.

In KEYNOTE-001, two randomized cohort evaluations of melanoma subjects receiving pembrolizumab at a dose of 2 mg/kg versus 10 mg/kg Q3W have been completed. The

clinical efficacy and safety data demonstrate a lack of clinically important differences in efficacy response or safety profile at these doses. For example, in Cohort B2, advanced melanoma subjects who had received prior ipilimumab therapy were randomized to receive pembrolizumab at 2 mg/kg versus 10 mg/kg Q3W. The overall response rate (ORR) was 26% (21/81) in the 2mg/kg group and 26% (25/79) in the 10 mg/kg group (full analysis set (FAS)). The proportion of subjects with drug-related adverse events (AEs), grade 3-5 drug-related AEs, serious drug-related AEs, death or discontinuation due to an AE was comparable between groups or lower in the 10 mg/kg group.

Available pharmacokinetic results in subjects with melanoma, NSCLC, and other solid tumor types support a lack of meaningful difference in pharmacokinetic exposures obtained at a given dose among tumor types. Population PK analysis has been performed and has confirmed the expectation that intrinsic factors do not affect exposure to pembrolizumab to a clinically meaningful extent. Taken together, these data support the use of lower doses (with similar exposure to 2 mg/kg Q3W) in all solid tumor indications. 2 mg/kg Q3W is being evaluated in NSCLC in PN001, Cohort F30 and PN010, and 200 mg Q3W is being evaluated in head and neck cancer in PN012, which are expected to provide additional data supporting the dose selection.

Selection of 200 mg as the appropriate dose for a switch to fixed dosing is based on simulation results indicating that 200 mg will provide exposures that are reasonably consistent with those obtained with 2 mg/kg dose and importantly will maintain individual patient exposures within the exposure range established in melanoma as associated with maximal clinical response. A population PK model, which characterized the influence of body weight and other patient covariates on exposure, has been developed using available data from 476 subjects from PN001. The distribution of exposures from the 200 mg fixed dose are predicted to considerably overlap those obtained with the 2 mg/kg dose, with some tendency for individual values to range slightly higher with the 200 mg fixed dose. The slight increase in PK variability predicted for the fixed dose relative to weight-based dosing is not expected to be clinically important given that the range of individual exposures is well contained within the range of exposures shown in the melanoma studies of 2 and 10 mg/kg to provide similar efficacy and safety. 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 tumor types and indication settings.

1.4. Rationale for this Combination

Until recently, a commonly held opinion was that chemotherapy and immunotherapy should not be combined because of the myelosuppressive effect of most cytotoxic agents. However, it has now become evident that chemotherapeutics can exert several beneficial effects on the immune system. Several studies demonstrated an “immunogenic cancer death” associated with certain cytotoxic chemotherapies. In particular, it has been demonstrated that oxaliplatin (OXP), but not cisplatin, induces immunogenic death of cancer cells [25]. Cancer cells undergo immunogenic apoptosis in response to OXP, meaning that their corpses are engulfed by dendritic cells (DC) and that tumor cell antigens are presented to tumor-specific CD8+ T cells, which then control residual tumor cells. One of the peculiarities of

immunogenic apoptosis is the early cell surface exposure of calreticulin (CRT). Pioneering work by Zitvogel et al. has shown that OXP induces immunogenic cell death, as upon treatment with OXP, tumor cells transport CRT to their cell surface. The exposure of CRT provides a signal that is recognized by DCs and ultimately results in phagocytosis of tumor cells [26]. Exposure of tumor cells to OXP also results in the release of high mobility group box 1 (HMGB1) protein [27], which activates DCs in a toll-like receptor-4 (TLR4)-dependent manner. This in turn activates DC and leads to cytotoxic T-lymphocyte (CTL) infiltration of the tumor [28]. OXP also reduces expression of PD-L2 resulting in enhanced antigen-specific proliferation and Th1 cytokine secretion [29].

Additionally, releasing antigens following effective therapy increases the cross presentation of these antigens, leading to increase in functional T lymphocytes activity in vivo [30]. Several mechanisms on how antigen presenting cells and other T cell inhibitory/stimulatory factors can interact following chemotherapy have been previously explored and discussed in detail [31]. Constant antigen presentation by APC, may lead to the upregulation of CTL stimulatory factors leading to activation of the immune response to antigen release [32].

While early phase studies [14, 22] included patients with colorectal cancer with no evidence of objective response, our proposal differs in the concept that it is evaluating the immunogenic effect of OXP on cell death leading to a magnified response when combined with pembrolizumab. Since mFOLFOX6 can induce an objective response (>30% tumor shrinkage per RECIST criteria) in nearly 50% of patients with metastatic colorectal cancer (mCRC) [33], this might lead to stimulating the immune response through OXP mediated immune cell death in addition to massive release of intracellular antigens from dying cancer cells leading to APCs chain activation.

The combination of OXP and 5FU (mFOLFOX6) represents a widely accepted standard of care for the treatment of patients with metastatic colorectal cancer, and is commonly used in the first line setting with or without biologic agents [34, 35]. Oxaliplatin demonstrated synergic effect in combination with 5FU and represented a paradigm shift in the management of patients with advanced colorectal cancer leading to superior response rate, progression free survival, and overall survival. Given the previous data referenced, there is a substantial rationale to combine pembrolizumab with mFOLFOX6 in patients with untreated locally advanced or metastatic colorectal cancer. Here we propose a phase II study of pembrolizumab plus mFOLFOX6 in subjects with untreated locally advanced or metastatic colorectal cancer.

2. STUDY OBJECTIVES

2.1. Primary Objective

- Estimate median progression free survival (mPFS) and compare to historical standard

  - **Primary Endpoint**
    - Median progression free survival as measured from the time of registration to the time of progression per RECIST v1.1 or subject death

2.2. Secondary Objectives

- Objective response rate (ORR)
- Disease control rate (DCR)
- Delayed response
- Overall Survival (OS)
- Toxicity of the combination of pembrolizumab plus mFOLFOX6
- Response Criteria per established Immune Related Response Criteria

  - **Secondary Endpoints**
    - ORR will be calculated with combining the number of subjects who achieve complete response and partial response per irRC criteria.
    - Disease control rate; defined as the sum of subjects with complete response, partial response and stable disease.
    - Delayed response will be evaluated in subjects who achieve stable disease on first tumor assessment but they exhibit an objective response on subsequent tumor evaluations per irRC.
    - Overall Survival will be calculated from the time of registration till the time of subject death
    - Toxicity of therapy will be assessed per CTCAE V4
    - Response rate per Immune Related Response Criteria (irRC)

2.3. Exploratory Objectives

- Assess the association between TLR4, MyD88, NF-kB expression by IHC and response rate.
- Assess Microsatellite status by IHC.
- Assess the association between HMGB1 blood level and response rate.
- Assess the association between PD-L1 expression and response rate.
- Blood samples will be stored in the HCRN Biorepository for future correlatives.
- Tissue samples will be stored in the HCRN Biorepository for future correlatives

3. ELIGIBILITY CRITERIA

3.1. Inclusion Criteria

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

1. Be willing and able to provide written informed consent for the trial and HIPAA authorization for release of personal health information.
    - **NOTE:** HIPAA authorization may be included in the informed consent or obtained separately.
2. Be ≥ 18 years of age on day of signing informed consent.
3. Have a performance status of 0 or 1 on the ECOG Performance Scale within 14 days prior to registration.
4. Have histological or cytological evidence of colorectal adenocarcinoma with confirmation of metastatic disease either by pathologic or radiologic findings.
5. Have identified tissue from an archival tissue sample (preferably from a metastasis, but sample from primary tumor allowable) or newly obtained core or excisional biopsy of a tumor lesion. See Section 9 for more details.
6. Have had no prior systemic therapy for advanced or metastatic disease. Prior adjuvant therapy should have been completed at least 9 months from documentation of metastatic disease. Prior palliative radiotherapy allowed if toxicities resolved to grade 1 or baseline.
7. Have measurable disease according to RECIST v1.1 obtained by imaging within 28 days prior to registration.
8. Hemoglobin ≥ 9 g/dL (transfusions are acceptable)
9. Absolute neutrophil count (ANC) ≥ 1.5×10^9/L
10. Platelets ≥ 100×10^9/L
11. Serum creatinine ≤ 1.5 × upper limit of normal (ULN), or measured or calculated creatinine clearance (estimated by Cockcroft-Gault formula below or measured) ≥ 50 mL/min
    - $\frac{\text{Males: (140 – Age in years)} \times \text{Actual Body Weight in kg}}{72 \times \text{Serum Creatinine (mg/dL)}}$
    - Females: Estimated creatinine clearance for males × 0.85
12. Serum total bilirubin ≤ 1.5 × ULN
13. Aspartate aminotransferase (AST, SGOT) and alanine aminotransferase (ALT, SGPT) ≤ 3 × ULN, unless evidence of liver metastases, then AST/ALT ≤ 5 x ULN
14. International Normalized Ratio (INR) **or** Prothrombin Time (PT) ≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
15. 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
16. Female subject of childbearing potential should have a negative urine or serum pregnancy test within 14 days prior to study registration. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required. Subjects of childbearing potential (regardless of sexual orientation, having undergone a tubal ligation, or remaining celibate by choice) are those who meet the following criteria:
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- Has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 12 consecutive months).

17. 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.

3.2. Exclusion Criteria

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

1. Has clearly resectable colon cancer liver metastases (CCLM), for example oligometastatic disease involving only one lobe of the liver. Subjects with suspected resectable CCLM should undergo evaluation by a liver surgeon prior to enrollment to document the incurable nature of their disease.
2. Is currently participating in or has participated in a study of an investigational agent or using an investigational device within 4 weeks of registration. Subjects are not permitted to participate in another investigational drug study while being treated on this protocol.
3. Is unable to receive a port or peripherally inserted central catheter (PICC).
4. Has a diagnosis of immunodeficiency or is receiving chronic steroid therapy of prednisone ≥ 10 mg daily or any equivalent dose of corticosteroids.
5. Has previously undergone organ or bone marrow transplantation and is on immunosuppressive therapy
6. Has had major surgery or significant traumatic injury within 4 weeks of study registration. Subject must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy. A diagnostic or research biopsy does not exclude subjects from enrollment. Placement of a vascular access device such as a Port-A-Cath is not considered major surgery.
7. Has baseline peripheral neuropathy/paresthesia grade ≥ 1.
8. Has a known additional malignancy within the past 3 years. Exceptions include treated localized basal cell or squamous cell carcinoma of the skin, in situ cervical or vulvar carcinoma that has undergone potentially curative therapy, superficial bladder tumors (Ta, Tis & T1), ductal carcinoma in situ (DCIS) of the breast and low grade prostate cancer (Gleason sore 6). Any cancer curatively treated > 3 years prior to registration with no clinical evidence of recurrence is permitted
9. Has known active central nervous system (CNS) metastases and/or carcinomatous 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 trial registration 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 registration.
10. 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. Exceptions to the rule:
    - Subjects with vitiligo
    - Subjects with resolved childhood asthma/atopy

- Subjects that require intermittent use of bronchodilators or local steroid injections
- Subjects with hypothyroidism stable on hormone replacement
- Subjects with Sjögren's syndrome

11. Has a history of pneumonitis that required steroids or current pneumonitis.
12. Has known history of active tuberculosis.
13. Has an active infection requiring systemic therapy (≥ grade 2) for more than 3 days within 1 week of enrollment.
14. 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 pembrolizumab.
15. Has known hypersensitivity to fluorouracil (5FU), oxaliplatin, or other platinum agents.
16. Known hypersensitivity to pembrolizumab or any of its excipients.
17. Has known dihydropyrimidine dehydrogenase deficiency (DPD) deficiency (testing not required)
18. 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).
19. Has known active Hepatitis B unless subject has been on antiviral agents for at least 2 months (baseline testing not required)
20. Has a known history of Human Immunodeficiency Virus (HIV) or Hepatitis C (baseline testing is not required).
21. Has received a live vaccine within 30 days prior to trial registration.
22. 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 site investigator.
23. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
24. Has any other psychological, familial, sociological or geographical condition potentially hampering compliance with the study protocol and follow-up schedule. Those conditions should be discussed with the subject before registration in the trial.

4. SUBJECT REGISTRATION

All subjects must be registered through Hoosier Cancer Research Network (HCRN) electronic data capture OnCore prior to starting protocol therapy. A subject is considered to be registered to the protocol when an "On Study" date has been entered into OnCore.

Subjects must begin therapy within **five business days** of the "On Study" date.

5. TREATMENT PLAN

5.1 Drug Administration

The treatment to be used in this trial is outlined below in Table 1 and will consist of: Pembrolizumab 200 mg will be administered as a 30 minute IV infusion, with a window of -5 and +10 minutes.

mFOLFOX6 premedications and chemotherapy will be administered 30 minutes after pembrolizumab. mFOLFOX is to be given at each site per their guidelines. Administration of mFOLFOX6 outside the suggested time will not be considered a deviation.

- Oxaliplatin 85 mg/m^2 IV with
- Leucovorin 400 mg/m^2 IV followed by
- 5FU 400 mg/m^2 bolus and then 2400 mg/m^2 via continuous infusion

See Section 5.2.3 for premedications recommendations for mFOLFOX6. Standard supportive care should be followed at each site per their guidelines. Subjects should receive *full supportive care*, including transfusions of blood and blood products, antibiotics, antiemetics, etc., when appropriate. The use of epoetin products is not allowed.

NOTE: Chemotherapy (mFOLFOX6) dose re-calculation on Day 1 is necessary for subjects who have >10% weigh change (gain or loss) from baseline. All BSA-based doses should use actual, not adjusted, weight.

Table 1: Drug Administration Schedule
Cycle = 28 days

Drug	Dose	Frequency of administration	Route of administration	Duration
Pembrolizumab	200 mg	every 3 weeks	30-minute IV infusion	up to 24 months
mFOLFOX6 premeds and chemo will be administered 30 minutes after pembrolizumab				
Oxaliplatin	85 mg/m^2	every 2 weeks	IV	
Leucovorin	400 mg/m^2	every 2 weeks	IV	
5FU	400 mg/m^2 bolus, then 2400 mg/m^2	every 2 weeks	IV continuous infusion	

5.2 Safety run-in cohort

The safety run-in cohort will include 6 subjects treated with 200 mg (fixed) IV infusion of pembrolizumab every 3 weeks plus standard-dose mFOLFOX6 given every 2 weeks. These first 6 subjects will be followed for 4 weeks for dose limiting toxicities (DLT) before enrolling an additional 24 subjects. If a DLT is observed in no more than 1 of 6 subjects, the trial will continue with enrolling subjects to the remainder of the phase II portion of the study. If 2 or more subjects experience DLT, 6 additional subjects will be enrolled at dose level -1. If no more than 1 DLT is observed, then phase II will enroll subjects at DL-1 for the total expected number of accrual. Otherwise, accrual will stop and the funder and sponsor-investigator will re-evaluate continuation of the trial.

Number of subjects with DLT	Action
≤1 of 6 subjects	Proceed with remainder of Phase II
≥2 of 6 subjects	MTD has been exceeded. Enroll 6 subjects at dose level -1 and re-evaluate.

A given dose cohort may be expanded up to a total of 12 subjects if further evaluation of the frequency of a given toxicity is warranted, based upon the observed safety profile in the 6 subjects already recruited in the cohort, and where the incidence of the confirmed DLT does not exceed 33%.

5.2.1 Dose limiting toxicity definition:

Dose limiting toxicity (DLT) is defined as one of the following treatment-related events occurring during the first 4 weeks of treatment:

- Any Grade 3-4 toxicity other than:
  - fatigue, or
  - nausea, vomiting, or diarrhea lasting less than 72 hours;
- Grade 3-4 thrombocytopenia or neutropenia that leads to treatment delays for > 14 days.
- Grade 4 febrile neutropenia if DPD deficiency is ruled out and subject requires delay for the next cycle >14 days
- Grade 3-4 Nausea, vomiting or diarrhea lasting 72 hours or longer despite maximal supportive care including anti emetics, intravenous hydration, anti-diarrhea agents and short term steroid use, if indicated.
- Asymptomatic grade 3-4 laboratory abnormalities will be discussed between the site investigator and sponsor-investigator before being assigned as DLT.

5.2.2 Dose Level -1

Pembrolizumab: 200 mg IV
Oxaliplatin: 65 mg/m^2 IV; Leucovorin: 400 mg/m^2 IV; 5FU: 320 mg/m^2 IV bolus then 5FU: 1920 mg/m^2 continuous infusion over 46 hours

Cohort	Pembrolizumab	Oxaliplatin	Leucovorin	5FU
0 (start)	200 mg IV	85 mg/m^2 IV	400 mg/m^2 IV	400 mg/m^2 IV bolus then 2400 mg/m^2
-1	200 mg IV	65 mg/m^2 IV	400 mg/m^2 IV	320 mg/m^2 IV bolus then 1920 mg/m^2

5.3 Phase II

If dose-limiting toxicity is observed in no more than 1 out of 6 subjects in the safety run-in cohort, the remainder of the subjects will be enrolled and treated at the current doses of pembrolizumab and mFOLFOX6.

All subjects will continue study treatment for up to 24 months, until disease progression (as defined below at Section 8) or unacceptable treatment-related toxicity occurs. Subjects will continue to be followed until progression of disease or death. Subjects with sustained CR, PR or SD at 24 months will stop therapy and be observed per study imaging schedule.

5.4 Treatment Schedules

Trial treatment should be administered on Day 1 of each cycle after all procedures/ assessments have been completed as detailed on the Study Schedule of Events (Section 7). Trial treatment may be administered up to 3 days before or after (±3 days) 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 (treatment cycle intervals may be increased due to toxicity as described in Section 5.4). Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

Please refer to the Study Procedures Manual (SPM) for specific instructions for pembrolizumab, reconstitution, preparation of the infusion fluid, and administration. mFOLFOX6 will be given per institutional standard of care. Below are recommendations for pre-medication of mFOLFOX6.

Recommended pre-medications, hydration and prophylactic medications for mFOLFOX6			
Agent	**Dose/Precautions**	**Route**	**Schedule**
Ondansetron (or formulary equivalent).May consider use of palonosetron or addition of neurokinin receptor antagonist for D1	24 mg Instruct subject to avoid exposure to cold (food, liquids, air) for 5 days following oxaliplatin	PO 30 minutes prior to oxaliplatin	Days 1
Prochlorperazine	10 mg	PO/IV q6h PRN for nausea or vomiting	Days 1-14
D5W	100 cc/h	IV during treatment for infusions in D5W and to flush lines between medications.	Days 1
Dexamethasone	12 mg	PO for nausea and vomiting unresponsive to above medications	per site investigator discretion
Loperamide (home instructions)[1]	4 mg at onset of diarrhea, then 2mg q2h prn until diarrhea stops for at least 12 hours	PO	Days 1-14

[1]For symptoms of diarrhea and/or abdominal cramping that occur at any time during a treatment cycle, subjects will be instructed to begin taking loperamide. Loperamide should be started at the earliest sign of (1) a poorly formed or loose stool or (2) the occurrence of 1 to 2 more bowel movements than usual in 1 day or (3) an increase in stool volume or liquidity. Loperamide should be taken in the following manner: 4 mg at the first onset of diarrhea, then 2 mg every 2 hours around the clock until diarrhea-free for at least 12 hours. Subjects may take loperamide 4 mg every 4 hours during the night. The maximum daily dose of loperamide is 16 mg/day. Subjects should be advised to obtain loperamide at the initial treatment visit so that they have sufficient supply on hand in case antidiarrheal support is required. Additional antidiarrheal measures may be used at the discretion of the site investigator. Subjects should be instructed to increase fluid intake to help maintain fluid and electrolyte balance during episodes of diarrhea.

5.5 Dose Modifications

The NCI Common Terminology Criteria for Adverse Events (CTCAE) v4 will be used to grade adverse events.

Subjects will be evaluated for adverse events (all grades), serious adverse events, and adverse events requiring study drug interruption or discontinuation at each study visit for the duration of their participation in the study.

5.5.1 Pembrolizumab Dose Modifications and Toxicity Management

The starting dose amount required to prepare the pembrolizumab infusion solution is a fixed dose of 200 mg and does not require calculation. On days when both chemotherapy and pembrolizumab are administered, if chemotherapy is held due to toxicity, pembrolizumab should also be held.

Pembrolizumab will be held for the following hematologic AEs if related:

- Hematologic toxicities
  - Any Grade 4
  - Grade 3 except neutropenia, anemia, thrombocytopenia and lymphopenia

Pembrolizumab will be held for the following non-hematologic AEs if related:

- Site investigators should consider holding pembrolizumab for Grade 2 toxicities with persistent symptoms unless specified in Table 2 below.
- Any ≥ Grade 3 toxicities including laboratory abnormalities. Exceptions include AST, ALT and bilirubin; Table 2 below should be followed in those instances.

Table 2: Dose modification guidelines for drug-related non-hematological adverse events

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Discontinue Subject
Diarrhea/Colitis	2-3	Toxicity resolves to Grade 0-1.	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue
AST, ALT, or Increased Bilirubin	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose.
	3-4	Permanently discontinue (see exception below)[1]	Permanently discontinue
Type 1 diabetes mellitus (if new onset) or Hyperglycemia	T1DM or 3-4	Hold pembrolizumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure.	Resume pembrolizumab when subjects are clinically and metabolically stable.
Hypophysitis	2-3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue
Hyperthyroidism	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue
Hypothyroidism	2-4	Therapy with pembrolizumab can be continued while treatment for the thyroid disorder is instituted	Therapy with pembrolizumab can be continued while treatment for the thyroid disorder is instituted.
Infusion Reaction	3-4	Permanently discontinue	Permanently discontinue

Pneumonitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	3-4	Permanently discontinue	Permanently discontinue
Renal Failure or Nephritis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	3-4	Permanently discontinue	Permanently discontinue
All Other Drug-Related Toxicity[2]	3 or Severe	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue

Note: Permanently discontinue for any severe or Grade 3 drug-related AE that recurs or any life-threatening event.
[1] For subjects with liver metastasis who begin treatment with Grade 2 AST or ALT, if AST or ALT increases by greater than or equal to 50% relative to baseline and lasts for at least 1 week then subjects should be discontinued.
[2] Subjects with intolerable or persistent Grade 2 drug-related AE may hold study medication at site investigator discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 12 weeks of the last dose.

Any delays will be recorded in the appropriate electronic case report form(s). If needed, a site investigator may consult with the sponsor-investigator by contacting the HCRN Project Manager.

If the toxicity does not resolve to Grade 0-1 within 4 weeks after last infusion, trial treatment should be discontinued per protocol guidelines. With site investigator and sponsor-investigator agreement, subjects with a laboratory adverse event still at Grade 2 after 4 weeks may continue treatment in the trial only if asymptomatic and controlled. See Section 11 for information on the management of adverse events.

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.

If a subject discontinues mFOLFOX6 treatment completely or any portion of this therapy (i.e. oxaliplatin) due to toxicity, the subject may elect to continue pembrolizumab with any portion of the original chemotherapy regimen or as a single-agent every 3 weeks per the protocol schedule for a total of 24 months, or may discontinue all study treatment and be treated at site investigator's discretion. If all therapy is discontinued, it is recommended that subjects not be treated with a second-line chemotherapy regimen until disease progression has been documented. Such subjects may continue to be imaged per the study guidelines until progression or complete withdrawal from the study.

5.5.2 mFOLFOX6 Dose Modifications and Toxicity Management

Missed doses of mFOLFOX6 will not be made up. Dose delays and treatment restarts will be made at the discretion of the site investigator according to institutional guidelines. Dose reduction of 5-FU and/or oxaliplatin is allowed and is recommended for grade 3 toxicities.

Subjects will receive mFOLFOX6 per institutional guidelines. Below are optional guidelines for dose modification and toxicity management. Subjects receiving oxaliplatin on this study

should be counseled to avoid cold drinks, chewing of ice chips, and exposure to cold water or air because the neurotoxicity often seen with oxaliplatin appears to be exacerbated by exposure to cold. The period of time during which the subject is at risk for these cold-induced sensory neuropathies is not well documented. Subjects should exercise caution regarding cold exposure during the treatment period. Peripheral sensory neuropathies can occur at any time after receiving oxaliplatin therapy.

- If a dose reduction beyond Level -3 is required for oxaliplatin, oxaliplatin will be discontinued. Continue 5-FU/leucovorin and pembrolizumab.
- If a dose reduction beyond level -3 is required for 5-FU, discontinue mFOLFOX6. Continue pembrolizumab.
- If more than one dose reduction applies, use the most stringent (i.e., the greatest dose reduction.)
- If mFOLFOX6 is delayed due to toxicity for ≥ 4 weeks, counting from the originally scheduled day of treatment that was held, consider 2 level dose reductions. Continue pembrolizumab at the same dose level.
- If pembrolizumab held due to toxicity not deemed related to mFOLFOX6, continue mFOLFOX6 therapy as scheduled.
- Missed doses of any of the drugs are not made up.

Dose Levels based on toxicity beyond DLT assessment period:

Agent	Level 0	Level –1	Level –2	Level –3
Oxaliplatin	85 mg/m^2	65 mg/m^2	50 mg/m^2	40 mg/m^2
5-FU Bolus	400 mg/m^2	320 mg/m^2	270 mg/m^2	230 mg/m^2
5-FU Infusion	2400 mg/m^2 over 46-48 hrs	1920 mg/m^2 over 46-48 hrs	1600 mg/m^2 over 46-48 hrs	1360 mg/m^2 over 46-48 hrs

Leucovorin dose is always 400 mg/m^2. If 5-FU is skipped, leucovorin must also be skipped.

Hematologic toxicities

- Dose modifications for hematologic toxicities are based on CBC on Day 1 of each cycle of mFOLFOX6. CBC may be collected within 72 hours of treatment (or 96 hours if due to holidays).
- **For ANC 1000-1199:** Delay mFOLFOX6 until ANC ≥ 1200, then resume at the previous doses of oxaliplatin and 5-FU.
- **For ANC < 1000:** Delay mFOLFOX6 until ANC ≥ 1200, then resume with one dose level reduction of oxaliplatin and 5-FU for all subsequent cycles.
- **For febrile neutropenia (defined as ANC < 1000 and T ≥ 38.5°C):** Delay mFOLFOX6 until fever has resolved and ANC ≥ 1200, then resume mFOLFOX6 with one dose level reduction of oxaliplatin and 5-FU for all subsequent cycles.
- **For platelets 50,000 – 74,999:** Delay mFOLFOX6 until platelets ≥ 75,000, then resume at the previous dose levels of oxaliplatin and 5-FU.
- **For platelets < 50,000:** Delay mFOLFOX6 until platelets ≥ 75,000, then resume with one dose level reduction of oxaliplatin and 5-FU for all subsequent cycles.

Gastrointestinal toxicities

- **For ≥ grade 2 diarrhea:** Delay mFOLFOX6 until diarrhea improves to < grade 2. Hold pembrolizumab until diarrhea improves to < grade 2.
- **Following grade 2 diarrhea at any time during a cycle:** Continue mFOLFOX6 at the previous dose levels of oxaliplatin and 5-FU.
- **Following grade 3 diarrhea at any time during a cycle:** Continue mFOLFOX6 with one dose level reduction of 5-FU for all subsequent cycles and the previous dose level of oxaliplatin.
- **Following grade 4 diarrhea at any time during a cycle:** Continue mFOLFOX6 with one dose level reduction of oxaliplatin and 5-FU for all subsequent cycles.

Oral Mucositis

- **For ≥ grade 2 oral mucositis present on Day 1 of a cycle,** delay mFOLFOX6 until mucositis improves to < grade 2. Continue pembrolizumab.
- **For grade 2 oral mucositis, at any time during a cycle,** resume/continue mFOLFOX6 at the previous dose level.
- **For grade 3 or 4 oral mucositis, despite optimal management, at any time during a cycle,** resume/continue mFOLFOX6 with one dose level reduction of 5-FU and the previous dose level of oxaliplatin.

Nausea or Vomiting

- **For grade ≥ 2 nausea or vomiting present on Day 1 of a cycle despite optimal antiemetic therapy,** delay mFOLFOX6 until nausea and vomiting improve to < grade 2. Continue pembrolizumab.
- **For grade 2 nausea or vomiting, at any time during a cycle,** resume/continue mFOLFOX6 at the previous dose levels.
- **For grade 3 nausea or vomiting, despite optimal antiemetic therapy, at any time during a cycle,** resume/continue mFOLFOX6 with one dose level reduction of oxaliplatin and the previous dose level of 5-FU.
- **For grade 4 nausea or vomiting, despite optimal antiemetic therapy, at any time during a cycle, r**esume/continue mFOLFOX6 with one dose level reduction of oxaliplatin and 5-FU.

Pulmonary Toxicities

- **For ≥ grade 3 cough, dyspnea, hypoxia, pneumonitis, or pulmonary infiltrates,** skip oxaliplatin until interstitial lung disease is ruled out. Continue 5-FU/leucovorin. Discontinue all protocol therapy if interstitial lung disease is confirmed.

Thrombotic microangiopathy

- **For ≥ grade 3 hemolytic uremic syndrome (HUS):** Discontinue oxaliplatin. Continue 5- FU/leucovorin and pembrolizumab.

Neurotoxicity
Toxicity Scale for the Sensory Neuropathies Associated with Oxaliplatin (using the Oxaliplatin Specific Neurotoxicity Scale)

	Symptoms
Grade 1	Paresthesias/dysesthesias* of short duration that resolve and do not interfere with function.
Grade 2	Paresthesias/dysesthesias* interfering with function, but not with activities of daily living (ADL)
Grade 3	Paresthesias/dysesthesias* with pain or with functional impairment that also interfere with ADL.
Grade 4	Persistent paresthesias/dysesthesias* that are disabling or life threatening.
	* May be cold-induced

- **For grade 2 neurotoxicity persisting between treatments:** Continue mFOLFOX6 with one dose level reduction of oxaliplatin for all subsequent cycles and the previous dose level of 5-FU. Continue pembrolizumab at the same dose level.
- **For grade 3 neurotoxicity resolving to ≤ grade 2 between treatments:** Continue mFOLFOX6 with one dose level reduction of oxaliplatin for all subsequent cycles and the previous dose level of 5-FU. Continue pembrolizumab at the same dose level.
- **For grade 3 neurotoxicity persisting between treatments:** Discontinue oxaliplatin. Continue 5-FU/leucovorin and pembrolizumab at the same dose level.
- **For grade 4 neurotoxicity:** Discontinue oxaliplatin. Continue 5-FU/leucovorin and pembrolizumab at the same dose level.
- **For pharyngolaryngeal dysesthesia:** Increase the duration of oxaliplatin infusion to 6 hours for all subsequent cycles.

Extravasation
Extravasation of oxaliplatin has been associated with necrosis; if extravasation is suspected, the infusion should be stopped and the drug administered at another site. Extravasation should be treated according to institutional guidelines.

Allergic Reactions
- **For grade 1 allergic reactions**: Decrease the infusion rate by 50% until symptoms resolve, then resume at the initial planned rate.
- **For grade 2 allergic reactions**: Stop infusion. Administer H1 and/or H2 blockers, and/or steroids according to institutional policy. Restart the infusion when symptoms resolve and pretreat before all subsequent doses. Treat according to institutional policy.
- **For grade 3 or grade 4 allergic reactions or anaphylaxis:** Stop the infusion. Discontinue oxaliplatin (if timing consistent with oxaliplatin hypersensitivity). Continue 5-FU and leucovorin and pembrolizumab. If grade 3 or 4 allergic reactions or anaphylaxis with first or second cycle, consider leucovorin as offending agent. Discontinue leucovorin and continue mFOLFOX6 (without leucovorin) and pembrolizumab.

Oxaliplatin-induced pharyngolaryngeal dysesthesia
Should a subject develop oxaliplatin-induced pharyngolaryngeal dysesthesia, her/his oxygen saturation should be evaluated via a pulse oximeter; if abnormal, an anxiolytic agent may be given and the subject observed in the clinic until the episode has resolved. Increase the duration of oxaliplatin to 6 hours for all subsequent treatments. Some overlap may exist between the manifestations of pharyngolaryngeal dysesthesia and hypersensitivity reactions. A table comparing the two is presented below

Comparison of the Symptoms and Treatment of Pharyngolaryngeal Dysesthesias and Platinum Hypersensitivity Reactions

Clinical Symptoms	**Pharyngo-Laryngeal Dysesthesias**	**Platinum Hypersensitivity**
Dyspnea	present	present
Bronchospasm	absent	present
Laryngospasm	absent	present
Anxiety	present	present
O_2 saturation	normal	decreased
Difficulty swallowing	present (loss of sensation)	absent
Pruritus	absent	present
Urticaria/rash	absent	present
cold-induced symptoms	yes	no
BP	normal or increased	normal or decreased
Treatment	anxiolytics, observation in a controlled clinical setting until symptoms abate or at the site investigator's discretion	oxygen, steroids, epinephrine, bronchodilators; fluids and vasopressors, if appropriate

Cardiovascular toxicities

- **For grade 3 or 4 cardiac ischemia/infarction:** Discontinue all protocol therapy (mFOLFOX6 and pembrolizumab).
- **For grade 3 or 4 cerebrovascular ischemia:** Discontinue all protocol therapy (mFOLFOX6 and pembrolizumab).

Other non-hematologic toxicities for mFOLFOX6
For other grade 3 or 4 non-hematologic toxicities considered related to mFOLFOX6, delay mFOLFOX6 until toxicity resolves to ≤ grade 1, then resume mFOLFOX6 at one dose level reduction of oxaliplatin and 5-FU (if toxicity is hand-foot syndrome or stomatitis, dose reduction of only 5-FU bolus and continuous infusion of 5-FU is permitted). Continue pembrolizumab.

Dose modifications for obese subjects
There is no clearly documented adverse impact of treatment of obese subjects when dosing is performed according to actual body weight. Therefore, **all dosing is to be determined solely by actual weight without any modification** unless explicitly described in the protocol. This will eliminate the risk of calculation error and the possible introduction of variability in dose administration. **Failure to use actual body weight in the calculation of drug dosages will be considered a major protocol deviation.** Site investigators who are uncomfortable with calculating doses based on actual body weight should recognize that doing otherwise would be a protocol violation.

The actual weight on the day of registration or the first day of treatment may be used for cycle 1 unless the change in the weight results in a change in calculated dose ≥10%, in which case the weight on the day of treatment should be used. Over the course of treatment it is not required to change the doses of 5-FU, leucovorin or oxaliplatin due to changes in weight unless the calculated dose changes by ≥10%.

5.6 Concomitant Medications/Vaccinations (allowed & prohibited)

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The site investigator should discuss any questions regarding this with the sponsor-investigator by contacting the HCRN project manager. The final decision on any supportive therapy or vaccination rests with the site investigator and/or the subject's primary physician. However, the decision to continue the subject on trial therapy or vaccination schedule requires the mutual agreement of the site investigator, the sponsor-investigator, and the subject. Please contact the HCRN project manager in such cases.

5.6.1 Acceptable Concomitant Medications

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

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered 30 days after the last dose of trial treatment should be recorded if associated with SAEs and events of clinical interest (ECIs).

5.6.2 Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase of this trial. The Exclusion Criteria describes other medications that are prohibited in this trial.

- Anti-cancer systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than Pembrolizumab
- Radiation therapy
  - Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed after consultation with sponsor-investigator.
- Live vaccines within 30 days prior to study registration and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chicken pox, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines

and are allowed; however, intranasal influenza vaccines (e.g. Flu-Mist®) are live attenuated vaccines, and are not allowed. Glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the sponsor-investigator. Please contact the HCRN project manager.

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

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

5.7 Supportive Care and Rescue Medications

5.7.1 Supportive Care Guidelines

Subjects should receive appropriate supportive care measures as deemed necessary by the site investigator including but not limited to the items outlined below:

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.

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

Diarrhea/Colitis:
Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).

- All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
- For Grade 2 diarrhea/colitis that persists greater than 3 days, administer oral corticosteroids.
- For Grade 3 or 4 diarrhea/colitis that persists > 1 week, treat with intravenous steroids followed by high dose oral steroids.
- When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or ≥ Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)

- For T1DM or Grade 3-4 Hyperglycemia
  - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.
  - Evaluate subjects with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

Hypophysitis:

- For Grade 2 events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
- For Grade 3-4 events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

Hyperthyroidism or Hypothyroidism:

Thyroid disorders can occur at any time during treatment. Monitor subjects for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.

- Grade 2 hyperthyroidism events (and Grade 2-4 hypothyroidism):
  - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
  - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.
- Grade 3-4 hyperthyroidism
  - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

Hepatic:

- For Grade 2 events, monitor liver function tests more frequently until returned to baseline values (consider weekly).
  - Treat with IV or oral corticosteroids
- For Grade 3-4 events, treat with intravenous corticosteroids for 24 to 48 hours.
- When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

Renal Failure or Nephritis:

- For Grade 2 events, treat with corticosteroids.
- For Grade 3-4 events, treat with systemic corticosteroids.
- When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

Management of Infusion Reactions:
Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

Table 3 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab.

Table 3 Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the site investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the site investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the site investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

5.8 Diet/Activity/Other Considerations

5.8.1 Diet

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

5.8.2 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. Women of child-bearing potential may be enrolled if they are willing to use two methods of birth control or are considered highly unlikely to conceive. Highly unlikely to conceive is defined as:

- Surgically sterilized, or
- Postmenopausal (a woman who is ≥ 45 years of age and has not had menses for > one year will be considered postmenopausal), or
- Not heterosexually active for the duration of the study.

The two birth control methods can either be two barrier methods or a barrier method plus a hormonal method to prevent pregnancy. Subjects should start using birth control from the point of registration up to 120 days after the last dose of study drug.

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 drug 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 for 120 days after the last dose of study drug. 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.

5.8.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on study, the subject will immediately be discontinued from protocol therapy. If treatment with pembrolizumab, 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 HCRN immediately 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 site investigator will make every effort to follow the outcome of the pregnancy and report the condition of the fetus or newborn to HCRN. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to HCRN and followed as described in the Adverse Event section 11.

5.8.4 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.

6. TREATMENT DISCONTINUATION

6.1. Reasons for Discontinuing Protocol Therapy

A subject will be discontinued from the treatment under the following circumstances:

- If there is evidence of progressive disease (based on immune related response criteria) as outlined in the Schedule of Events. Progression per RECIST 1.1 is NOT a criteria for removal from study, unless the site investigator determines that removal from protocol is in the subject's best interest.
- If a situation other than progression of disease arises and the site investigator determines a change of therapy would be in the best interest of the subject.
- If the drug(s) exhibit(s) an unacceptable adverse event.
- If a subject becomes pregnant or is unwilling to use appropriate birth control techniques as outlined in the inclusion criteria (See Section 3).
- If there is a treatment interruption for greater than 4 weeks due to treatment related adverse event.
- Subjects can stop participating at any time. However, if they decide to stop participating in the study, subjects will continue to be followed for disease progression and survival.
- Twenty-four months of uninterrupted protocol therapy.

6.2. Reasons for Withdrawal from Study

- Subject withdraws consent for participation
- Termination of the study
- Lost to follow-up
- Death

7. STUDY SCHEDULE OF EVENTS

Footnotes on next page Cycle = 28 days

Examination	Screening		Cycles 1–2		Cycles 3–4		Cycle 5+		Disease progression	End of Treatment	Follow up
	-28 days	-14 days	Day 1 (±3)	Day 15 (±3)	Day 1 (±3)	Day 15 (±3)	Day 1 (±3)	Day 15 (±3)		30 days (± 7) after last dose	
REQUIRED ASSESSMENTS											
Informed Consent and Eligibility		X									
Demographics, Medical History, Ht		X									
Physical Examination		X	X	X	X		X			X	
Vital Signs (BP, wt) and ECOG PS[11]		X	X	X	X	X	X	X		X	
AEs, Con Meds, ECI's[11]		X	X	X	X	X	X	X		X	
LABORATORY ASSESSMENTS											
Comprehensive Metabolic Panel[1]		X	X[1]	X	X	X	X	X		X	
Calculated Creatinine Clearance		X								X	
CBC with Differential and platelet[2]		X	X[2]	X	X	X	X	X		X	
PT, PTT, INR		X									
CEA[9]			X		X		X				
CA19-9			X		X[10]						
TSH, T3 and T4[3]		X	X		X		X			X	
Pregnancy Test (Urine or Serum)[4]		X									
DISEASE ASSESSMENT											
CT or MRI of Chest, Abd and Pelvis[5]	X				X		X			X	
TREATMENT EXPOSURE											
mFOLFOX6 every 2 weeks			X	X	X	X	X	X			
Pembrolizumab every 3 weeks[11]			C1D1	C1D22	C2D15	C3D8	C4D1	C4D22	C5D15	C6D8; up to 24 months of treatment	
CORRELATIVE STUDIES											
MANDATORY serum collection[6]			X							X	
MANDATORY whole blood collection[6]			X	X	X					X	
Tissue sample for PD-LI and IHC[7]			X						X		
FOLLOW-UP											
For progression, start of additional cancer treatment, and survival[8]											X

Footnotes:

[1]Comprehensive metabolic profile to include will include: serum chemistries (creatinine, glucose, total protein, blood urea nitrogen [BUN], total carbon dioxide [CO_2 or bicarbonate], albumin, total bilirubin, alkaline phosphatase, and aspartate transaminase [AST] and alanine transaminase [ALT]) and electrolytes (total calcium, chloride, potassium, sodium). **NOTE:** For Cycle 1 Day 1 only, required tests do not need to be repeated if done within 7 days prior.

[2] Complete blood cell profile to include hemoglobin, hematocrit, red blood cell count, WBC [total and differential], absolute neutrophil count and platelet count. **NOTE:** For Cycle 1 Day 1 only, required tests do not need to be repeated if done within 7 days prior

[3] TSH, T3, T4 at screening then every 4 weeks during treatment with study medication through Cycle 5. After cycle 5 thyroid testing will be done every other cycle (C7, C9, etc).

[4]Women of child bearing potential ONLY

[5]Chest/Abdomen/Pelvic CT scans to be done every 8 weeks starting C1D1. Screening CT scan done within 28 days from C1D1 is adequate as a baseline imaging. For EOT visit, radiological assessment is needed if > 28 days from last assessment only if subject EOT due to toxicity.

[6] MANDATORY correlative serum samples are to be collected at pre-dose C1D1, pre-dose C2D1 and at End of Treatment. MANDATORY whole blood samples are to be collected at pre-dose C1D1, pre-dose C1D15, pre-dose C3D1 (after 2 cycles of treatment or at first response assessment) and at End of Treatment. Refer to the SPM for collection, processing, labeling and shipping instructions.

[7] MANDATORY: Have identified tissue from an archival tissue sample (preferably from a metastasis, but sample from primary tumor allowable) or newly obtained core or excisional biopsy of a tumor lesion. OPTIONAL biopsy for submission of unstained slides for IHC testing are requested at the time the subject progresses. OPTIONAL submission of tissue obtained from surgery for those subjects that have surgery during study treatment or follow up. See Section 9 for more details. See Study Procedures Manual for collection, labeling and shipping instructions.

[8]Subjects who discontinue treatment without evidence of tumor progression will be followed for progression with imaging studies performed every three months (±7 days), or until new treatment is initiated. Every effort should be made to perform the same imaging modality (e.g., CT or MRI) throughout the follow-up period. In addition, the site investigator or designees will make every possible attempt to contact the subject or family every three months (±7 days) for two years from completion of protocol therapy, every six months (±7 days) for years 3-5, and annually thereafter (±2 weeks) to obtain the survival information of the subject and start date of additional anticancer treatment.

[9]Subjects will have CEA done every 2months while on treatment.

[10]Subjects will have CA19-9 at baseline and at the time of first scan while on treatment.

[11] On Days in which Pembrolizumab ONLY is given, the following assessments are required: Blood pressure, weight [kg] and ECOG performance status. Adverse events and concomitant medications should also be recorded. This includes ECI's.

7.1 Screening

Within 28 days prior to registration for protocol therapy:

- Radiological assessment (CT or MRI of chest, abdomen, and pelvis) with tumor measurements
- Prior to the subject being registered to the study, an available archived tumor block (preferably from a metastasis, but sample from primary tumor is allowable) must be identified. If an archived tumor tissue sample is not available, subjects may consent to a newly obtained core or excisional biopsy of a tumor lesion prior to treatment C1D1 after all other eligibility criteria has been met. See SPM for collection, labeling and shipping instructions.

Within 14 days prior to registration for protocol therapy unless otherwise noted:

- Eligibility evaluation (review of inclusion and exclusion criteria)
- Medical history and Height
- Physical examination
- Record Adverse Events and concomitant medications. Including ECI's.
- Vitals Signs (including blood pressure), Weight [kg] and ECOG PS
- Laboratory Assessments:
  - Complete metabolic profile (CMP) will include: serum chemistries (creatinine, glucose, total protein, blood urea nitrogen [BUN], total carbon dioxide [CO2 or bicarbonate], albumin, total bilirubin, alkaline phosphatase, and aspartate transaminase [AST] and alanine transaminase [ALT]) and electrolytes (total calcium, chloride, potassium, sodium).
  - CBC (hemoglobin, hematocrit, red blood cell count, WBC [total and differential], absolute neutrophil count and platelet count)
  - Calculated Creatinine Clearance
  - Urine pregnancy test for female subjects of childbearing potential (If urine pregnancy results cannot be confirmed as negative, a serum β-human chorionic gonadotropin test will be required)
  - PT, PTT and INR
  - TSH, T3, T4

7.2. On Treatment

Cycles 1 and Cycle 2 Day 1 (± 3 days) unless otherwise noted:
On Day 1 of each treatment cycle, the following assessments/treatment administrations will occur: (**NOTE:** For Cycle 1 Day 1 only, required tests do not need to be repeated if done within 7 days prior)

- Physical examination
- Record Adverse Events and concomitant medications. Including ECI's.
- Vital signs, Weight [kg] and ECOG PS
- Laboratory Assessments:
  - CMP will include: serum chemistries (creatinine, glucose, total protein, BUN, total CO2, albumin, total bilirubin, alkaline phosphatase, and AST and ALT) and electrolytes (total calcium, chloride, potassium, sodium)

  - CBC (hemoglobin, hematocrit, red blood cell count, WBC [total and differential], absolute neutrophil count and platelet count)
  - CEA- prior to C1D1 ONLY
  - CA19-9- prior to C1D1 ONLY
  - TSH, T3, T4
  - MANDATORY serum samples are to be collected at pre-dose C1D1 and pre-dose C2D1. Refer to the SPM for collection, processing, labeling and shipping instructions.
  - MANDATORY whole blood samples are to be collected at pre-dose C1D1. Refer to the SPM for collection, processing, labeling and shipping instructions.
- Have provided tissue from an archival tissue sample (preferably from a metastasis, but sample from primary tumor allowable) or newly obtained core or excisional biopsy of a tumor lesion.
- Pembrolizumab IV is given every 3 weeks. First dose C1D1
- mFOLFOX6 IV is given every 2 weeks. First dose C1D1

Cycles 1 and Cycle 2 Day 15 (± 3 days) unless otherwise noted:

- Physical exam
- Record Adverse Events and concomitant medications. Including ECI's.
- Vital signs, Weight [kg] and ECOG PS
- Laboratory Assessments:
  - CMP will include: serum chemistries (creatinine, glucose, total protein, BUN, total CO2, albumin, total bilirubin, alkaline phosphatase, and AST and ALT) and electrolytes (total calcium, chloride, potassium, sodium)
  - CBC (hemoglobin, hematocrit, red blood cell count, WBC [total and differential], absolute neutrophil count and platelet count)
  - Mandatory whole blood samples are to be collected at pre-dose C1D15. Refer to the SPM for collection, processing, labeling and shipping instructions.
- Pembrolizumab IV C2D15
- mFOLFOX6 a IV

Cycles 3 and Cycle 4 Day 1 (± 3 days) unless otherwise noted:

On Day 1 of each treatment cycle, the following assessments/treatment administrations will occur:

- Physical examination
- Record Adverse Events and concomitant medications. Including ECI's.
- Vital signs, Weight [kg] and ECOG PS
- Laboratory Assessments
  - CMP will include: serum chemistries (creatinine, glucose, total protein, BUN, total CO2, albumin, total bilirubin, alkaline phosphatase, and AST and ALT) and electrolytes (total calcium, chloride, potassium, sodium)
  - CBC (hemoglobin, hematocrit, red blood cell count, WBC [total and differential], absolute neutrophil count and platelet count)
  - CEA- prior to C3D1
  - CA19-9- prior to C4D1 or with first set of scans while on treatment.
  - TSH, T3, T4

  - MANDATORY whole blood samples are to be collected pre-dose C3D1 (after 2 cycles of treatment or at first response assessment). Refer to the SPM for collection, processing, labeling and shipping instructions.
- CT or MRI of chest, abdomen/pelvis: pre-dose C3D1 (after 2 cycles of treatment or at first response assessment).
- CT or MRI of chest, abdomen/pelvis after 4 cycles (PRIOR to 5TH CYCLE)
- Pembrolizumab IV C4D1
- mFOLFOX6 IV

Cycle 3 and Cycle 4 Day 15 (± 3 days) unless otherwise noted:

- Record Adverse Events and concomitant medications. Including ECI's.
- Vital signs, Weight [kg] and ECOG PS
- Laboratory Assessments
  - CMP will include: serum chemistries (creatinine, glucose, total protein, BUN, total CO2, albumin, total bilirubin, alkaline phosphatase, and AST and ALT) and electrolytes (total calcium, chloride, potassium, sodium)
  - CBC (hemoglobin, hematocrit, red blood cell count, WBC [total and differential], absolute neutrophil count and platelet count)
- mFOLFOX6 IV

Cycle 5 and subsequent Cycles Day 1 (± 3 days) unless otherwise noted:

On Day 1 of each treatment cycle, the following assessments/treatment administrations will occur:

- Physical examination
- Record Adverse Events and concomitant medications. Including ECI's.
- Vital signs, Weight [kg] and ECOG PS
- Laboratory Assessments
  - CMP will include: serum chemistries (creatinine, glucose, total protein, BUN, total CO2, albumin, total bilirubin, alkaline phosphatase, and AST and ALT) and electrolytes (total calcium, chloride, potassium, sodium)
  - CBC (hemoglobin, hematocrit, red blood cell count, WBC [total and differential], absolute neutrophil count and platelet count)
  - CEA
- CT or MRI of chest, abdomen/pelvis after 4 cycles (PRIOR to 5TH CYCLE)
- mFOLFOX6 IV

Cycle 5 and subsequent Cycles Day 15 (± 3 days) unless otherwise noted:

- Vital signs, Weight [kg] and ECOG PS
- Record Adverse Events and concomitant medications. Including ECI's.
- Laboratory Assessments
  - CMP will include: serum chemistries (creatinine, glucose, total protein, BUN, total CO2, albumin, total bilirubin, alkaline phosphatase, and AST and ALT) and electrolytes (total calcium, chloride, potassium, sodium)
  - CBC (hemoglobin, hematocrit, red blood cell count, WBC [total and differential], absolute neutrophil count and platelet count)

- mFOLFOX6 IV

Pembrolizumab ONLY infusion days:

- Record Adverse Events and concomitant medications. Including ECI's.
- Vital signs[kg] and ECOG PS
- Pembrolizumab IV every 3 weeks

7.3. Restaging Evaluations

A restaging CT or MRI of the chest, abdomen, and pelvis will be performed after every two cycles of treatment (i.e., approximately every 8 weeks). For subjects on an increased dosing interval (every four weeks) due to pneumonitis (as per section 5.2.3), the frequency of restaging imaging studies should be discussed with the sponsor-investigator by contacting the HCRN project manager. Every effort should be made to perform the same imaging modality (e.g., CT or MRI) throughout the study.

7.4. Determination of Progression/Post-progression imaging

irRC will be adapted as follows to account for the unique tumor response seen in this class of therapeutics.

After the first documentation of progression per irRC, if the subject is clinically stable, confirmatory scans should be performed 4-6 weeks later. It is at the discretion of the site investigator to keep a clinically stable subject on study therapy or to stop study therapy until repeat imaging performed.

Clinical Stability is defined as:

1. Absence of symptoms and signs indicating clinically significant progression of disease (including worsening of laboratory values) indicating disease progression.
2. No decline in ECOG PS
3. Absence of rapid progression of disease or progressive tumor at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention.

Subjects who are deemed clinically unstable are not required to have repeat imaging for confirmation of progression.

If progression is confirmed, the subject will be discontinued from study therapy. If progression is not confirmed, the subject should resume/continue study therapy and have their next scan approximately eight weeks from the date of the scan that first showed progression.

When feasible, subjects should not be discontinued until progression is confirmed.

7.5. Progression/End of Treatment (EOT)

Treatment will be discontinued if there is evidence of disease progression per irRC (after confirmatory CT scan) or unacceptable toxicity or if the subject stops participating or withdraws from the study.

At least 30 days (+7) of last dose of study drug

- Physical examination
- Vital signs including Blood pressure, Weight [kg] and ECOG PS
- Laboratory assessments
    - CMP will include: serum chemistries (creatinine, glucose, total protein, BUN, total CO2, albumin, total bilirubin, alkaline phosphatase, and AST and ALT) and electrolytes (total calcium, chloride, potassium, sodium)
    - CBC (hemoglobin, hematocrit, red blood cell count, WBC [total and differential], absolute neutrophil count and platelet count)
    - Calculated Creatinine Clearance
    - MANDATORY serum samples are to be collected at end of treatment. Refer to the SPM for collection, processing, labeling and shipping instructions.
    - MANDATORY whole blood samples are to be collected at End of Treatment. Refer to the SPM for collection, processing, labeling and shipping instructions.
    - OPTIONAL biopsy for submission of unstained slides from Time of Progression. In addition, subjects that undergo surgery for tumor resection at any time during study treatment or follow up will be asked to provide tissue for research. Refer to the SPM for collection, labeling and shipping instructions.
- Record Adverse Events and concomitant medications. Including ECI's.
- Subjects will continue to be followed until resolution or stabilization of any treatment-related toxicities.
- Radiological assessments (only if > 28 days since last assessment) for subjects taken off study due to toxicity ONLY.

7.6. Follow-up

- Subjects who discontinue treatment without evidence of tumor progression will be followed for progression with imaging studies performed every three months (±7 days), or until new treatment is initiated. Every effort should be made to perform the same imaging modality (e.g., CT or MRI) throughout the follow-up period.
- The site investigator or designees will make every possible attempt to contact the subject or family every three months (±7 days) for two years from completion of protocol therapy, every six months (±7 days) for years 3-5, and annually thereafter (±2 weeks), to obtain the survival information of the subject and start date of additional anticancer treatment.

8. CRITERIA FOR DISEASE EVALUATION

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

8.1. Immune Related Response Criteria

This study will utilize the Immune Related Response Criteria (irRC) for secondary endpoints. These response criteria were developed to overcome the variable and unusual

patterns of response to immunotherapeutic agents, in particular, ipilimumab [1]. The development of the guidelines were prompted by observations, mostly in subjects with metastatic melanoma, of initial disease progression followed by later response, late responses, and mixed responses with an overall decrease in tumor burden.

8.2. Antitumor response based on total measurable tumor burden

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

Table 4: Comparison of WHO and irRC criteria

	WHO	irRC
New, measurable lesions	Always represent PD	Incorporated into tumor burden
New, non-measurable lesions	Always represent PD	Do not define progression (but preclude irCR)
Non-index lesions	Changes contribute to defining BOR of CR, PR, SD, and PD	Contribute to defining irCR (complete disappearance required)
CR	Disappearance of all lesions in two consecutive observations not less than 4 wk apart	Disappearance of all lesions in two consecutive observations not less than 4 wk apart
PR	≥50% decrease in SPD of all index lesions compared with baseline in two observations at least 4 wk apart, in absence of new lesions or unequivocal progression of non-index lesions	≥50% decrease in tumor burden compared with baseline in two observations at least 4 wk apart
SD	50% decrease in SPD compared with baseline cannot be established nor 25% increase compared with nadir, in absence of new lesions or unequivocal progression of non-index lesions	50% decrease in tumor burden compared with baseline cannot be established nor 25% increase compared with nadir
PD	At least 25% increase in SPD compared with nadir and/or unequivocal progression of non-index lesions and/or appearance of new lesions (at any single time point)	At least 25% increase in tumor burden compared with nadir (at any single time point) in two consecutive observations at least 4 wk apart

8.3. Time-point response assessment using irRC

Percentage changes in tumor burden per assessment time point describe the size and growth kinetics of both conventional and new, measurable lesions as they appear. At each tumor assessment, the response in index and new, measurable lesions is defined based on the change in tumor burden (after ruling out irPD). Decreases in tumor burden must be assessed relative to baseline measurements (i.e., the SPD of all index lesions at screening). The irRC were derived from WHO criteria and, therefore, the thresholds of response remain the same. However, the irRC response categories have been modified from those of WHO criteria as detailed in Tables 8 and 9.

8.4. Overall response using the irRC

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

Definition of Index Lesions Response Using irRC

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

- **irPartial Response (irPR):** Decrease, relative to baseline, of 50% or greater in the sum of the products of the 2 largest perpendicular diameters of all *index* and all new measurable lesions (ie, Percentage Change in Tumor Burden). Note: the appearance of new measurable lesions is factored into the overall tumor burden, but does not automatically qualify as progressive disease until the SPD increases by ≥25% when compared to SPD at nadir.

- **irStable Disease (irSD):** Does not meet criteria for irCR or irPR, in the absence of progressive disease.

- **irProgressive Disease (irPD):** At least 25% increase Percentage Change in Tumor Burden (i.e., taking sum of the products of all *index* lesions and any new lesions) when compared to SPD at nadir.

Definition of Non-Index Lesions Response Using irRC

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

- **irPartial Response (irPR) or irStable Disease (irSD):** *non-index* lesion(s) are not considered in the definition of PR, these terms do not apply.

- **irProgressive Disease (irPD):** Increases in number or size of *non-index* lesion(s) does not constitute progressive disease unless/until the Percentage Change in Tumor Burden increases by 25% (i.e., the SPD at nadir of the index lesions increases by the required amount).

Impact of New Lesions on irRC

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

Definition of Overall Response Using irRC

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

- **Immune-Related Complete Response (irCR):** Complete disappearance of *all* tumor lesions (index and non-index together with no new measurable/unmeasurable lesions) for at least 4 weeks from the date of documentation of complete response.

- **Immune-Related Partial Response (irPR):** The sum of the products of the two largest perpendicular diameters of all index lesions is measured and captured as the SPD baseline. At each subsequent tumor assessment, the sum of the products of the two largest perpendicular diameters of all index lesions and of new measurable lesions are added together to provide the Immune Response Sum of Product Diameters (irSPD). A decrease, relative to baseline of the irSPD compared to the previous SPD baseline, of 50% or greater is considered an immune Partial Response (irPR).

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

  - At least a 25% increase in the sum of the products of all index lesions and new measurable lesions (irSPD) over the baseline SPD calculated for the index lesions.

Immune-Related Best Overall Response Using irRC (irBOR)
irBOR is the best confirmed irRC overall response over the study as a whole, recorded between the date of first dose until the last tumor assessment before subsequent therapy (except for local palliative radiotherapy for painful bone lesions) for the individual subject in the study. For the assessment of irBOR, all available assessments per subject are considered.

irCR or irPR determinations included in the irBOR assessment must be confirmed by a second (confirmatory) evaluation meeting the criteria for response and performed no less than 4 weeks after the criteria for response are first met.

Table 5: Derivation of irRC overall responses

Measurable response	Nonmeasurable response		Overall response
Index and new, measurable lesions (tumor burden),*%	Non-index lesions	New, nonmeasurable lesions	Using irRC
↓100	Absent	Absent	irCR‡
↓100	Stable	Any	irPR‡
↓100	Unequivocal progression	Any	irPR‡
↓≥50	Absent/Stable	Any	irPR‡
↓≥50	Unequivocal progression	Any	irPR‡
↓<50 to <25↑	Absent/Stable	Any	irSD
↓<50 to <25↑	Unequivocal progression	Any	irSD
≥25	Any	Any	irPD‡

*Decreases assessed relative to baseline (scan prior to start of any protocol therapy), including measurable lesions only

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

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

8.5.1. Measurable disease

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

8.5.2. Measurable lesions

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

8.5.3 Non-measurable lesions

All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥ 10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

NOTE: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

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

8.5.4 Malignant lymph nodes

To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

8.5.5 Target lesions

All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions, recorded, and measured at baseline. Target lesions should be selected based on their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion that can be measured reproducibly should be selected.

A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

8.5.6 Non-target lesions

All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as non-target lesions and should be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

8.6. Response Criteria

8.6.1. Evaluation of target lesions

Complete Response (CR)	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
Partial Response (PR)	At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters
Progressive Disease (PD)	At least a 20% increase in the sum of the 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 progressions).

Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study

8.6.2. Evaluation of non-target lesions

Complete Response (CR)	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) Note: If tumor markers are initially above the upper normal limit, they must normalize for a subject to be considered in complete clinical response.
Non-CR/ Non-PD	Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits
Progressive Disease (PD)	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the site investigator should prevail in such circumstances, and the progression status should be confirmed later by the sponsor-investigator.

8.6.3. Evaluation of best overall response

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/ Non-PD	No	PR
	Not evaluated	No	PR
PR	Non-CR/ Non-PD/ not evaluated	No	PR
SD	Non-CR/ Non-PD/ not evaluated	No	SD
PD	Any	Yes or No	PD
Any	PD*	Yes or No	PD
Any	Any	Yes	PD

*In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Subjects with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having

"symptomatic deterioration." Every effort should be made to document the objective progression even after discontinuation of treatment.

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

8.7 Definitions for Response Evaluation – RECIST version 1.1

8.7.1 First Documentation of Response
The time between initiation of therapy and first documentation of PR or CR.

8.7.2 Confirmation of Response
To be assigned a status of complete or partial response, changes in tumor measurements must be confirmed by repeat assessments performed no less than four weeks after the criteria for response are first met.

8.7.3 Duration of Response
Duration of overall response—the period measured from the time that measurement criteria are met for complete or partial response (whichever status is recorded first) until the date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since treatment started).

8.7.4 Duration of Overall Complete Response
The period measured from the time that measurement criteria are met for complete response until the first date that recurrent disease is objectively documented.

8.7.5 Objective response rate
The objective response rate is the proportion of all subjects with confirmed PR or CR according to RECIST v1.1, from the start of treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the start of treatment).

8.7.6 Time to Progression:
A measurement from the date of registration until the criteria for disease progression is met as defined by RECIST 1.1. Subjects who have not progressed or have died due to any cause will be right-censored at the date of the last disease evaluation or date of death.

8.7.7 Progression Free Survival
A measurement from the date of registration until the criteria for disease progression is met as defined by RECIST 1.1 or death occurs. Subjects who have not progressed will be right-censored at the date of the last disease evaluation.

8.7.8 Overall Survival
Overall survival is defined by the date of registration to date of death from any cause.

9. BIOLOGICAL SPECIMEN PARAMETERS FOR CORRELATIVES

As part of the correlative research we will assess the relationship between potential immune biomarker and response to the combination of pembrolizumab plus chemotherapy.

Potential immune biomarkers include Toll-Like Receptor 4 (TLR4) pathway including the expression level of TLR4, MyD88 and NF-kB as well as PD-1/PD-L1 expression level in the tumor.

9.1 Unstained Slides for PD-L1 Testing

9.1.1 MANDATORY: An archival tissue sample (preferably from a metastasis, but sample from primary tumor allowable) or newly obtained core or excisional biopsy of a tumor lesion will be used for PD-L1 testing.

9.1.2 Archival tissue sample for submission must be identified during the screening process. If not, available, the subject will undergo a new biopsy

9.1.3 A fine needle aspirate, frozen sample, plastic embedded sample, cell block, clot, bone, bone marrow or cytologic specimen are not acceptable for PD-L1 analysis.

9.1.4 A tissue sample from a pre-treatment biopsy will not be submitted until the subject is registered to the trial.

9.1.5 Submission of unstained slides for PD-L1 expression will be analyzed at a central lab using a Merck-specified assay

9.1.6 See SPM for collection, labeling and shipping instructions.

9.2 Unstained Slides for IHC Testing

9.2.1 MANDATORY: An archival tissue sample (preferably from a metastasis, but sample from primary tumor allowable) or newly obtained core or excisional biopsy of a tumor lesion will be used for IHC testing.

9.2.2 Archival tissue sample for submission must be identified during the screening process. If not, available, the subject will undergo a new biopsy

9.2.3 A tissue sample from a pre-treatment biopsy will not be submitted until the subject is registered to the trial.

9.2.4 OPTIONAL AT PROGRESSION: Submission of unstained slides at time of progression for IHC testing. Potential immune biomarkers include Toll-Like Receptor 4 (TLR4) pathway including the expression level of TLR4, MyD88 and NF-Kb

9.2.5 See SPM for collection, labeling and shipping instructions.

9.3 MANDATORY submission of Whole Blood and Serum Sample

9.3.1 MANDATORY serum samples are to be collected at pre-dose C1D1, pre-dose C2D1 and at End of Treatment.

9.3.2 MANDATORY whole blood samples are to be collected at pre-dose C1D1, pre-dose C1D15, pre-dose C3D1 (or at first response assessment; after 2 cycles of treatment) and at End of Treatment.

9.3.3 See SPM for collection, labeling and shipping instructions.

9.4 OPTIONAL submission of tissue from Surgery

If patients undergo surgical resection during study treatment or follow up, formalin fixed paraffin embedded tissue will be retrieved, or 10 unstained slides will be retrieved for correlative studies.

10. DRUG INFORMATION

10.1 Drug Name

Pembrolizumab (MK-3475), SCH 900475 (Anti-PD-1)
Please see Investigator's Brochure for detailed information regarding Pembrolizumab

10.1.1 Chemical Name

Humanized X PD-1_mAb (H409A11) IgG4

10.1.2 Investigational Product

The site investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

Clinical Supplies will be provided by Merck as summarized below:

Product Name & Potency	Dosage Form
Pembrolizumab (MK-3475) 100 mg/ 4mL	Solution for Injection

10.1.3 Packaging and Labeling Information

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

10.1.4 Clinical Supplies Disclosure

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

10.1.5 Returns and Reconciliation

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

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

10.1.6 Storage and Handling Requirements

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label. Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site. Clinical supplies may not be used for any purpose other than that stated in the protocol.

10.1.7 Adverse Events

Please see Investigator's Brochure for complete details regarding adverse events related to Pembrolizumab

Pembrolizumab is generally well tolerated and demonstrates a favorable safety profile in comparison to chemotherapy. Pembrolizumab is an immunomodulatory agent, and based on this mechanism of action, immune mediated adverse events are of primary concern. Important identified risks for pembrolizumab are of an immune mediate nature, including: pneumonitis, colitis, thyroid disorders (hypothryoidism/hyperthyroidism), hepatitis, hypophysitis, Type I diabetes mellitis, uveitis, and nephritis. After a recent review of data, events newly characterized as identified risks also include pancreatitis, myositis, and severe skin reaction; these are included in the reference safety information in the current IB. The majority of immune-mediated adverse events were mild to moderate in severity, were manageable with appropriate care, and rarely required discontinuation of therapy. In addition to the previously noted identified risks, infusion-related reactions are a risk but are not considered immune mediated; these are also further described in the current IB.

10.2 mFOLFOX6

Qualified personnel who are familiar with procedures that minimize undue exposure to themselves and to the environment should undertake the preparation, handling, and safe disposal of chemotherapeutic agents in a self-contained, protective environment.

Discard unused portions of injectable chemotherapeutic agents that do not contain a bacteriostatic agent or are prepared with unpreserved diluents (i.e., Sterile Water for Injection USP or 0.9% Sodium Chloride for Injection USP) within eight hours of vial entry to minimize the risk of bacterial contamination.

The total administered dose of cytotoxic chemotherapy may be rounded up or down within a range of 5% of the actual calculated dose.

It is not necessary to change the doses of 5-FU, leucovorin or oxaliplatin due to changes in weight unless the calculated dose changes by ≥10% from baseline.

10.2.1 Oxaliplatin [Eloxatin]

Please refer to the package insert for complete product information.

Availability

Oxaliplatin is commercially available as an aqueous solution in vials containing 50 mg and 100 mg at a concentration of 5 mg/mL. The vials do not contain any preservative and they are intended for single use.

Storage and Stability

Intact vials should be stored at room temperature. Solutions diluted in D5W are **stable for 6 hours at room temperature or 24 hours under refrigeration.**

Preparation

The calculated dose of oxaliplatin should be diluted for infusion with 250 mL to 500 mL D5W. Oxaliplatin should not be diluted with a sodium chloride solution. Needles, syringes, catheters or IV administration sets containing aluminum should not be used with oxaliplatin. As with other platinum compounds, contact with aluminum may result in a black precipitate.

Administration

Oxaliplatin will be administered by intravenous infusion over 120 minutes prior to or concurrent with leucovorin. Infusion time may be prolonged (up to 6 hours) in subjects experiencing pharyngolaryngeal dysesthesia.

Oxaliplatin is unstable in the presence of chloride or alkaline solutions. **Do NOT** mix or administer oxaliplatin with saline or other chloride-containing solutions. **Do NOT** administer other drugs or solutions in the same infusion line. Flush IV lines/catheters with Dextrose 5% in Water both before and after oxaliplatin administration.

Toxicity

The most commonly observed oxaliplatin toxicities include neurotoxicity, GI toxicity, and myelosuppression. Three neurotoxicity syndromes have been seen: acute sensory neuropathy develops within hours to 2 days after oxaliplatin administration. Symptoms include paresthesias, dysesthesias, and hypoesthesia of the hands, feet, and perioral region. Jaw spasm, abnormal tongue sensation, dysarthria, eye pain and a sensation of chest pressure have also been noted. Acute sensory neuropathy symptoms may be exacerbated by exposure to cold temperature or cold objects. Symptoms are reversible, usually resolving within 14 days and commonly recurring with further dosing. This syndrome has been observed in about 56% of subjects receiving oxaliplatin with 5-FU and leucovorin.

Acute pharyngolaryngeal dysesthesia is reported to occur in 1-2% of subjects. This syndrome is characterized by a subjective sensation of difficulty breathing or swallowing without laryngospasm or bronchospasm or objective evidence of hypoxia. Avoidance of cold drinks, food and air is suggested in order to minimize pharyngolaryngeal dysesthesia. Antianxiety agents (e.g., lorazepam) may be used to treat pharyngolaryngeal dysesthesias once oxygen saturation has been documented to be normal.

Peripheral neuropathy persisting > 14 days is characterized by paresthesias, dysesthesias, and hypoesthesia. Abnormalities in proprioception may also be seen. Symptoms of persistent neuropathy may improve upon discontinuation of oxaliplatin.

Gastrointestinal toxicities include nausea, vomiting (oxaliplatin is considered to be moderately emetogenic) and diarrhea.

Neutropenia is reported in 73% of subjects receiving oxaliplatin with 5-FU and leucovorin (44% grade 3 or 4). Grade 3 or 4 thrombocytopenia is reported to occur in 4% of subjects receiving the combination.

Allergic reactions, similar to those seen with other platinum compounds, have also been observed in subjects treated with oxaliplatin. Reactions range from rash to anaphylaxis.

Rarely, oxaliplatin has been associated with pulmonary fibrosis, which may be fatal. Oxaliplatin should be discontinued in the presence of unexplained pulmonary symptoms (e.g. nonproductive cough, dysphagia) or pulmonary infiltrates until interstitial lung disease or pulmonary fibrosis have been ruled out.

Recent reports of oxaliplatin extravasation suggest that tissue necrosis may result and that oxaliplatin should be considered a vesicant. No standard treatment exists for oxaliplatin extravasation although heat and sodium thiosulfate have both been suggested. Veno-occlusive disease (VOD) of the liver is a rare complication associated with oxaliplatin and 5-FU. Clinical manifestations of VOD include hepatomegaly, ascites, and jaundice. Histologically, VOD is characterized by diffuse damage in the centrilobular zone of the liver. Sequelae of VOD include hepatomegaly, splenomegaly, portal hypertension, and esophageal varices. A recent analysis of resected liver metastases in 153 subjects indicated histological findings consistent with VOD in 6/27 subjects who received 5-FU alone, 4/17 subjects who received 5-FU and irinotecan, 20/27 subjects who received 5-FU and oxaliplatin, and 14/16 who received 5-FU, oxaliplatin and irinotecan. The remaining 66 subjects had not received chemotherapy prior to resection. There were no such findings in these subjects.

10.2.2 5-Fluorouracil (5-FU; fluorouracil)

Please refer to the package insert for complete product information.

Availability

5-FU is commercially available as a 50 mg/mL solution for injection in 10 mL, 20 mL, 50 mL and 100 mL vials.

Preparation

Inspect for precipitate; if found, agitate or gently heat in water bath. Bolus injections are prepared using undiluted drug.

46-48 hour infusion of 5-FU should be prepared for administration via ambulatory infusion pump according to the individual institution's standards. These solutions may be prepared in D5W or 0.9% NaCl. 5-FU should not be mixed in the same solution with most parenteral antiemetics.

Storage and Stability

Intact vials should be stored at room temperature and protected from light. Slight yellow discolor does not usually indicate decomposition. Stability in ambulatory pumps varies according to the pump, manufacturer of drug, concentration and diluent. Please refer to appropriate reference sources for additional information.

Administration

In this study, 5-FU is administered as a 400 mg/m^2 IV bolus followed by 2400 mg/m^2 by IV infusion over 46 to 48 hours. The bolus is administered after leucovorin, and the 46-48 hour infusion follows immediately after the bolus.

Toxicity

Nausea, diarrhea, vomiting (mild); stomatitis: 5-8 days after treatment initiation; myelosuppression: granulocytopenia (9-14 days); thrombocytopenia (7-14 days); Alopecia; loss of nails; hyperpigmentation; photosensitivity; maculopapular rash; palmar–plantar erythrodysesthesias: (42-82% receiving continuous infusion); CNS effects: cerebral ataxia (rare); cardiotoxicity: MI, angina; asymptomatic S–T changes 68%; ocular effects: excessive lacrimation and less commonly, tear duct stenosis.

Drug Interactions

Leucovorin enhances the cytotoxicity of 5-FU by forming a more stable tertiary complex with thymidylate synthase. Concomitant administration of 5-FU with warfarin has been reported to result in increased INR/prolonged prothrombin time. Subjects receiving both drugs should be followed with weekly INRs.

10.2.3 Leucovorin Calcium (Folinic Acid)

Please refer to the package insert for complete product information.

In the case of a leucovorin shortage, levo-leucovorin may be administered (dose to be at site investigator's discretion). Other dosing alternatives may also be used during leucovorin shortage such as leucovorin 20mg/m^2.

Availability
Leucovorin calcium is commercially available in: 50 mg, 100 mg, 200 mg, 350 mg and 500 mg vials for reconstitution, and as a solution for injection in 50 mL vials at a concentration of 10 mg/mL.

Storage and Stability
Intact vials should be stored at room temperature and protected from light. Solutions reconstituted with BWI are stable for at least 7 days at room temperature. Solutions diluted for infusion are stable for 24 hours at room temperature and 4 days under refrigeration.

Preparation
Leucovorin may be reconstituted with Bacteriostatic Water for Injection (BWI), Sterile Water For Injection, or bacteriostatic NaCl or NaCl. Solutions should be further diluted in D5W, 0.9% NaCl or Ringers solution for infusion over two hours.

Administration
Leucovorin will be administered as a 400 mg/m^2 IV infusion over 2 hours after oxaliplatin administration and immediately before 5-FU. Leucovorin may also be administered concurrently with oxaliplatin as a separate IV infusion.

Toxicity
The only adverse reactions associated with leucovorin are allergic reactions. These are extremely uncommon.

11. ADVERSE EVENTS

11.1 Definitions of Adverse Events

11.1.1 Adverse Event
Adverse event (AE) means any untoward medical occurrence in a study subject administered an investigational drug that does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with participation in an investigational study, whether or not considered related to the investigational product. An AE is any sign, symptom, or diagnosis that appears or changes in intensity during the course of the study. An AE may be an intercurrent illness or injury that impairs the well-being of the subject.

Abnormal laboratory values or diagnostic test results constitute AEs only if they induce clinical signs or symptoms or require treatment or further diagnostic tests.

11.1.2 Serious Adverse Event

A serious adverse event is any untoward medical occurrence resulting in one or more of the following:

- Results in death: **NOTE:** deaths due unequivocally to progression are not SAEs.
- Is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed in the definition above). Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions not resulting in hospitalization; or the development of drug dependency or drug abuse.

11.2 Adverse Event Reporting

11.2.1 Site Requirements for Recording and Reporting Adverse Events

Adverse events (AEs) will be recorded from the time of consent and for at least 30 days after last dose of study drug, regardless of whether or not the event(s) are considered related to the study drug. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) v4 will be utilized for AE reporting. A copy of the CTCAE v4 can be downloaded from the CTEP website at http://ctep.cancer.gov.

This study must be conducted in compliance with FDA regulations, local safety reporting requirements and reporting requirements of the sponsor-investigator.

11.2.2 Site Requirements for Reporting Serious Adverse Events

Site investigators and other site personnel must report any SAE to HCRN **within one business day** of discovery of the event. This includes events both related and unrelated to the study drug.

Site investigators will report a follow up to a SAE, 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 the last dose of study drug, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to Merck product.

The definition of "related" being that there is a reasonable possibility the drug caused the adverse experience.

Unrelated	The Adverse Event is ***clearly not related*** to the study drug
Unlikely	The Adverse Event is ***doubtfully related*** to the study drug
Possible	The Adverse Event ***may be related*** to the study drug
Probable	The Adverse Event is ***likely related*** to the study drug
Definite	The Adverse Event is ***clearly related*** to the study drug

Additionally, SAEs considered by a site investigator to be related to Merck product that is brought to the attention of the site investigator at any time outside of the time period specified in the previous paragraph also must be reported immediately to HCRN. HCRN will report these events to Merck.

The completed SAE/ECI Submission Form must be faxed to HCRN **within one business day** of discovery of the event. The site investigator is responsible for informing the IRB and/or the Regulatory Authority of the SAE as per local requirements. All AEs and SAEs will be recorded in the subject's medical record and on the appropriate study specific eCRF form within the EDC system. In addition, all SAEs will be reported on the SAE/ECI Submission Form and submitted to HCRN per guidelines outlined in this section.

The original copy of the SAE/ECI Submission Form and the fax confirmation sheet (or equivalent) must be kept within the Trial Master File at the study site.

Follow-up information will be faxed to HCRN, using a new SAE/ECI Submission Form stating that this is a follow-up to the previously reported SAE and giving the date of the original report. Each re-occurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, whether the blind was broken or not (if applicable), and whether the subject continued or withdrew from study participation.

11.2.3 Definition of an Overdose for This Protocol and Reporting of Overdose to HCRN

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, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

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

If a dose of 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 one business day** to HCRN. HCRN will report such events to Merck Global Safety **within one business day** of notification of the event. (Attn: Worldwide Product Safety; FAX 215 993-1220)

11.2.4 Reporting of Pregnancy and Lactation to HCRN

Although pregnancy and lactation are not considered adverse events, it is the responsibility of site 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 after the last dose of study drug. 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 one business day** to HCRN. HCRN will report such events to Merck Global Safety **within one business day** of receiving notification of the event. (Attn: Worldwide Product Safety; FAX 215-993-1220)

11.2.5 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be recorded as such on SAE Submission Form and reported **within one business day** to HCRN. HCRN will report these events **within one business day** of receipt to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220).

ECIs that occur in any subject from the date of first dose through 90 days following the last dose of study drug, or the initiation of a new anticancer therapy, whichever is earlier, whether or not related to the Merck's product, must be reported as outlined above.
Events of clinical interest for this trial include:

- An overdose of Merck product, as defined above, that is not associated with clinical symptoms or abnormal laboratory results.
- 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.

11.2.6 Hoosier Cancer Research Network Requirements for Reporting SAEs to Merck

HCRN will submit all SAEs received from sites to Merck & Co., Inc. **within one business day** of receipt of the SAE Submission Form and to regulatory authorities (FDA) per federal guidelines.

HCRN will submit SAE reports and any other relevant safety information to the Merck Global Safety facsimile number: +1-215-993-1220. Follow-up information will be provided as reasonably requested.

11.2.7 Sponsor-Investigator Responsibilities

HCRN will send a SAE summary to the sponsor-investigator **within 1 business day** of receipt of SAE Submission Form from a site. The sponsor-investigator will promptly review the SAE summary and assess for expectedness and relatedness.

11.2.8 Reporting to the Food and Drug Administration (FDA)

The FDA deemed this protocol IND exempt after review of the submission packet 24FEB2015.

As such, HCRN will assist the sponsor-investigator with compliance with requirements for informed consent under 21 CFR 50.20 and initial/continuing Institutional Review Board review under 21 CFR Part 56. In addition to complying with the applicable provisions of sections 402(i) and 402(j) of the Public Health Service Act (PHS Act) (42 U.S.C. §§ 282(i) and (j)), which was amended by Title VIII of the Food and Drug Administration Amendments Act of 2007 (FDAAA) (Public Law No. 110-85, 121 Stat. 904).

12. STATISTICAL METHODS

12.1 General Considerations

This is a multi-institutional, single arm, open-label, phase II study, including a safety run-in cohort, of the combination of pembrolizumab plus mFOFLOX6 in subjects with untreated locally advanced or metastatic colorectal cancer. After a safety run-in cohort of 6 subjects at the tolerated dose level, 24 additional subjects will be enrolled. These 30 subjects will be analyzed as a single cohort for subsequent efficacy and safety analyses. PFS and OS will be estimated by Kaplan-Meier method. Median PFS (mPFS) and OS (mOS) will be estimated with 80% confidence intervals (CIs). The response rates will be estimated with their Agresti-Coull 95% CIs. Duration of response will be summarized by descriptive statistics. Frequencies and proportions will be used to describe toxicities. Common Terminology Criteria for Adverse Events (CTCAE) v4 will be used for assessment of acute and late toxicities during the follow-up period. Logistic regressions will be used for the exploratory objectives studying potential predictors of responses.

12.2 Study Design

This is a multi-institutional, single arm, open-label, phase II study with a safety run-in cohort. No randomization or blinding will be involved.

12.3 Definition of Primary Endpoint

Median progression free survival (mPFS), measured from the time of registration to the time of signs of progression per RECIST 1.1 or subject death depending on whichever occurs first.

12.4 Definitions of Secondary Endpoints

- Objective response rate (ORR), calculated with combining the number of subjects who achieve complete response and partial response per irRC criteria
- Disease control rate (DCR), defined as the sum of subjects with complete response, partial response and stable disease.
- Delayed response, evaluated in subjects who achieve stable disease on first tumor assessment but they exhibit an objective response on subsequent tumor evaluations per irRC.
- Overall Survival (OS), calculated from the time of registration till the time of subject death
- Toxicity of therapy will be assessed per CTCAE V4.0
- Response rate per Immune Related Response Criteria (irRC)

12.5 Definitions of Exploratory Endpoints

- Assess the association between TLR4, MyD88, NF-kB expression by IHC and response rate.
- Assess Microsatellite status by IHC
- Assess the association between HMGB1 blood level and response rate.
- Assess the association between PD-L1 expression and response rate.
- Blood samples will be stored at the HCRN for future correlatives.
- Tissue samples will be stored at the HCRN for future correlatives

12.6 Analysis Plan for Primary Objectives/Aims

PFS will be estimated by Kaplan-Meier method. In particular, median PFS will be estimated with its two-sided 90% CI.

12.7 Analysis Plan for Secondary Objectives/Aims

ORR, DCR, delayed response, irRC and toxicities will be summarized by frequency and percentages with Agresti-Coull 95% confidence intervals. OS will be estimated using Kaplan-Meier method.

12.8 Analysis Plan for Exploratory Objectives

Logistic regressions will be used to evaluate the associations between risk factors and responses. For the stored blood samples, appropriate statistical methods will be determined according to potential future correlatives

12.9 Interim analysis

No interim analysis will be performed.

12.10 Subgroup Analysis

Subgroup analyses will be performed based on PD-1/PD-L1 expressions. Both efficacy endpoints and toxicity endpoints will be evaluated in the subgroup analyses.

12.11 Criteria for Stopping Study

The trial will be terminated if there is any definite pembrolizumab-related death despite maximum supportive therapy.

12.12 Analysis Datasets

Population	Definition
Enrolled	This will comprise all subjects who meet the eligibility criteria and are registered onto the study. This data set will be used for subject characteristics summaries.
Evaluable	This will comprise all patients who receive at least one dose of trial drug and either undergo at least one post-baseline assessment or die before any evaluation. This data set will be used for response rates and OS evaluations.
Intention-to-treat (ITT)	This will comprise all subjects who meet the eligibility criteria and are registered onto the study irrespective of their compliance to the planned course of treatment. This data set will be used for the primary objective analysis.
Safety	This will comprise all subjects who receive at least one dose of trial drug. This data set will be used for toxicities evaluations.

12.13 Sample Size/Accrual/Study Duration/Replacement Rules

The primary objective of this early Phase II study is to estimate mPFS. Historically, first line FOLFOX achieves a median PFS (mPFS) as 9.0 months [37]. If there are moderate evidences that adding Pembrolizumab will increase mPFS from 9- to around 14-months, a full scale randomized Phase II study will be considered to formally test whether the improvement is meaningful and statistically significant.

The sample size N=30 is determined based on budget/recruitment considerations and precision of estimation. We expect that these 30 subjects will be uniformly accrued within 12-months with an additional 24-months follow up. Kaplan-Meier method will be used to estimate PFS. mPFS and its two-sided 80% CI will be calculated. Consequently, the half-width of the CI from the lower bound to the point estimate of mPFS will be around 4.5-months, which is smaller than the difference between 14- and 9-months. We consider this precision level will allow a decision to be made whether to pursue further studies.

12.14 Subject Characteristics and Significant Protocol Violations

Subject's characteristics will be summarized by descriptive statistics. Significant protocol violations will be documented and reported.

12.15 Concomitant Medications

Concomitant medications will be documented and managed accordingly.

12.16 Disposition
The number of enrolled subjects will be summarized in flow chart with frequency of completion and discontinuation. The subjects discontinued from study medication and their corresponding information will be listed.

13. TRIAL MANAGEMENT
This study will be conducted in accordance with the IU Simon Cancer Center's (IUSCC) Data and Safety Monitoring Plan. This study will have a Data Safety Monitoring Board as described below and a DSMB Charter.

In addition HCRN data and safety monitoring activities include:

- Conduct review of clinical trial for progress and safety
- Review of all adverse events requiring expedited reporting as defined in the protocol
- Provide sponsor-investigator with trial progress and safety information monthly.
- Notification of participating sites of adverse events requiring expedited reporting and subsequent committee recommendations for study modifications

13.1 Data and Safety Monitoring Board
This study will have a Data and Safety Monitoring Board (DSMB) that will review and monitor study progress, toxicity, safety and other data from this trial. The DSMB is separate from the IU Simon Cancer Center (IUSCC) Data and Safety Monitoring Committee (DSMC). The board is chaired by an independent medical oncologist external to this trial. Questions about subject safety or protocol performance will be addressed with the sponsor-investigator, statistician and study team members. Should any major concerns arise; the DSMB will offer recommendations regarding whether or not to suspend the trial.

The DSMB will meet after the first 6 subjects are treated with at least one dose of study drug and observed for a minimum of 4 weeks after first dose of study drug. The DSMB will then meet twice a year thereafter to review accrual, toxicity, response and reporting information. Information to be provided to the DSMB may include: subject accrual, treatment regimen information, adverse events and serious adverse events reported by category, summary of any deaths on study, audit and/or monitoring results. During DSMB's initial review of the 1st six subjects, accrual to the trial will be halted until completion of review.

The DSMB will provide a recommendation to the team after all information is reviewed. This information will also be provided to the site investigator sites for submission to their respective IRB according to the local IRB's policies and procedures.

13.1.1 Early Study Closure
At any time during the conduct of the trial, if it is the opinion of the sponsor-investigator that the risks (or benefits) to the subjects warrant early closure of the study, this recommendation should be made in writing to the DSMB Alternatively, the DSMB may initiate suspension or early closure of the study based on its regular review of the sponsor-investigator reports.

13.2 On-site Monitoring

Monitoring visits to the trial sites will be made periodically during the trial, to ensure all aspects of the protocol are followed. Source documents will be reviewed for verification of agreement with data as submitted via the data collection system. The site investigator/institution guarantee access to source documents by HCRN or its designee and appropriate regulatory agencies.

The trial site may also be subject to quality assurance audit by Merck & Co., Inc. or its designee as well as inspection by appropriate regulatory agencies.

It is important for the site investigator and their relevant personnel to be available during the monitoring visits and possible audits and for sufficient time to be devoted to the process.

13.3 Compliance with Trial Registration and Results Posting Requirements

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

14. DATA HANDLING AND RECORD KEEPING

14.1 Case Report Forms and Submission

This study will utilize electronic case report form (eCRF) in the Hoosier Cancer Research Network (HCRN) electronic data capture (EDC) system. Access to the data through EDC system is restricted by user accounts and assigned roles. Once logged into the EDC system with a user ID and password, the EDC system defines roles for each user, which limits access to appropriate data. User information and passwords can be obtained by contacting HCRN at (317) 921-2050.

Generally, clinical data will be electronically captured in the EDC system and correlative results will be captured in the EDC system or other secure database. If procedures on the study calendar are performed for standard of care, at minimum, that data will be captured in the source document. Select standard of care data will also be captured in the EDC system, according to study-specific objectives. Please see the Data Management Plan for further details.

The completed dataset is the sole property of the sponsor-investigator's institution. It should not be made available in any form to third parties, except for authorized representatives of appropriate Health/Regulatory Authorities, without written permission from the sponsor-investigator and HCRN.

14.2 Record Retention

To enable evaluations and/or audits from Health Authorities/HCRN, the site investigator agrees to keep records, including the identity of all subjects (sufficient information to link records; e.g., hospital records), all original signed informed consent forms, copies of all source documents, and detailed records of drug disposition. All source documents are to remain in the subject's clinic file. To comply with international regulations, the records should be retained by the site investigator in compliance with regulations.

During data entry, range and missing data checks will be performed remotely. The checks to be performed will be documented in the Data Management Plan for the study. A summary report (QC Report) of these checks together with any queries resulting from manual review of the eCRFs will be generated for each site and transmitted to the site and the site monitor. Corrections will be made by the study site personnel. This will be done on an ongoing basis.

14.3 Confidentiality

There is a slight risk of loss of confidentiality of subject information. All records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available.

Information collected will be maintained on secure, password protected electronic systems. Paper files that contain personal information will be kept in locked and secure locations only accessible to the study team. Samples that are collected will be identified by a subject study number assigned at the time of registration to the trial. Any material issued to collaborating researchers will be anonymized and only identified by the subject study number.

Subjects will be informed in writing that some organizations including the sponsor-investigator and his/her research associates, HCRN, Merck & Co., Inc., IRB, or government agencies, like the FDA, may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

If the results of the study are published, the subject's identity will remain confidential.

14.4 Changes to the Protocol

Study procedures will not be changed without the mutual agreement of the sponsor-investigator, HCRN, and Merck & Co., Inc.

If it is necessary for the study protocol to be amended, the amendment or a new version of the study protocol (amended protocol) will be generated by the HCRN and must be approved by each IRB, Merck & Co., Inc., and if applicable, also the local regulatory authority. Local requirements must be followed.

If a protocol amendment requires a change to the Informed Consent Form, then the IRB must be notified. Approval of the revised Informed Consent Form by the IRB is required before the revised form is used.

The site investigator is responsible for the distribution of these documents to his or her IRB, and to the staff at his or her center. The distribution of these documents to the regulatory authority will be handled according to local practice.

Merck & Co., Inc.'s willingness to supply study drug is predicated upon the review of the protocol. HCRN agrees to provide written notice to Merck & Co., Inc. of any modifications to the protocol or informed consent.

15. ETHICS

15.1 Ethics Review

The final study protocol, including the final version of the Informed Consent Form, must be approved in writing by an IRB. The site investigator must submit written approval to the HCRN office before he or she can enroll any subject into the study. The site investigator is responsible for informing the IRB of any amendment to the protocol in accordance with local requirements. In addition, the IRB must approve all advertising used to recruit subjects for the study. The protocol must be re-approved by the IRB annually, as local regulations require.

Progress reports and notifications of serious unexpected adverse drug reactions will be provided to the IRB according to local regulations and guidelines.

The site investigator is also responsible for providing the IRB with reports of any serious adverse drug reactions from any other study conducted with the investigational product. Merck & Co., Inc. will provide this information to the sponsor-investigator. These reports will be forwarded to participating sites for submission to their Institutional Review Boards per their guidelines.

15.2 Ethical Conduct of the Study

The study will be performed in accordance with ethical principles originating from the Declaration of Helsinki, which are consistent with ICH Good Clinical Practice guidelines, and applicable regulatory requirements.

15.3 Informed Consent Process

The site investigator will ensure the subject is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study. Subjects must also be notified they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided.

The subject's signed and dated informed consent must be obtained before conducting any procedure specifically for the study. The site investigator must store the original, signed Written Informed Consent Form. A copy of the signed Informed Consent Form must be given to the subject.

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17. APPENDIX

17.1. ECOG Performance Status

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

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

17.2. Common Terminology Criteria for Adverse Events v4 (CTCAE)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) v4 will be utilized for adverse event reporting. (http://ctep.cancer.gov/reporting/ctc.html)

17.3. Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 Criteria for Evaluating Response in Solid Tumors

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

* As published in the European Journal of Cancer:

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

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