Protocol Title

A phase II multi-center study evaluating combination immunotherapy for advanced cholangiocarcinoma with pembrolizumab and Sylatron (peginterferon alfa-2b)

Sponsor Investigator
Aiwu Ruth He, MD, PhD
Lombardi Comprehensive Cancer Center
Georgetown University
3800 Reservoir Road, NW
Washington DC, 20007
Tel: 202-444-8642
Fax:202-444-9429
arh29@georgetown.edu

Co-Investigators

Iyer Renuka MD
Roswell Park Cancer Institute
646 Main St, Buffalo, NY 14202
Telephone (716) 845-2300
Renuka.Iyer@RoswellPark.org

Davendra P. S. Sohal, MD, MPH
Cleveland Clinic Clinical Genomics Program
Taussig Cancer Institute
9500 Euclid Avenue, R35
Cleveland, OH - 44195
Telephone: (216) 444-8258
sohald@ccf.org

Statistician
Hongkun Wang, PhD
4000 Reservoir Road, NW
Washington DC, 20057
202-687-4146
Hongkun.Wang@georgetown.edu

Trial Management Provided by
Hoosier Cancer Research Network, Inc.
500 N. Meridian, Suite 100
Indianapolis, IN 46204

Trial Supported by: Merck Sharp and Dohme Corp. ("Merck")
Investigational New Drug (IND) # 129815

Initial Protocol Version Date: 12/9/15
Protocol Amendment Version Date:
30DEC2016
10FEB2017
11APR2017
07JUN2017
19SEP2017
15DEC2017

PROTOCOL SIGNATURE PAGE

A phase II multi-center study evaluating combination immunotherapy for advanced cholangiocarcinoma with pembrolizumab and Sylatron (peginterferon alfa-2b)

VERSION DATE: 15DEC2017

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, 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 institutional review board(s).

_____ _____

Signature of Site Investigator Date

_____

Site Investigator Name (printed)

_____

Site Investigator Title

_____

Name of Facility

_____

Location of Facility (City and State)

PLEASE COMPLETE AND EMAIL A COPY TO HCRN

SYNOPSIS

TITLE	A phase II multi-center study evaluating combination immunotherapy for advanced cholangiocarcinoma with pembrolizumab and Sylatron (peginterferon alfa-2b)
SHORT TITLE	Phase II study evaluating pembrolizumab and sylatron for the treatment of advanced cholangiocarcinoma
PHASE	II
OBJECTIVES	Primary Objectives: • Assess objective response rate of patients receiving pembrolizumab and Sylatron combination therapy based RECIST 1.1. Secondary Objectives: • Assess progression free survival of patients receiving pembrolizumab and Sylatron. • Assess overall survival of patients receiving pembrolizumab and Sylatron • Assess the objective response rate of patients receiving pembrolizumab and Sylatron combination therapy based on irRECIST. • Assess the safety and tolerability of combined pembrolizumab and Sylatron therapy Exploratory Objectives: • Determine the effect of Sylatron on anti- programmed death ligand-1 (PD-L1) level, immune score as determined by the density of CD3 and CD8 in the tumor interior and invasive margin(s) of the tumor, and the clonality of the T-cell repertoire. • Determine the effect of Sylatron on anti-cancer immunity by measuring proliferation of CD8+ T cells, Granzyme B+ cells and proliferation of tumor cells, and correlating these changes with clinical response as determined by RECIST 1.1.
STUDY DESIGN	This is an open-label, single-arm, multicenter Phase II safety and efficacy study of combination therapy with pembrolizumab and Sylatron (Peginterferon alpha-2b) in patients with advanced cholangiocarcinoma who have progressed on or cannot tolerate frontline chemotherapy.

ELIGIBILITY CRITERIA

Inclusion Criteria:

1. Be willing and able to provide written informed consent/assent for the trial.
2. Be ≥ 18 years of age on day of signing informed consent.
3. Patients must have received 1 line of prior systemic therapy for metastatic or resectable disease (i.e. patients may have received adjuvant gemcitabine and then later gemcitabine/cisplatin for recurrent metastatic disease)
4. Histological confirmation of cholangiocarcinoma.
5. Have measurable disease based on RECIST 1.1.
6. Be willing to provide tissue from a newly obtained core or excisional biopsy of a tumor lesion. Newly obtained is defined as a specimen obtained up to 6 weeks (42 days) prior to initiation of treatment on Day 1. Subjects for whom newly obtained samples cannot be provided (e.g. inaccessible or subject safety concern) may submit an archived specimen only upon agreement from the sponsor-investigator.
7. Have a performance status of 0 or 1 on the ECOG Performance Scale.
8. Demonstrate adequate organ function as defined in
9. Table 1. All screening labs will be performed within 28 days of registration.

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥ 1,500 /μL
Platelets	≥ 100,000 / μL
Hemoglobin	≥ 9 g/dL or ≥ 5.6 mmol/L without transfusion or EPO dependency (within 7 days of assessment)
Renal	
Serum creatinine **OR** Measured or calculated CrCl (GFR can also be used in place of creatinine or CrCl)	≤ 1.5 X upper limit of normal (ULN) **OR** ≥ 60 mL/min for subject with creatinine levels > 1.5 x institutional ULN
Hepatic	
Serum total bilirubin	≤ 2.0 X ULN
AST (SGOT) and ALT (SGPT)	≤ 3.0 X ULN **OR** ≤ 5 X ULN for subjects with liver metastases
Albumin	≥ 2.5 mg/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤ 1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants ≤ 1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants

	10. Female subject of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required. 11. Female subjects of childbearing potential should be willing to use adequate 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. Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year. 12. Male subjects should agree to use an adequate method of contraception starting with the first dose of study therapy through 120 days after the last dose of study therapy. **Exclusion Criteria** 1. Has symptomatic congestive heart failure, unstable angina pectoris, symptomatic or poorly controlled cardiac arrhythmia. Symptomatic heart failure (NYHA Class II-IV) 2. A history of anaphylaxis to peginterferon alfa-2b or interferon alfa-2b 3. Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of treatment. 4. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment. 5. Has a known history of active TB (Bacillus Tuberculosis) 6. Hypersensitivity to pembrolizumab or any of its excipients. 7. Has had a prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.

	8. Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent. • **NOTE:** Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study. • **NOTE:** If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy. 9. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer. 10. 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 the first dose of trial treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to trial treatment. This exception does not include carcinomatous meningitis, which is excluded regardless of clinical stability. 11. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids, or immunosuppressive drugs). Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment. 12. Has known history of, or any evidence of active, non-infectious pneumonitis. 13. Has an active infection requiring systemic therapy. 14. 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

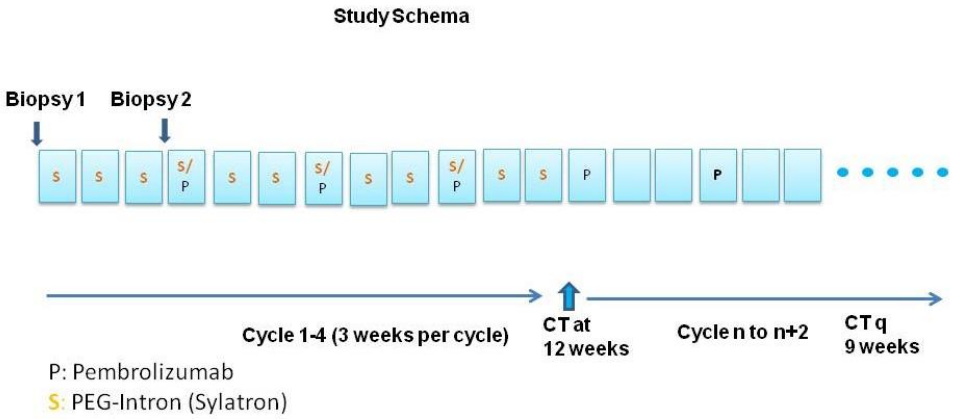
	duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the site investigator. 15. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial. 16. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment. 17. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent. 18. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies). 19. Has known active Hepatitis B without HBV treatment (HBV infection with ongoing HBV treatment is allowed); has persistent chronic Hepatitis C infection (successfully treated HCV infection is allowed). 20. Has received a live vaccine within 30 days of planned start of study therapy. **Note:** Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however, intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed. 21. Has history of bipolar disorder or major depression as identified by the enrolling physician. See Study Procedures Manual (SPM) for additional information. 22. Has history of not tolerating interferon treatment. 23. Has known serious neuropsychiatric condition.

STATISTICAL CONSIDERATIONS	The primary objective of the study is to test that addition of Sylatron will improved the response rate of pembrolizumab from 17% to 35% in patients with advanced cholangiocarcinoma. The sample size is based on the number of patients that are anticipated to complete the primary objective. We propose that the addition of Sylatron to a pembrolizumab regimen will improve the response rate of pembrolizumab from 17% to 35%. Simon's two-stage optimum design will be used to test the null hypothesis of P <= 0.17 versus the alternative hypothesis of P >= 0.350. At the first stage, 16 patients will be treated with the combination of Sylatron and pembrolizumab. The trial will be terminated if 3 or fewer respond. If there are at least 4 responders, the trial will go on to the second stage and 28 patients will be enrolled to a total of 44 patients. If the total number responding among the 44 patients is less than or equal to 11, the null hypothesis will not be rejected and the combination of Sylatron plus pembrolizumab will not be investigated further. Using this statistical method, there is a 0.046 (4.6%) probability of erroneously concluding that this drug combination is effective when it actually is not (targeted value of 0.05). If the drug is actually effective, there is a 0.191 (1.9%) probability of concluding that it is not (targeted value of 0.2). The power of the design is 80% with a one-sided significance level of 0.05. We anticipate that 2 patients will be recruited per month and total time for study recruitment will be 24 months, with each patient followed for a minimum of 6 months, the anticipated time to complete the study will be 24 – 30 months. Allowing for a 4% drop out rate, a total of 46 patients will be enrolled for this trial.
TOTAL NUMBER OF SUBJECTS	N = 44
ESTIMATED ENROLLMENT PERIOD	Estimated 24 months
ESTIMATED STUDY DURATION	Estimated 30 months

TABLE OF CONTENTS

SCHEMA

1. Sylatron at 200mcg will be administered subcutaneously weekly starting Cycle 1 Day 1.
2. Pembrolizumab will be administered intravenously at 200mg every 3 weeks starting Cycle 2 Day 1 (Week 4).



1. BACKGROUND AND RATIONALE

1.1 Cholangiocarcinoma

Cholangiocarcinoma has been steadily increasing in incidence worldwide over the last decade [9]. It is difficult to establish the diagnosis of early stage cholangiocarcinoma as symptoms are not prominent until the disease is far advanced. Patients with unresectable tumors have a dismal prognosis, with a median survival time of nine months [10.11]. Surgical resection is considered to be the most viable approach to attempting a "cure" for cholangiocarcinoma but even after surgery, five-year survival rates range from only 25% to 48% due to incomplete resection and subsequent high recurrence rate (~50%) [12–14]. New approaches are urgently needed to prevent tumor recurrence after surgical resection in an attempt to further improve patient survival.

1.1.1 Chemotherapy and Cholangiocarcinoma

A recent randomized and controlled trial (ABC-02 study) proved that gemcitabine combined with cisplatin (GC-therapy) performed better than gemcitabine monotherapy for cholangiocarcinoma [15]. However, the benefit of GC-therapy is modest with progression free survival (PFS) of 8.1 months of and overall survival (OS) of 11.7 months in patients treated in ABC-02 study. Furthermore, there is no effective second line therapy for patients with advanced cholangiocarcinoma. In a Phase II study of second line gemcitabine monotherapy for cholangiocarcinoma patients refractory to 5-fluorouracil treatment, the median time to progression (TTP) was 1.6 months (95% CI: 1.3-1.9 months), and the median overall survival (OS) time was 4.1 months (95% CI: 2.7-5.5 months) [16]. A retrospective analysis of metastatic gallbladder cancer and cholangiocarcinoma that were treated second-line FOLFOX4 chemotherapy, after disease progression from gemcitabine based first line chemotherapy, showed stable disease in 22% of patients, and the median disease free survival (DFS) and OS were 96 (13 weeks) and 138 (19 weeks) days respectively.

1.2 Pembrolizumab

Pembrolizumab (MK-3475) is a potent humanized IgG4 mAb with high specificity of binding to the PD-1 receptor, thus inhibiting its interaction with PD-L1 and PD-L2. Based on preclinical in vitro data, pembrolizumab has high affinity and potent receptor blocking activity for PD-1. Pembrolizumab has an acceptable preclinical safety profile and is being advanced for clinical development as an IV immunotherapy for advanced malignancies.

1.2.1 Pharmaceutical and Therapeutic Background

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

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related

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

Pembrolizumab (MK-3475) is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Keytruda™ (pembrolizumab) has recently been approved in the United Stated for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if the melanoma is BRAF V600 mutation positive, a BRAF inhibitor.

1.2.2 Preclinical and Clinical Trial Data

1.2.2.1 Non-Clinical Toxicology Summary of Results

In the 1-month and 6-month toxicology study in cynomologus monkeys, pembrolizumab, intravenously administered once a week and once every other week respectively up to a dose of 200 mg/kg, resulted in no adverse treatment-related effects. In tissue cross-reactivity studies of pembrolizumab in human and monkey tissues, the expected on-target staining of mononuclear leukocytes membranes was demonstrated in both species. Off-target cross-reactivity staining was also noted in both species but was limited to the cytoplasm of various cell types/tissues and the

stroma (extracellular connective tissue matrix), and was considered related to experimental methodological artifacts, i.e. tissue processing for IHC, which are well recognized limitations of tissue cross-reactivity studies and, thus not considered toxicologically relevant. No reproductive or developmental toxicity studies are planned with pembrolizumab. Therefore, inclusion of women of childbearing potential in clinical trials should be avoided in accordance with the study protocol and applicable regulatory guidance (e.g., ICH M3(R2): Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals).

1.2.2.2 Clinical Summary of Results

As of 18-Oct-2013, 1,000 patients have been treated with pembrolizumab at several dose schedules, including 10 mg/kg every 2 weeks. Pembrolizumab has been generally well tolerated, as expected based on preclinical findings and other anti-PD-1 monoclonal antibodies. As of 18-Oct-2013 no serious infusion reactions have been reported in PN001, however, as the potential exists in anti-PD-1 monoclonal antibodies, investigators should be vigilant to this possibility. Less than 1% of patients assayed thus far have had confirmed positive ADA samples and among these, no clear impact on exposure has been observed. There is no contraindication to further clinical investigation with pembrolizumab. Pharmacokinetics were as expected, based on pembrolizumab being an IgG mAb, and preclinical data support dosing once every 2 or 3 weeks. Pembrolizumab monotherapy induces an ORR of between 25% and 27% in patients with ipilimumab-exposed melanoma by central independent RECIST and oncology review/investigator assessed irRC, respectively. Pembrolizumab monotherapy induces an ORR of 39% and 43% in patients with ipilimumab-naive melanoma by central independent RECIST and oncology review/investigator assessed irRC, respectively. These responses are remarkably durable. The preliminary 1-year survival rate for patients who receive pembrolizumab, many of whom have had multiple prior therapies, including ipilimumab, is 81%. Pembrolizumab monotherapy induces an ORR of 21% and 24% in patients with previously-treated NSCLC by central independent RECIST and investigator assessed irRC, respectively, with these responses also remarkably durable. Preliminary data suggest that higher levels of PD-L1 expression in tumors of NSCLC are associated with increased activity (ORR 67% by investigator assessed irRC and 57% by central independent RECIST); additional data are required to define the optimal PD-L1 assay cut-off point. The most commonly experienced treatment emergent AEs are reportedly fatigue (43.8%), nausea (26.7%), cough (25.3%), pruritus (24.6%), diarrhea (22.3%) and rash (21.5%). Immune-related adverse events were reported in 21.4% of melanoma patients; most of these events (15.8%) were considered drug-related by the investigator. The most commonly reported, immune-related adverse events across the dose-schedules are rash (3.2%), pruritus (2.9%), vitiligo (2.9%), hypothyroidism (2.7%), arthralgia (2.2%), diarrhea (2.2%), and pneumonitis (1.9%). Review of the overall benefit:risk ratio of pembrolizumab favors enrollment of eligible patients into clinical trials of pembrolizumab. The preliminary data suggest that a dose of pembrolizumab at 2 mg/kg Q3W is appropriate for patients with melanoma.

1.2.2.3 Dosage and Administration

Pembrolizumab is provided as a white to off white lyophilized powder (50 mg/vial) or as a liquid solution (100 mg/vial) in Type I glass vials intended for single use only. Pembrolizumab Powder for Solution for Infusion, 50 mg/vial, is reconstituted with sterile water for injection prior to use. Pembrolizumab is formulated with L-histidine as buffering agent, polysorbate 80 as surfactant,

sucrose as stabilizer/tonicity modifier, and hydrochloric acid (HCl) and/or sodium hydroxide (NaOH) for pH adjustment (if necessary). The drug product is stored as a stable lyophilized powder or liquid solution under refrigerated conditions (2°C - 8°C). The product after reconstitution with sterile water for injection and the liquid drug product is a clear to opalescent solution which may contain extraneous and proteinaceous particulates. The reconstituted product and liquid product is intended for IV administration. The reconstituted drug product solution and liquid drug product can be further diluted with normal saline in IV containers made of polyvinyl chloride (PVC) or non-PVC material. Reconstituted vials should be immediately used to prepare the infusion solution in the IV bag and the infusion solution should be immediately administered. If not used immediately, vials and/or IV bags may be stored at 2-8 °C for up to a cumulative time of 20 hours. If refrigerated, the vials and/or IV bags should be allowed to equilibrate to room temperature prior to subsequent use. Pembrolizumab solutions may be stored at room temperature for a cumulative time of up to 4 hours. This includes room temperature storage of reconstituted drug product solution and liquid drug product in vials, room temperature storage of infusion solution in the IV bag and the duration of infusion.

1.3 Sylatron

SYLATRON™ is an alpha interferon indicated for the adjuvant treatment of melanoma with microscopic or gross nodal involvement within 84 days of definitive surgical resection, including complete lymphadenectomy. Sylatron (Peginterferon alfa-2b) is a pleiotropic cytokine; the mechanism by which it exerts its effects in patients with melanoma is unknown. The FDA-recommended dose for this indication is 6 mcg/kg/week subcutaneously for 8 doses, followed by 3 mcg/kg/week subcutaneously for up to 5 years.

1.3.1 Pharmaceutical and Therapeutic Background

The pharmacokinetics of Sylatron was studied in 32 patients receiving adjuvant therapy for melanoma with the agent according to the recommended dose and schedule (6 mcg/kg/week for 8 doses, followed by 3 mcg/kg/week thereafter). At a dose of 6 mcg/kg/week once weekly, the geometric mean Cmax was 4.4 ng/mL (CV 51%) and the geometric mean AUCtau was 430 ng•hr/mL (CV 35%) at week 8. The mean terminal half-life was approximately 51 hours (CV 18%). The mean accumulation from week 1 to week 8 was 1.7. After administration of 3 mcg/kg/week once weekly, the mean geometric Cmax was 2.5 ng/mL (CV 33%) and the geometric mean AUCtau was 228 ng•hr/mL (CV 24%) at week 4. The mean terminal half-life was approximately 43 hours (CV 19%). Renal clearance accounts for approximately 30% of total Sylatron clearance. The effect of renal impairment on the pharmacokinetics of Sylatron was studied in 24 subjects with normal or impaired renal function after a single 4.5 mcg/kg dose. Compared to subjects with normal renal function (CLcr > 80 mL/min/1.73 m^2), the geometric mean AUClast to Sylatron increased by 1.4-fold in subjects with moderate renal impairment (CLcr 30 to 50 mL/min/1.73m^2) and 2.1-fold in subjects with severe renal impairment (CLcr < 30 mL/min/1.73m^2) or ESRD requiring dialysis. No clinically meaningful amounts of Sylatron were removed during hemodialysis following a single 1 mcg/kg dose in subjects with renal impairment.

1.3.2 Clinical Summary of Results:

The data described below reflect exposure to Sylatron in 608 patients with surgically resected, AJCC Stage III melanoma. Sylatron was studied in an open label, multicenter, randomized, observation controlled trial. The median age of the population was 50 years with 10% of patients 65 years or older, and 42% being female. Fourteen percent of patients completed the 5 year treatment schedule.

Patients randomized to Sylatron were to receive total doses of 48 mcg/kg (6 mcg/kg subcutaneous once weekly for 8 doses), and 780 mcg/kg (3 mcg/kg subcutaneous once weekly until disease recurrence or for up to 5 years), as tolerated. The median total dose received was 42 mcg/kg (range: 6 to 78 mcg/kg) for the first 8 doses, and 136 mcg/kg (range: 1 to 774 mcg/kg) for doses 9 to 260.

Because clinical trials are not all conducted under identical conditions, adverse reaction rates observed in the clinical trials of one drug cannot be directly compared to adverse reaction rates in the clinical trials of another drug, and may not reflect the rates observed in clinical practice. In the current combination phase II study, since Sylatron is administrated to potentiate the effect of pembrolizumab, the total doses of 2400 mcg will be administered over 12 week period of time, which is less than 20% of the median total dose of Sylatron administered in a patient who weighed 70kg and was enrolled in the Sylatron phase III melanoma registration study. Therefore, patients enrolled in the current combination phase II study are not expected to have the level of side effects and toxicities as patients experienced in the phase III melanoma study as described below.

Serious adverse events were reported in 199 (33%) patients who received Sylatron and 94 (15%) patients in the observation group. The most common adverse reactions experienced by Sylatron-treated patients were fatigue (94%), increased ALT (77%), increased AST (77%), pyrexia (75%), headache (70%), anorexia (69%), myalgia (68%), nausea (64%), chills (63%), and injection site reaction (62%). The most common serious adverse reactions were fatigue (7%), increased ALT (3%), increased AST (3%), and pyrexia (3%) in the Sylatron-treated group vs. <1% in the observation group for these reactions.

Thirty three percent of patients receiving Sylatron discontinued treatment due to adverse reactions. The most common adverse reactions present at the time of treatment discontinuation were fatigue (27%), depression (17%), anorexia (15%), increased ALT (14%), increased AST (14%), myalgia (13%), nausea (13%), headache (13%), and pyrexia (11%).

1.4 Rationale for the Study

1.4.1 Overall Rationale

If we are to further improve the survival of patients with cholangiocarcinoma, new approaches are urgently needed to prevent tumor recurrence after surgical resection of cholangiocarcinomas. Pembrolizumab is a monoclonal antibody that binds to the programmed death-1 (PD-1) receptor and blocks its interaction with ligands PD-L1 and PD-L2, thereby releasing PD-1 pathway-mediated inhibition of the immune response, including a strong anti-tumor immune response. Prognostic impact of tumor-infiltrating immune cells on biliary tract cancer was studied in a

retrospective study of 375 patients with cholangiocarcinoma. [19] High levels of tumor infiltrating lymphocytes in cholangiocarcinoma were associated with good clinical outcome. Therapies that target the PD-1 receptor have shown yet unmatched rates of lasting clinical responses in patients with a number of cancer types. [1-5]. The response rate of pembrolizumab in patients with advanced cholangiocarcinoma was 17% in a phase I study (internal communication with research team in Merck, unpublished data). Interferon alpha 2b has been shown to increase tumor immune infiltrates [20,21]. We propose to combine Sylatron with pembrolizumab to improve the response rate of pembrolizumab. We hypothesize that Sylatron will improved the response rate of pembrolizumab from 17% to 35% by increasing the immune cell infiltrate into the tumor.

1.4.2 Rationale – Effect of Pembrolizumab on Immune Function

Studies reveal a direct causal relationship between cancer and immune dysfunction, whereby tumor cells and their microenvironment are able to evade immune attack by exploiting various immunoregulatory mechanisms in a process termed cancer immunoediting (22). Regulatory pathways that limit the immune response to cancer are becoming increasingly well characterized and provide new strategies in cancer therapy. Monoclonal antibodies targeting PD-1 that boost the immune system are being developed for the treatment of a number of cancers. On September 4, 2014, the U.S. Food and Drug Administration granted accelerated approval to pembrolizumab (KeytrudaTM, Merck Sharp & Dohme Corp.) for the treatment of patients with unresectable or metastatic melanoma (23). Pembrolizumab is a monoclonal antibody that binds to the PD-1 receptor and blocks its interaction with ligands PD-L1 and PD-L2, thereby releasing PD-1 pathway-mediated inhibition of the immune response, including anti-tumor immune response.

Evasion of the immune surveillance and metastatic spread can be achieved through a number of complex mechanisms. Targeting multiple sites should overcome tumor immune evasion. IFNs activate immune cells, such as natural killer cells and macrophages, and they increase host defenses by up-regulating tumor antigen presentation by virtue of increasing the expression of major histocompatibility complex (MHC) antigens. Blockade of tumor immune checkpoints by pembrolizumab augments anti-tumor immunity. Activation of IFN signaling in combination with the blockade of immune-inhibitory receptors should increase the exposure of cholangiocarcinoma to cellular immune surveillance, and enhance anti-tumor activity.

The B7-H1/PD-1 pathway has recently been found to contribute to immune evasion of cancer cells from host immune system. Expression of B7-H1 and PD-1 was found to be up-regulated in cholangiocarcinoma tissues compared with adjacent tissues. Tumor-related B7-H1 expression was significantly correlated with both tumor differentiation and pTNM stage and was inversely correlated with CD8+ tumor infiltrative lymphocytes (TILs) but not CD4+ TILs. TILs in primary carcinoma showed a high level of apoptosis suggesting that promotion of anti-tumor immunity may be a novel therapeutic strategy in the treatment of cholangiocarcinoma [24]. Therapies that boost host anti-tumor activity have shown promising activity against cholangiocarcinoma. A phase II study evaluating the efficacy of intravenous 5-FU and subcutaneous IFN-α for cholangiocarcinoma produced a 34% response rate, the median time to disease progression was 9.5 months, and the median survival time was 12 months [25]. The response rate of pembrolizumab in patients with advanced cholangiocarcinoma was 17% in a phase I study (internal communication with research team in Merck, unpublished data).

IFN-α has been used to treat certain types of leukemias and lymphomas, skin melanomas, and Kaposi sarcoma. IFNs activate immune cells, such as natural killer cells and macrophages, and thus increase host defenses by up-regulating antigen presentation by virtue of increasing the expression of major histocompatibility complex (MHC) antigens. In addition, IFN-□ also induces the immune-inhibitory interaction between PD-L1 and PD-1 receptors [26]. Interconnections between IFNs and immune-inhibitory receptors serve as targets for combination immunotherapies.

1.4.3 Rationale – Pembrolizumab Dose Selection/Regimen/Modification

An open-label Phase I trial (Protocol 001) was conducted to evaluate the safety and clinical activity of single agent pembrolizumab. The dose escalation portion of 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 MTD has been identified to date. Recent data from other clinical studies within the pembrolizumab program has shown that a lower dose of pembrolizumab and a less frequent schedule may be sufficient for target engagement and clinical activity.

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

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of pembrolizumab were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. Pembrolizumab has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg Q3W body weight based regimen are anticipated to remain well within the established exposure margins of 0.5 – 5.0 for pembrolizumab in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W vs. the proposed dose regimen of 2 mg/kg Q3W (i.e. 5-fold higher dose and exposure). The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

The rationale for further exploration of 2 mg/kg and comparable doses of pembrolizumab in solid tumors is based on: 1) similar efficacy and safety of pembrolizumab when dosed at either 2 mg/kg or 10 mg/kg Q3W in melanoma patients, 2) the flat exposure-response relationships of pembrolizumab for both efficacy and safety in the dose ranges of 2 mg/kg Q3W to 10 mg/kg Q3W, 3) the lack of effect of tumor burden or indication on distribution behavior of

pembrolizumab (as assessed by the population PK model) and 4) the assumption that the dynamics of pembrolizumab target engagement will not vary meaningfully with tumor type.

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

A fixed dose regimen will simplify the dosing regimen to be more convenient for physicians and to reduce potential for dosing errors. A fixed dosing scheme will also reduce complexity of the trial at treatment facilities and reduce wastage.

1.4.3.1 The safety of combining pembrolizumab and Sylatron

The safety of combining pembrolizumab and Sylatron has been evaluated in a phase I study. This safety and dose-seeking study of combination pembrolizumab and Sylatron for patients (≥ 18) with recurrent inoperable AJCC stage III and metastatic stage IV melanoma. Pembrolizumab is given 2 mg/kg q3 weeks for 2 years while Sylatron is given SC at 3 dose levels (1, 2 and 3 mcg/kg) to identify the recommended phase 2 dose (RP2D) prior to dose expansion up to 32 patients. Results: Twelve patients have been enrolled in this trial (4 patients at each dose level). No dose limiting toxicity has been observed to date. Most common side adverse events were grade 1 liver toxicities (n = 4), grade 1 neutropenia (n = 4), grade 1 skin rash (n = 3) and grade 1 anemia (n = 2). The only grade 3/4 toxicities were lymphopenia (n = 2) and hyponatremia (n = 1). Six out of 12 patients were evaluated for clinical responses at week 12 and we observed 1CR, 4SD and 1PD with mixed clinical responses (NCT02112032)[27]. In this current phase II study, the dosage of pembrolizumab and Sylatron are selected based on the data from the phase I study. Pembrolizumab will be administered at 200mg q 3 weeks, which is close to the dosage of pembrolizumab at 3mg/kg for a patient of 70 kg; Sylatron will be administered at 200mcg weekly, which is close to the dose of Sylatron at 3mcg/kg for a patient of 70 kg.

1.4.4 Rationale - Efficacy Endpoints

As stated in the Overall Rationale, prognostic impact of tumor-infiltrating immune cells on biliary tract cancer was studied in a retrospective study including a cohort of 375 patients with cholangiocarcinoma.[19] High levels of tumor infiltrating lymphocytes in cholangiocarcinoma were associated good clinical outcome. Therapies that target the PD-1 receptor have shown unprecedented rates of durable clinical responses in patients with various cancer types. [1-5] Pre-existing CD8+ T cells distinctly located at the invasive tumor margin are associated with expression of the PD-1/PD-L1 immune inhibitory axis and seems to predict response to therapy.[8] In previous retrospective study, 48.4% of cases showed high levels of CD8+ T lymphocytes and 36.3% of cases had high levels of CD4+ T lymphocyte infiltrates [28]. An increase in tumor immune infiltrate may be a strategy to improve the responsiveness of cancer to anti-PD-1 treatment. The response rate of pembrolizumab in patients with advanced cholangiocarcinoma was 17% in a phase I study (internal communication with research team in Merck, unpublished data). Interferon alpha 2b has shown to increase the tumor immune

infiltrates.[20,21] We propose to combine Sylatron and pembrolizumab to improve the response rate of pembrolizumab. Interferon alpha 2b in combination with 5-Flurouracil produced a response rate of 34% in patients with cholangiocarcinoma in a phase II study. However, 5-Flurouracil as single agent induced a response rate of 32% in patients with advanced cholangiocarcinoma in another phase II study. Interferon alpha 2b is likely to have minimal activity against cholangiocarcinoma as a single agent. We hypothesize that Sylatron will improved the response rate of pembrolizumab from 17% to 35% by increasing the immune cell infiltrate into the tumor.

After first-line treatment with gemcitabine-based regimen, there is no standard second-line chemotherapy for patients with metastatic gallbladder cancer and cholangiocarcinoma. A retrospective analysis of metastatic gallbladder cancer and cholangiocarcinoma that were treated with second-line FOLFOX4 chemotherapy after disease progression on gemcitabine-based first line chemotherapy, showed stable disease in 22% of patients, and the median disease free survival (DFS) and OS were 96 days (13 weeks) and 138 days (19 weeks), respectively [18]. Immune checkpoint inhibitors have shown durable disease control in multiple types of cancer, e.g., melanoma, renal cell carcinoma, non small cell lung cancer, etc. We propose that a PFS of 24 weeks and OS of 40 weeks in patients who receive the combination of pembrolizumab and Sylatron after cancer progression or intolerance of frontline treatment will be considered clinical meaningful.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Objectives

2.1.1 Primary Objective

Assess objective response rate of patients receiving pembrolizumab and Sylatron combination therapy based RECIST 1.1.

Hypothesis:
The addition of Sylatron to a pembrolizumab regimen will improve the response rate of pembrolizumab from 17% to 35% in patients with advanced cholangiocarcinoma based on RECIST1.1.

2.1.2 Secondary Objectives

- Assess progression free survival of patients receiving pembrolizumab and Sylatron.
- Assess overall survival of patients receiving pembrolizumab and Sylatron
- Assess the objective response rate of patients receiving pembrolizumab and Sylatron combination therapy based irRECIST.
- Assess the safety and tolerability of combined pembrolizumab and Sylatron therapy.

Hypothesis:
We propose that a PFS of 24 weeks and an OS of 40 weeks is clinically meaningful in patients who receive the combination of pembrolizumab and Sylatron following failure on frontline therapy (due to cancer progression or intolerance of treatment).

2.1.3 Correlative/Exploratory Objectives

- Determine the effect of Sylatron on anti- programmed death ligand-1 (PD-L1) level, immune score as determined by the density of CD3 and CD8 in the tumor interior and invasive margin(s) of the tumor, and the clonality of the T-cell repertoire.
- Determine the effect of Sylatron on anti-cancer immunity by measuring proliferation of CD8+ T cells, Granzyme B+ cells and proliferation of tumor cells, and correlating these changes with clinical response as determined by RECIST 1.1.

Hypothesis:
Therapies that target the programmed death-1 (PD-1) receptor have shown unprecedented rates of durable clinical responses in a sub-group of patients with various cancer types [1-5]. Biomarkers in patient tumor that will predict response to anti-PD-1 therapy are being actively investigated. One mechanism by which cancer tissues limit the host immune response is via upregulation of PD-1 ligand (PD-L1) and its ligation to PD-1 on antigen-specific CD8+ T cells [6, 7]. Recent research has reported that tumor regression after therapeutic PD-1 blockade requires pre-existing CD8+ T cells that are negatively regulated by PD-1/PD-L1-mediated adaptive immune resistance [8]. We propose that Sylatron will increase the response of pembrolizumab by increasing the tumor infiltrating CD3 and CD8 cells, and the level of PD-L1 and PD-1 expression in immune cells and cancer cells. In this study, we will first test the effect of Sylatron on tumor infiltrating lymphocytes, the level of PD-1 and PD-L1 expression, and clonality of the T-cell repertoire by comparing the tumor biopsies from baseline and three weeks of Sylatron treatment. Then we will examine whether the change of tumor infiltrating lymphocytes, the level of PD-1 and PD-L1 expression from Sylatron treatment correlates with clinical response in patient treated with pembrolizumab and Sylatron combination therapy.

2.2 Endpoints

2.2.1 Primary Endpoint

- The objective response rate is the proportion of all subjects with confirmed PR or CR according to RECIST 1.1, from the start of treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the start of treatment).

2.2.2 Secondary Endpoints

- PFS defined as 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.
- OS is defined as the time of registration until death as a result of any cause.
- Objective response rate is defined as the sum of complete response (CR) + confirmed partial response (PR) and will be determined as per irRECIST.
- Grade 3 and 4 toxicities as defined by the NCI Common Terminology Criteria for Adverse Events (NCI CTCAE) v4.

2.2.3 Exploratory Endpoints

- The density of CD3 and CD8 in the tumor interior and invasive margin of the tumor, and the clonality of the T-cell repertoire.
- Proliferation of CD8+ T cells, Granzyme B+ cells and proliferation of tumor cells.

3. ELIGIBILITY CRITERIA

This trial is a multi-institutional study, enrolling patients with cholangiocarcinoma, manifesting as either intrahepatic, extrahepatic or gallbladder cancer, that is unresectable, metastatic, or has either failed to respond to or demonstrated progression despite prior therapy. Patients must be, in the opinion of the site investigator, an appropriate candidate for experimental therapy. Patients should be evaluated for the need to undergo biliary drainage by stent placement prior to study participation. Patients should have adequate biliary drainage with no unresolved biliary obstruction.

The target recruitment for this study is 44 patients. As the trial is opening at several centers, recruitment is anticipated to take 21 months, and the anticipated time to complete follow-up of all patients will be 36 months.

3.1 Inclusion Criteria

Subject must meet all of the following applicable inclusion criteria to participate in this study:

1. Be willing and able to provide written informed consent/assent for the trial.

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

3. Patients must have received 1 line of prior systemic therapy for metastatic or resectable disease (i.e. patients may have received adjuvant gemcitabine and then later gemcitabine/cisplatin for recurrent metastatic disease)

4. Histological confirmation of cholangiocarcinoma.

5. Have measurable disease based on RECIST 1.1.

6. Be willing to provide tissue from a newly obtained core or excisional biopsy of a tumor lesion. Newly obtained is defined as a specimen obtained up to 6 weeks (42 days) prior to initiation of treatment on Day 1. Subjects for whom newly obtained samples cannot be provided (e.g. inaccessible or subject safety concern) may submit an archived specimen only upon agreement from the sponsor-investigator.

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

8. Demonstrate adequate organ function as defined in Table 1. All screening labs will be performed within 28 days of registration.

Table 1: Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥ 1,500 /μL
Platelets	≥ 100,000 / μL
Hemoglobin	≥ 9 g/dL or ≥ 5.6 mmol/L without transfusion or EPO dependency (within 7 days of assessment)
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤ 1.5 X upper limit of normal (ULN) **OR** ≥ 60 mL/min for subject with creatinine levels > 1.5 x institutional ULN
Hepatic	
Serum total bilirubin	≤ 2.0 X ULN
AST (SGOT) and ALT (SGPT)	≤ 3.0 X ULN **OR** ≤ 5 X ULN for subjects with liver metastases
Albumin	≥ 2.5 mg/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤ 1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants ≤ 1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a]Creatinine clearance should be calculated per institutional standard.	

9. Female subject of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

10. Female subjects of childbearing potential should be willing to use adequate birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication (Reference Section 5.5.2). Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.

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

3.2 Subject Exclusion Criteria

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

1. Has symptomatic congestive heart failure, unstable angina pectoris, symptomatic or poorly controlled cardiac arrhythmia. Symptomatic heart failure (NYHA Class II-IV)

2. Has a history of anaphylaxis to peginterferon alfa-2b or interferon alfa-2b

3. Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of treatment.

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

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

6. Hypersensitivity to pembrolizumab or any of its excipients.

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

8. Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.
    a. **NOTE:** Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study.
    b. **NOTE:** If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

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

10. 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 the first dose of trial treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to trial treatment. This exception does not include carcinomatous meningitis, which is excluded regardless of clinical stability.

11. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids, or immunosuppressive drugs). Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

12. Has known history of, or any evidence of active, non-infectious pneumonitis.

13. Has an active infection requiring systemic therapy.

14. 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.

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

16. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment.

17. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent.

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

19. Has known active Hepatitis B without HBV treatment (HBV infection with ongoing HBV treatment is allowed); has persistent chronic Hepatitis C infection (successfully treated HCV infection is allowed).

20. Has received a live vaccine within 30 days of planned start of study therapy.
    NOTE: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however, intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.

21. Has history of bipolar disorder or major depression as identified by the enrolling physician. See SPM for additional information.

22. Has history of not tolerating interferon treatment.

23. Has known serious neuropsychiatric condition.

24. Has a history of organ or stem cell transplantation

4. SUBJECT REGISTRATION

All subjects must be registered through HCRN's electronic data capture (EDC) system. A subject is considered registered when an "On Study" date is entered into the EDC system. Subjects must be registered prior to starting protocol therapy.

5. TREATMENT PLAN

5.1 Pre-medication and Hydration

There are no pre-medications for sylatron or pembrolizumab

5.2 Pembrolizumab and Sylatron Administration

Each cycle = 21 days or 3 weeks. Sylatron will be administered at 200mcg subcutaneously every week for up to 12 weeks (3 weeks as single agent, and 9 weeks in combination of pembrolizumab) starting Cycle 1 Day 1. The dose of sylatron may be reduced to 120mcg weekly, or 60mcg weekly if patients experience unacceptable toxicities at the higher dose levels. Patients will be premedicated with acetaminophen 500 to 1000 mg orally 30 minutes prior to the first dose of sylatron and as needed for subsequent doses. Injection sites will be rotated. During the Cycle 1 Day 1 visit, subjects will be provided education on how to manage sylatron injections at home.

Pembrolizumab will be administered intravenously as a 30 minute infusion at a dose of 200 mg every 3 weeks starting Week 4 (Cycle 2 Day 1). **All trial treatments will be administered on an outpatient basis.** On the days that sylatron and pembrolizumab are given together, sylatron should be administered prior to the pembrolizumab infusion.

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

With concerns about toxicity while using long-term sylatron, if no additional benefit is seen beyond 12 weeks of treatment on top of that expected from pembrolizumab alone, patients will discontinue sylatron treatment after they have received 12 weeks of this therapy (3 weeks as single agent and 9 weeks in combination with pembrolizumab). Pembrolizumab should be continued in the absence of unacceptable toxicity (based on CTCAE v4) until disease progression-based on RECIST 1.1. In patients who had responded to the combination therapy, when patient shows disease progression while on single agent pembrolizumab treatment, sylatron may be resumed at the discretion of the site investigator after discussion with the sponsor-investigator.

Medication Administration (Cycle = 21 days or 3 weeks)

Drug	Dose	Route	Schedule	Regimen Treatment Period	Cycle
Sylatron	200mcg	Subcutaneous injection	Q1W starting C1D1	Day 1, 8 and 15 of each cycle (weekly)	21 days
Pembrolizumab	200mg	Intravenous Infusion (IV) over 30 minutes	Q3W starting C2D1	Day 1 of each cycle	21 days
Dosing interruptions are permitted in the case of medical/surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). Subjects should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the sponsor-investigator. The reason for interruption should be documented in the subject's chart and electronic case report forms (eCRFs).					

5.3 Treatment Beyond Radiological Disease Progression with Pembrolizumab

Accumulating clinical evidence indicates that some subjects treated with immune system stimulating agents may develop disease progression by conventional response criteria before demonstrating clinical objective responses and/or stable disease. This phenomenon was observed in the Phase I study of nivolumab, CA209003. Two hypotheses explain this phenomenon. First, enhanced inflammation within tumors could lead to an increase in tumor size, which would appear as enlarged index lesions and as newly visible small non-index lesions. Over time, both the malignant and inflammatory portions of the mass may then decrease leading to overt signs of clinical improvement. Alternatively, in some individuals, the kinetics of tumor growth may initially outpace anti-tumor immune activity. With sufficient time, the anti-tumor activity will dominate and become clinically apparent. Therefore, subjects will be allowed to continue study therapy after an initial investigator-assessed RECIST 1.1 defined progression as long as they meet the following criteria:

1. Site investigator assessed clinical benefit, and
2. Subject is tolerating treatment.

These criteria aim to ensure the risk/benefit for continuing treatment will continue to favor the subjects. The assessment of clinical benefit should take into account whether the subject is clinically deteriorating and unlikely to receive further benefit from continued treatment. Palliative radiotherapy or surgical resection of isolated lesions is permitted in these subjects and will not preclude the continued treatment with pembrolizumab.

All decisions to continue treatment beyond initial progression must be discussed with the sponsor-investigator and documented in the study records. Subjects should discontinue study therapy upon further evidence of further progression, defined as an additional 10% or greater increase in tumor burden volume from time of initial progression (including all target lesions and new measurable lesions). New lesions are considered measurable at the time of initial progression if the longest diameter is at least 10 mm (except for pathological lymph nodes, which must have a short axis of at least 15 mm). Any new lesion considered non-measurable at the time of initial progression may become measurable and therefore included in the tumor

burden measurement if the longest diameter increases to at least 10 mm (except for pathological lymph nodes, which must have an increase in short axis to at least 15 mm).

For statistical analyses that include the site investigator-assessed progression date, subjects who continue treatment beyond initial site investigator-assessed, RECIST 1.1-defined progression will be considered to have site investigator-assessed progressive disease at the time of the initial progression event.

5.4 Discontinuation of Pembrolizumab after CR

Discontinuation of treatment may be considered for subjects who have attained a confirmed CR that have been treated for at least 24 weeks with pembrolizumab and had at least two treatments with pembrolizumab beyond the date when the initial CR was declared. Subjects who then experience radiographic disease progression may be eligible for up to one year of additional treatment with pembrolizumab at the discretion of the site investigator if no cancer treatment was administered since the last dose of pembrolizumab, the subject meets the safety parameters listed in the Inclusion/Exclusion criteria, and the trial is open. Subjects will resume therapy at the same dose and schedule at the time of initial discontinuation.

5.5 Concomitant Medications

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. The final decision on any supportive therapy or vaccination rests with the site investigator and/or the subject's primary physician.

5.5.1 Allowed 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 (eCRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the eCRF.

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs.

5.5.2 Prohibited Concomitant Medications

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

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy
  - **NOTE:** Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed at the site investigator's discretion.
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids 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.

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. Subjects may receive other medications that the investigator deems to be medically necessary. The Exclusion Criteria describes other medications which are prohibited in this trial. There are no prohibited therapies during the Post-Treatment Follow-up Phase.

5.6 Supportive Care

Subjects should receive appropriate supportive care measures as deemed necessary by the site investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined below. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the site investigator determines the events to be related to pembrolizumab.

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event.

Pneumonitis

- For **Grade 2 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.
- For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
- Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.

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 patients 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 patients 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 liothyronine, 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 2 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab.

Table 2: **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 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 hr	**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 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 1.5 hr (± 30 minutes) prior to infusion of pembrolizumab (MK-3475) with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
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 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.		

5.7 Diet and Other Considerations

5.7.1 Diet

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

5.7.2 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm. Non-pregnant, non-breast-feeding women may be enrolled if they are willing to use adequate birth control or are considered highly unlikely to conceive. Highly unlikely to conceive is defined as 1) surgically sterilized, or 2) postmenopausal (a woman who is ≥45 years of age and has not had menses for greater than 1 year will be considered postmenopausal), or 3) not heterosexually active for the duration of the study. Birth control methods can be barrier methods or hormonal method to prevent pregnancy. Subjects should start using birth control from study Visit 1 throughout the study period up to 120 days after the last dose of study therapy.

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

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

5.7.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab, the subject will immediately be removed from the study. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Hoosier Cancer Research Network (HCRN) **within 1 business day** of becoming aware of the event. HCRN will then report to Merck **within 1 business day** if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn).

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to 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 to Merck and followed as described above.

5.7.4 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. TOXICITIES AND DOSE DELAYS/DOSE MODIFICATIONS

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

Subjects enrolled in this study will be evaluated clinically and with standard laboratory tests before and at regular intervals during their participation in this study as specified in Study Calendar & Evaluations.

Subjects will be evaluated for adverse events (all grades), serious adverse events, and adverse events requiring study drug interruption or discontinuation as specified in Study Calendar & Evaluations.

6.1 Dose Delay of Pembrolizumab

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per Table 3.

Table 3: Dose Modification and Toxicity Guidelines of pembrolizumab for Immune-related AEs Associated with Pembrolizumab

General instructions:

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

Immune-related AEs	Toxicity grade or conditions (CTCAEv4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment • Add prophylactic antibiotics for opportunistic infections
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus). • Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.
	Grade 4	Permanently discontinue		

Immune-related AEs	Toxicity grade or conditions (CTCAEv4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
AST / ALT elevation or Increased bilirubin	Grade 2	Withhold	• Administer corticosteroids (initial dose of 0.5- 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable
	Grade 3 or 4	Permanently discontinue	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold	• Initiate insulin replacement therapy for participants with T1DM • Administer anti-hyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated.	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hyperthyroidism	Grade 2	Continue	• Treat with non-selective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders.
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hypothyroidism	Grade 2-4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyroinine) per standard of care	• Monitor for signs and symptoms of thyroid disorders.
Nephritis and Renal dysfunction	Grade 2	Withhold	• Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper.	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		

Immune-related AEs	Toxicity grade or conditions (CTCAEv4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
Myocarditis	Grade 1 or 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
All other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	• Based on type and severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Gullain-Barre Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		

1. Withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician.

NOTE:

For participants with Grade 3 or 4 immune-related endocrinopathy where withhold of pembrolizumab is required, pembrolizumab may be resumed when AE resolves to ≤ Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM).

6.2 Dose Modification of Sylatron

Table 4: Dose Modification Guidelines of Sylatron for Drug-Related Adverse Events

Resume treatment at reduced dose (see Table 5)	Withhold treatment	Permanently discontinue treatment
Absolute Neutrophil Count (ANC) $\geq 0.5x10^9/L$	Absolute Neutrophil Count (ANC) $< 0.5x10^9/L$	Persistent or worsening severe neuropsychiatric disorders toxicity
Platelet Count (PLT) $\geq 50x10^9/L$	Platelet Count (PLT) $< 50x10^9/L$	New or worsening retinopathy
Non-hematologic toxicity has completely resolved or improved to Grade 1	Non-hematologic toxicity Grade ≥ 3	Grade 4 non-hematologic toxicity
ECOG PS 0-1	ECOG PS ≥ 2	Inability to tolerate a dose of 60mcg/week

Table 5: Sylatron Dose Modifications

Dose level -1	120 mcg weekly
Dose level -2	60 mcg weekly

6.3 Subject Withdrawal/Protocol Therapy Discontinuation

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

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

- The subject or legal representative (such as a parent or legal guardian) withdraws consent.
- Confirmed radiographic disease progression
  NOTE: A subject may be granted an exception to continue on treatment with confirmed radiographic progression if there is objective clinical benefit, please see Section 5.3
- Unacceptable adverse experienced
- Intercurrent illness that prevents further administration of treatment
- Site investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements
  The subject is lost to follow-up
- Protocol therapy interrupted for > 12 weeks
- Completed 24 months of uninterrupted treatment with pembrolizumab or 35 administrations of pembrolizumab, whichever is later. **NOTE:** 24 months of pembrolizumab is calculated from the date of first dose.
- Administrative reasons

The End of Treatment and Follow-up visit procedures are listed in Section 7. After the end of treatment, each subject will have follow up visit (1) at 30 days after last dose of study treatment. Serious adverse events will be collected for 90 days after last dose of study treatment (follow up visit 2). Follow up for patients that have not experienced progression will occur every 9 weeks ± 7 days. This may be done by phone call or other avenues as appropriate. Every effort should be made to obtain information on radiology scans or other types of disease assessment documentation. Once a subject experiences confirmed disease progression or starts a new anti-cancer therapy, the subject moves into the survival follow-up phase and should be contacted by telephone every 12 weeks ± 7 days to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.

6.4 Subject Replacement Strategy

If all four sites have more eligible patients than the available slots, the order of enrollment will rotate among the four sites. Otherwise the patients will be enrolled according to the order they become eligible for enrollment.

6.5 Clinical Criteria for Early Trial Termination

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

1. Quality or quantity of data recording is inaccurate or incomplete
2. Poor adherence to protocol and regulatory requirements
3. Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects
4. Plans to modify or discontinue the development of the study drug. In the event of Merck decision to no longer supply study drug, ample notification will be provided so that appropriate adjustments to subject treatment can be made.

7. STUDY CALENDAR & EVALUATIONS

Study Evaluation Cycle = 21 days	Screening	On Treatment Cycle 1-n	End of Treatment Visit[11]	Safety Follow Up Visit[12]	Long Term Follow Up [13]
	≤ 28 days	Day 1 ± 5 days	± 7 days	30 days after EOT/study drug ± 7 days	Every 12 weeks after Visit 2
REQUIRED ASSESSMENTS					
Informed Consent	X				
Medical History[1]	X				
Diagnosis and Staging[1]	X				
Physical Exam[2]	X	X[10]	X	X	
Vital signs and ECOG Performance Status[3]	X	X	X	X	
Baseline ophthalmologic assessment[4]	X				
AEs & concomitant medications	X	X	X	X	
LABORATORY ASSESSMENTS					
Complete Blood Cell Count with diff (CBC)	X	X[10]	X	X	
Comprehensive Metabolic Profile (CMP) and amylase	X	X[10]	X	X	
PT/INR and aPTT	X	X[10]	X	X	
Thyroid Function (TSH, free T4, T3)	X	X[10]	X	X	
Urinalysis	X	X[10]	X	X	
Pregnancy test (serum or urine) (WOCBP)[5]	X				
DISEASE ASSESSMENT					
CT of chest[6]	X	X			X
CT or MRI of abdomen and pelvis[6]	X	X			X
MRI Brain[6]	X	X			X
TREATMENT EXPOSURE					
Sylatron[7]		X			
Pembrolizumab[7]		X			
SPECIMEN COLLECTION					
Archival and Newly Obtained Tumor Tissue[8]	X	X[8]			
Blood Samples[9]	X	X	X[9]	X[9]	
FOLLOW-UP					
Survival Status, Subsequent Therapy					X

Key to Footnotes

[1] During the medical history other information to be collected includes smoking history, alcohol history, diagnosis and staging and how the healthcare provider and/or subject learned about this study. Diagnosis and staging to include: reviewing pathology report and screening scans. Special attention should be given to the assessment of past or current depression or suicidal tendencies. In addition, if a subject has substance abuse issues, they are at higher risk of relapse. If concerns are identified during this visit, the appropriate referral to psychiatric services should be made to further assess. See SPM for additional information.

[2] Patients will be screened for depression, suicidal tendencies and possible relapse of substance abuse at each physical exam. If a concern is noted, the patient will be referred for the appropriate mental health evaluation. In addition, cardiac issues will be assessed per SOC and if concerns are identified, appropriate testing should be completed to fully assess the subject. This may include an EKG, ECHO/MUGA or cardiology referral.

[3] Vital signs to include blood pressure, weight, and height (screening only) and ECOG performance status

[4] A baseline ophthalmologic assessment will be done in subjects with pre-existing retinopathy, or history of retinal vein or artery thrombosis.

[5] For women of childbearing potential (WOCBP): urine or serum βhCG, within 28 days of registration and only if clinically appropriate. If a urine test is done and it is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

[6] Radiology Imaging for disease assessment will be performed at the following timepoints: screening, at the end of Cycle 3 (9 weeks of combination therapy) then every 9 weeks (3 Cycles). Tumor imaging will be performed after the last on-study scan only for patients who do NOT have radiographic progression: Radiology Imaging should occur at 30 days, then every 9 weeks thereafter until disease (radiographic) progression. MRI of the brain should only be done if the patient is suspected to have a brain lesion.

[7] Sylatron will start on C1D1 and continue weekly. During the C1D1 clinic visit, subjects will be trained on how to manage sylatron administration at home. Pembrolizumab will start C2D1 and continue every 3 weeks. Please see Section 5 for details regarding sylatron and pembrolizumab administration. A window of ± 5 days is allowed to accommodate for schedule conflicts. Laboratory testing should be performed on the day of study treatment administration.

[8] Archival tissue will be identified during screening and is required if available for all subjects. All subjects will have a biopsy performed of a safely accessible tumor before starting treatment (within 42 days of starting treatment on this protocol) and 3 weeks later prior to initiation of pembrolizumab (prior to Cycle 2 Day 1). Subjects for whom newly obtained samples cannot be provided (e.g. inaccessible tumor for biopsy or subject safety concerns) may omit both biopsies provided documentation of reason is clear in the medical record.

[9] Serial blood samples will be collected to support biomarker research at the following timepoints: Prior to treatment (1) C1D1, (2) C2D1, (3) C5D1 (4) C7D1 and (5) End of Treatment/Safety Follow Up Visit. See CLM for additional details.

[10] If screening (baseline) labs and physical exam were performed within 7 days of Cycle 1 Day 1 of treatment, these do not need to be repeated. All laboratory assessments should be done prior to treatment.

[11] An end of treatment (EOT) visit will be performed for the following situations: (1) if patient has progressed and a discussion of scans/next treatment is needed or (2) if a patient has experienced toxicities that necessitates study treatment discontinuation. Special attention should be given to the assessment of current depression, suicidal tendencies or relapse of substance abuse. If concerns are identified during this visit, the appropriate referral to psychiatric services should be made to further assess. There may be certain situations in which this visit will take place locally and discussion regarding next steps would occur via phone call. In this case the research specific blood samples would not be done until the safety follow up visit.

[12] A safety follow up visit will be performed about 4 weeks after the EOT visit or last dose of study drug. Special attention should be given to the assessment of current depression or suicidal tendencies in addition to relapse of substance abuse. If concerns are identified during this visit, the appropriate referral to psychiatric services should be made to further assess. After completion of the EOT visit, subjects should be contacted on a monthly basis until 180 days after their last dose of study drug to assess for issues with mood or behavior. If a concern is identified the patient will be referred to the appropriate mental health resources for evaluation. If research specific blood samples were not done during the EOT visit, they should be drawn during this visit. This visit may take place locally if the patient had an EOT visit at the study facility and study specific blood samples were drawn at that time.

[13] Long Term Follow up will occur every 12 weeks ± 7 days. This may be done by phone call or other avenues as appropriate. Every effort should be made to obtain information on radiology scans or other types of disease assessment documentation. Follow up will continue until death, withdrawal of consent, or the end of the study, whichever occurs first.

Hematology	Chemistry	Urinalysis	Other
Hematocrit	Albumin	Blood	Serum β-human chorionic gonadotropin†
Hemoglobin	Alkaline phosphatase	Glucose	(β-hCG)†
Platelet count	Alanine aminotransferase (ALT)	Protein	PT (INR)
WBC (total and differential)	Aspartate aminotransferase (AST)	Specific gravity	aPTT
Red Blood Cell Count	Total protein	Microscopic exam *(If abnormal)*	Total triiodothyronine (T3)
Absolute Neutrophil Count	Carbon Dioxide ‡	results are noted	Free thyroxine (T4)
Absolute Lymphocyte Count	*(CO_2 or bicarbonate)*	Urine pregnancy test †	Thyroid stimulating hormone (TSH)
	Sodium		Blood for correlative studies
	Calcium		
	Chloride		
	Glucose		
	Phosphorus		
	Potassium		
	Blood Urea Nitrogen		
	creatinine		
	Total Bilirubin		
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*		
	amylase		

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

‡ If considered standard of care in your region.

8. BIOSPECIMEN STUDIES AND PROCEDURES

Biomarkers in patient tumors that can predict response to anti-PD-1 therapy are being actively investigated. One mechanism by which cancer tissues limit the host immune response is via upregulation of PD-1 ligand (PD-L1) and its ligation to PD-1 on antigen-specific CD8+ T cells [6, 7]. Recent research has reported that tumor regression after therapeutic PD-1 blockade requires pre-existing CD8+ T cells that are negatively regulated by PD-1/PD-L1-mediated adaptive immune resistance. [8] We propose that Sylatron will increase the response of pembrolizumab by increasing the tumor infiltrating CD3 and CD8 cells, and the level of PD-L1 and PD-1 expression in immune cells and cancer cells. In this study, we will first test the effect of Sylatron on tumor infiltrating lymphocytes, the level of PD-1 and PD-L1 expression, and clonality of the T-cell repertoire by comparing the tumor biopsies from baseline and three weeks of Sylatron treatment. Then we will correlate the change of PD-1, PD-L1 levels, immune score, and clonality of the T-cell repertoire from Sylatron treatment with clinical response.

Immunohistochemical (IHC) staining: The level of PD-1 and PD-L1 expression in tumor biopsy will be measured by immunohistochemical (IHC) staining at Merck laboratories. Immunohistochemical (IHC) staining, Digital image acquisition and analysis: The density of intratumoral total (CD3+) and cytotoxic (CD8+) T lymphocytes will be measured in the tumor interior (TI) and in the invasive margin (IM) of the three biopsied tumor samples. Immune cell densities will be obtained by immunohistochemistry and quantified using a biomarker imaging system in tandem with Image J processing software at Lombardi Comprehensive Cancer Center. Immune cell density in the TI and IM was converted to a binary score (0 as Low, 1 as High), with a cutoff threshold determined by the median density of CD3+ and CD8+ cells. We previously carried out a study on the association of intratumoral CD3 and CD8 cell density with recurrence free survival in patients with surgically resected Hepatocellular Carcinoma.

Next Generation Sequencing for T-cell receptor clonality: TCR sequencing and clonality quantification was performed as previously described [29,30] from tumor samples preserved using RNAlater (Qiagen) and stored at −80°C. DNA was isolated by mincing followed by extraction utilizing a DNeasy kit (Qiagen). TCRβ CDR3 regions were amplified and sequenced using the survey ImmunoSeq assay in a multiplexed PCR method using 45 forward primers specific to TCR Vβ gene segments and 13 reverse primers specific to TCR Jβ gene segments. The experiments of measuring T-cell receptor clonality will be carried out at Lombardi Comprehensive Cancer Center.

PCR template abundance estimation: In order to estimate the average read coverage per input template in our multiplex PCR and sequencing approach, we will employ a set of approximately 850 unique types of synthetic TCR analog, comprising each combination of Vβ and Jβ gene segments.[30] These molecules will be included in each PCR reaction at very low concentration so that most unique types of synthetic template are not observed in the sequencing output. Using the known concentration of the synthetic template pool, we will simulate the relationship between the number of observed unique synthetic molecules and the total number of synthetic molecules will be added to reaction (this is very nearly one-to-one at the low concentrations we employed). These molecules then allowed us to calculate for each PCR reaction the mean number of sequencing reads obtained per molecule of PCR template, and thus to estimate the

number of rearranged T cell receptors per diploid genome (i.e., level of TIL infiltration) in the input material. The PCR analysis will be carried out in Lombardi Comprehensive Cancer Center.

8.1 Tumor Biopsies

Archival tissue will be requested for all subjects. All subjects will have a biopsy performed of a safely accessible tumor before starting treatment (within 42 days of starting treatment on this protocol) and 3 weeks later prior to initiation of pembrolizumab (prior to Cycle 2 Day 1). Subjects for whom newly obtained samples cannot be provided (e.g. inaccessible tumor for biopsy or subject safety concerns) may omit both biopsies provided documentation of reason is clear in the medical record. In these instances, submission of an archived specimen only will suffice.

Analysis of molecular characteristics of baseline and on-treatment tumor samples. All patients will have biopsies performed of safely accessible tumors before starting treatment (within 42 days of starting treatment on this protocol) and 3 weeks later. Preferred biopsy is core biopsy; five 20-gauge core needle biopsies (diameter about 2 mm) with a throw length of approximately 2 cm will provide adequate tissue for study. Available paraffin embedded tumor tissue from prior biopsies or excisions will also be collected as pre-treatment comparator samples. Preferred archival material includes tissue obtained in the same manner as specified in this trial and with no systemic anti-tumor treatment received by the patient between the biopsy and their enrollment on this protocol. However, other archival tissue may also be collected.

Each set of core biopsies will be divided into 5 portions and processed as below for later analysis:

1) FFPE for histology and IHC (20% of the specimen, or 1 core biopsy)

2) Quick-frozen in OCT for immunohistology and protein studies (40% of the specimen, or 2 core biopsy): embedded in optimum cutting temperature (OCT) solution, frozen in liquid nitrogen, and then stored at -80° C.

3) Placed in RNA-later for RNA and RT-PCR (40% of the specimen, or 2 core biopsy).

Of note, patients who are on chronic anticoagulation will be required to hold anticoagulation prior to the biopsies being performed. Patients on warfarin must hold treatment for 5 days, but will be on low-molecular weight heparin (LMWH), 1mg/kg subcutaneously twice a day. The LMWH will continue until the last biopsy is complete. Patients may then resume warfarin the day after the last biopsy. Additionally, patients on LMWH will hold (*i.e.*, not receive) the dose of LMWH the morning of the procedure, but will resume the LMWH the evening of the day of the biopsy.

8.2 Blood Collections

Plasma, serum, and peripheral blood for PMBCs will be collected at the five scheduled time points: prior to treatment (1) C1D1, (2) C2D1, (3) C5D1 (4) C7D1 and (5) End of Treatment. PBMCs will be isolated by Ficoll gradient centrifugation and cryopreserved in 10% DMSO at -80°C for subsequent evaluation.

8.3 Storage of Biospecimens
Remaining specimens will be stored for future research once protocol described biospecimen-based studies are complete. This option will be presented to subjects in the informed consent.

8.4 Confidentiality of Biospecimens
Samples that are collected will be identified by a subject's sequence ID assigned at the time of registration to the trial. Any material issued to collaborating researchers will be anonymized and only identified by the subject's sequence ID.

9. CRITERIA FOR DISEASE EVALUATION
The following sections describe the recommended method to track disease response for solid tumor trials as per the Response Evaluation Criteria In Solid Tumors (RECIST) 1.1 (see Eisenhauer EA et al. *New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1).* Eur J Can, 2009.45:p.228-247).

9.1 Measurable Disease
Measurable disease is defined as the presence of at least one measurable lesion. 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).

9.1.1 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.

9.2 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.

9.3 Target Lesions
All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that

lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

9.4 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 also 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.

9.5 Evaluation of Target Lesions

NOTE: In addition to the information below, also see section 4.3.2 in the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee, version 1.1 (Eur J Cancer 45;2009:228-247) for special notes on the assessment 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
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
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).

9.6 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 at a later time by the sponsor investigator.

9.7 Evaluation of Best Overall Response

NOTE: Revise table if response rate is primary objective of the study, and confirmation of response is required. See Eur J Cancer 45;2009:228-247 for complete details.

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/ Non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD/ or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	Non-evaluable
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.

9.8 Definitions for Response Evaluation – RECIST 1.1

9.8.1 First Documentation of Response

The time between initiation of therapy and first documentation of PR or CR.

9.8.2 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.

9.8.3 Objective Response Rate
The objective response rate is the proportion of all subjects with confirmed PR or CR according to RECIST 1.1, from the start of treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the start of treatment).

9.8.4 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.

9.8.5 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.

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

9.9 Immune-Related RECIST (irRECIST)

9.9.1 Measurable Lesion Definitions and Target Lesion Selection
Follow the definitions from RECIST 1.1. Measurable lesions must be accurately measured in at least one dimension with a minimum size of:

- 10 mm in the longest diameter by CT or MRI scan (or no less than double the slice thickness) for non-nodal lesions and ≥15 mm in short axis for nodal lesions
- 10 mm caliper measurement by clinical exam
- 20 mm by chest X-ray
- 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 and five cutaneous index lesions) is calculated.
- Baseline: Target and Non-Target Lymph Node Lesion Definitions - Follow the definitions from RECIST 1.
- Baseline: Bone Lesions- Follow the definitions from RECIST 1.1. Regardless of the imaging modality blastic bone lesions will not be selected as target lesions. Lytic or mixed lytic-blastic lesions with a measurable soft tissue component ≥10 mm can be selected as target lesions.
- Baseline: Cystic and necrotic lesions as target lesions that are partially cystic or necrotic can be selected as target lesions. The longest diameter of such a lesion will be added to the Total Measured Tumor Burden (TMTB) of all target lesions at baseline. If other lesions with a non-liquid/non-necrotic component are present, those should be preferred.
- Baseline: Lesions with prior local treatment during target lesion selection the radiologist will consider information on the anatomical sites of previous intervention (e.g. previous irradiation, RF-ablation, TACE, surgery, etc.). Lesions undergoing prior intervention will

not be selected as target lesions unless there has been a demonstration of progress in the lesion

9.9.2 Non-measurable Lesion Definitions

Follow definitions from RECIST 1.1

9.9.3 Non-target lesions will include

- Measurable lesions not selected as target lesions
- All sites of non-measurable disease, such as neoplastic masses that are too small to measure because their longest uninterrupted diameter is < 10 mm (or < two times the axial slice thickness), ie. the longest per-pendicular diameter is ≥10 and < 15 mm.
- Other types of lesions that are confidently felt to represent neoplastic tissue, but are difficult to measure in a reproducible manner. These include bone metastases, leptomeningeal metastases, malignant ascites, pleural or pericardial effusions, ascites, inflammatory breast disease, lymphangitis cutis/pulmonis, cystic lesions, ill-defined abdominal masses, skin lesions, etc.

9.9.4 Target and Non-Target Lymph Node Lesion Definitions

Follow definitions from RECIST 1.1

9.9.5 Non-Target Lesion Selection

All lesions or sites of disease not recorded as target lesions should be recorded as non-target lesions at baseline. There is no limit to the number of non-target lesions that can be recorded at baseline.

9.9.6 Bone Lesions

Follow definitions from RECIST 1.1

Regardless of the imaging modality blastic bone lesions will not be selected as target lesions. Lytic or mixed lytic-blastic lesions with a measurable soft tissue component ≥10 mm can be selected as target lesions.

9.9.7 Cystic and Necrotic Lesions as Target Lesions

Lesions that are partially cystic or necrotic can be selected as target lesions. The longest diameter of such a lesion will be added to the Total Measured Tumor Burden (TMTB) of all target lesions at baseline. If other lesions with a non-liquid/non-necrotic component are present, those should be preferred.

9.9.8 Lesions with Prior Local Treatment

During target lesion selection the radiologist will consider information on the anatomical sites of previous intervention (e.g. previous irradiation, RF-ablation, TACE, surgery, etc.). Lesions undergoing prior intervention will not be selected as target lesions unless there has been a demonstration of progress in the lesion.

9.9.9 Follow-up

- Only index and measurable new lesions are taken into account. The longest diameters of non-nodal target and new non-nodal measurable lesions, and short axes of nodal target

and new nodal measurable lesions will be recorded. Together they determine the Total Measured Tumor Burden (TMTB) at follow-up.

- Definition of Measurable New Lesions: In order to be selected as new measurable lesions (≤ 2 lesions per organ, ≤ 5 lesions total, per timepoint), new lesions must meet criteria as defined for baseline target lesion selection and meet the same minimum size requirements of 10 mm in long diameter and minimum 15 mm in short axis for new measurable lymph nodes. New measurable lesions shall be prioritized according to size, and the largest lesions shall be selected as new measured lesions.
- Non-Target Lesion Assessment- The RECIST 1.1 definitions for the assessment of non-target lesions apply. The response of non-target lesions primarily contributes to the overall response assessments of irCR and irNon-CR/Non-PD (irNN). Non-target lesions do not affect irPR and irSD assessments. Only a massive and unequivocal worsening of non-target lesions alone, even without progress in the TMTB is indicative of irPD.
- New Non-Measurable Lesions Definition and Assessment: All new lesions not selected as new measurable lesions are considered new non-measurable lesions and are followed qualitatively. Only a massive and unequivocal progression of new non-measurable lesions leads to an overall assessment of irPD for the timepoint. Persisting new non-measurable lesions prevent irCR

9.9.10 Evaluation of Best Overall Response per irRECIST

% Change in Sum of the Diameters	Target Lesion Definition	Non-Target Lesion Definition	New Measurable Lesions	New Unmeasurable Lesions	Overall Modified RECIST Timepoint Response
-100% [a]	CR	CR	No	No	CR
-100% [a]	CR	Non-CR or not all evaluated	No	No	PR
≤ - 30%	PR	Any	Yes or no	Yes or no	PR
> -30% to <+20%	SD	Any	Yes or no	Yes or no	SD
Not all evaluated	Not evaluated	Any	Yes or no	Yes or no	NE
≥ ± 20%	PD	Any	Yes or no	Yes or no	PD

CR =complete response; NE= not evaluable; PD=progressive disease; PR =partial response; RECIST =Response Evaluation Criteria in Solid Tumors; SD= stable disease.

[a] When lymph nodes are included as target lesions, the % change in the sum of the diameters may not be 100% even if complete response criteria are met since a normal lymph node is defined as having a short axis of < 10 mm. Any pathological lymph nodes (whether target or

non-target) must have reduction in short axis to < 10 mm in order to meet the definition of CR. Including Measurable New Lesions When Present.

10. DRUG INFORMATION

10.1 Pembrolizumab (Keytruda®)

Pembrolizumab is a potent and highly selective humanized mAb of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Keytruda™ (pembrolizumab) has recently been approved in the United Stated for the treatment of subjects with unresectable or metastatic melanoma and disease progression following ipilumumab and, if BRAF V600 mutation positive, a BRAF inhibitor. It is also approved for the treatment of subjects with PD-L1 positive metastatic NSCLC who have disease progression on or after platinum chemotherapy. Please refer to the current version of the Keytruda® prescribing information and the pembrolizumab Investigator's Brochure (IB) for additional information regarding this drug.

10.1.1 Supplier/How Supplied

Merck will supply pembrolizumab at no charge to subjects participating in this clinical trial, as summarized in the table below.

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

10.1.2 Preparation

Please refer to the Pharmacy Manual for a comprehensive description of pembrolizumab preparation.

10.1.3 Storage and Stability

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

Clinical supplies may not be used for any purpose other than that stated in the protocol.

10.1.4 Handling and Disposal

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

10.1.5 Dispensing, Returns and Reconciliation

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

10.1.6 Use in Pregnancy and Nursing Women

Based on its mechanism of action, pembrolizumab can cause fetal harm when administered to a pregnant woman. Subjects who are pregnant or planning to become pregnant are not eligible for enrollment. 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.

10.1.7 Adverse Events

Please refer to the current Investigator's Brochure for a complete list of adverse events.

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, hypophysitis (including hypothryoidism/hyperthyroidism), hepatitis, Type I diabetes mellitis, uveitis, and nephritis, myositis, Guillain-Barre syndrome, pancreatitis, and severe skin reaction toxic epidermal necrolysis (TEN), some with fatal outcome). A new important risk of myocarditis has been identified; cases with fatal outcome have been reported.

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 Sylatron

SYLATRON, peginterferon alfa-2b, is a covalent conjugate of recombinant alfa-2b interferon with monomethoxy polyethylene glycol (PEG). The average molecular weight of the PEG portion of the molecule is 12,000 daltons. The average molecular weight of the SYLATRON molecule is approximately 31,000 daltons. The specific activity of pegylated interferon alfa-2b is approximately 0.7 x 108 international units/mg protein.

Interferon alfa-2b is a protein with a molecular weight of 19,271 daltons produced by recombinant DNA techniques. It is obtained from the bacterial fermentation of a strain of Escherichia coli bearing a genetically engineered plasmid containing an interferon gene from human leukocytes.

Each vial contains200 mcg of peginterferon alfa-2b as a sterile, white to off-white lyophilized powder, and dibasic sodium phosphate anhydrous (1.11 mg), monobasic sodium phosphate dihydrate (1.11 mg), polysorbate 80 (0.074 mg), and sucrose (59.2 mg).

10.2.1 Supplier/How Supplied

Merck will supply sylatron at no charge to subjects participating in this clinical trial, as summarized in the table below.

Product Name & Potency	Dosage Form
Sylatron	Lyophilized Powder for Injection

10.2.2 Preparation

Reconstitute SYLATRON with 0.7 mL of Sterile Water for Injection, USP.

SYLATRON Single-Use Vial		Diluent (Sterile Water for Injection, USP)		Deliverable Product and Volume	Final Concentration
200 mcg*	**add**	**0.7 mL**	**=**	**200 mcg in 0.5 mL**	**40 mcg/0.1 mL**

Swirl gently to dissolve the lyophilized powder. **DO NOT SHAKE.**

- Visually inspect the solution for particulate matter and discoloration prior to administration. Discard if solution is discolored, cloudy, or if particulates are present.
- Do not withdraw more than 0.5 mL of reconstituted solution from each vial.
- Administer SYLATRON subcutaneously. Rotate injection sites.
- If reconstituted solution is not used immediately, store at 2°-8°C (36°-46°F) for no more than 24 hours. Discard reconstituted solution after 24 hours. **DO NOT FREEZE.**
- For single-use only. **DISCARD ANY UNUSED PORTION.**

10.2.3 Storage and Stability

SYLATRON should be stored at 25°C (77°F); excursions permitted to 15°-30°C (59-86°F) [see USP Controlled Room Temperature]. DO NOT FREEZE.

10.2.4 Handling and Disposal

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

10.2.5 Dispensing, Returns and Reconciliation

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

10.2.6 Use in Pregnancy and Nursing Women

There are no adequate and well-controlled studies of SYLATRON in pregnant women. Nonpegylated interferon alfa-2b was an abortifacient in Macaca mulatta (rhesus monkeys) at 15 and 30 million international units (IU)/kg (estimated human equivalent of 5 and 10 million IU/kg, based on body surface area adjustment for a 60-kg adult). The estimated Intron A human

equivalent dose of 5 to 10 million IU/kg daily is approximately equal to a human equivalent dose of 79 to 158 mcg/kg/week of SYLATRON. Use SYLATRON during pregnancy only if the potential benefit justifies the potential risk to the fetus.

It is not known whether the components of SYLATRON are excreted in human milk. Studies in mice have shown that mouse interferons are excreted in breast milk. Because of the potential for adverse reactions from the drug in nursing infants, a decision must be made whether to discontinue nursing or discontinue the SYLATRON treatment, taking into account the importance of the therapy to the mother.

10.2.7 Adverse Events

Please refer to the current prescribing information for sylatron for additional details. Most common adverse reactions (>60%) are: fatigue, increased ALT, increased AST, pyrexia, headache, anorexia, myalgia, nausea, chills, and injection site reaction. (6.1)

11 ADVERSE EVENTS

The descriptions and grading scales found in the NCI CTCAE v4 will be utilized for AE assessment. A copy of the CTCAE v4 can be downloaded from the CTEP website at http://ctep.cancer.gov. All forms for AE/SAE recording and reporting can be found in the Study Procedure Manual or in the EDC system (Documents and Information Tab).

11.1 Definitions

11.1.1 Adverse Event (AE)

An AE is any untoward medical occurrence whether or not considered related to the study drug that appears to change in intensity during the course of the study. The following are examples of AEs:

- Unintended or unfavorable sign or symptom
- A disease temporally associated with participation in the protocol
- 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

Hospitalization for elective surgery or routine clinical procedures that are not the result of an AE (e.g., surgical insertion of central line) should not be recorded as an AE.

Disease progression should not be recorded as an AE, unless it is attributable to the study regimen by the site investigator.

11.1.2 Serious Adverse Event (SAE)

A SAE is an adverse event that:

- Results in death. **NOTE:** Death due to disease progression should not be reported as a SAE, unless it is attributable by the site investigator to the study drug(s)
- 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 for > 24 hours or prolongation of existing hospitalization. **NOTE:** Hospitalization for anticipated or protocol specified procedures such as administration of chemotherapy, central line insertion, metastasis interventional therapy, resection of primary tumor, or elective surgery, will not be considered serious adverse events.
- 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.

11.1.3 Unexpected Adverse Event

For this study, an AE is considered unexpected when it varies in nature, intensity or frequency from information provided in the current IB, prescribing information or when it is not included in the informed consent document as a potential risk. Unexpected also refers to AEs that are mentioned in the IB as occurring with a class of drugs or are anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

11.1.4 Relatedness

AEs will be categorized according to the likelihood that they are related to the study drug(s). Specifically, they will be categorized using the following terms:

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

11.2 Reporting

11.2.1 Adverse Events

- AEs will be recorded from time of signed informed consent until 30 days after discontinuation of study drug(s).
- AEs will be recorded regardless of whether or not they are considered related to the study drug(s).
- All AEs will be recorded in the subject's medical record and on the appropriate study specific eCRF form within the EDC system.
- AEs considered related to study drug(s) will be followed until resolution to ≤ Grade 1 or baseline, deemed clinically insignificant, and/or until a new anti-cancer treatment starts, whichever occurs first.

11.2.2 Serious Adverse Events (SAEs)

11.2.2.1 Site Requirements for Reporting SAEs to HCRN

- SAEs will be reported from time of signed informed consent until 90 days after discontinuation of study drug(s).
- SAEs will be reported on the SAE Submission Form **within 1 business day** of discovery of the event.
- SAEs include events related and unrelated to the study drug(s).
- All SAEs will be recorded in the subject's medical record and on the appropriate study specific eCRF form within the EDC system.
- All SAEs regardless of relation to study drug will be followed until resolution to ≤ Grade 1 or baseline and/or deemed clinically insignificant and/or until a new anti-cancer treatment starts, whichever occurs first.

The site will submit the completed SAE Submission Form to HCRN **within 1 business day** of discovery of the event**.** The form may be submitted to HCRN electronically to safety@hoosiercancer.org. The site investigator is responsible for informing the IRB and/or other local regulatory bodies as per local requirements.

The original copy of the SAE Submission Form and the email correspondence must be kept within the study file at the study site.

Once the SAE has resolved (see resolution guidelines listed in 11.2.2.1), sites must submit a follow-up SAE Submission Form within a reasonable timeframe to HCRN electronically to safety@hoosiercancer.org.

11.2.2.2 HCRN Requirements for Reporting SAEs to Merck

Hoosier Cancer Research Network will report any possibly related and unexpected SAE to Merck **within one business day** of receipt of the SAE/ECI Submission Form and to regulatory authorities (FDA) per federal guidelines. Follow-up information will be provided to Merck as it is received from site. Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220).

11.2.2.3 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.

11.3 HCRN Responsibilities to FDA

HCRN will manage the Investigational New Drug Application (IND) associated with this protocol on behalf of the sponsor-investigator. HCRN will cross-reference this submission to the Merck's parent IND at the time of submission. Additionally, HCRN will submit a copy of these documents to Merck at the time of submission to FDA.

For protocols conducted under an IND, HCRN will be responsible for all communication with the FDA in accordance with 21CFR312 including but not limited to the 7 and 15 Day Reports, as well as an Annual Progress Report. Additionally, HCRN will submit a copy of these reports to Merck at the time of submission to FDA.

11.4 IND Safety Reports Unrelated to this Trial

Merck will provide to HCRN IND safety reports from external studies that involve the study drug(s) per their guidelines. HCRN will forward safety reports to the sponsor-investigator who will review these reports and determine if revisions are needed to the protocol or consent. HCRN will forward these reports to participating sites **within 1 business day** of receiving the sponsor-investigator's review. Based on the sponsor-investigator's review, applicable changes will be made to the protocol and informed consent document (if required). All IND safety reports will also be made available to sites via the EDC system.

Upon receipt from HCRN, site investigators (or designees) are responsible for submitting these safety reports to their respective IRBs, as per their IRB policies.

11.5 Reporting of Pregnancy and Lactation to the Sponsor-Investigator and to Merck

Although pregnancy and lactation are not considered adverse events, it is the responsibility of 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 of completing the trial. 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 adverse events. If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported **within 24 hours** to HCRN. HCRN will report such events to the sponsor-investigator and Merck Global Safety **within 1 business day** of receiving notification of the event. (Attn: Worldwide Product Safety; FAX 215-993-1220)

11.6 Events of Clinical Interest

Events of Clinical Interest (ECI) described below must be recorded as such on the SAE/ECI Submission Form and reported **within 24 hours** to safety@hoosiercancer.org at HCRN. HCRN will report these events within **one business day** of receipt to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220).

Events of clinical interest for this trial include:

1. an overdose of Funder's product that is not associated with clinical symptoms or abnormal laboratory results. For purposes of this trial, an overdose will be defined as any dose exceeding the prescribed dose for pembrolizumab greater than 10mg/kg over the prescribed dose. No specific information is available on the treatment of overdose of pembrolizumab. In the event of overdose, pembrolizumab should be discontinued and 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.
2. an elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

***NOTE**: These criteria are based upon available regulatory guidance. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology.

12 STATISTICAL METHODS

12.1 Study Design

This is an open-label, single-arm, multicenter Phase II safety and efficacy study of combination therapy with pembrolizumab and Sylatron (Peginterferon alpha-2b) in patients with advanced cholangiocarcinoma who have progressed on or cannot tolerate frontline chemotherapy.

12.2 Endpoints

12.2.1 Definition of Primary Endpoint

- Efficacy endpoint: objective response rate: defined as the proportion of subjects who achieve the best response (CR and PR) determined by RECIST1.1.

12.2.2 Definition of Secondary Endpoints

- PFS: defined as time from registration till the patient's disease progression or death from any cause whichever occurs first (those for whom event of progression or death not observed will be censored).
- OS: defined as time from registration till the patient's death from any cause (patient who are still alive at the end of the study will be censored).

- Objective response rate: defined as the proportion of subjects who achieve the best response (CR and PR) determined by irRECIST.
- Safety and tolerability of the combination of Sylatron and pembrolizumab as assessed by CTCAE v4. Will include patients' toxicity profile, adverse event, serious adverse event, serious adverse even leading to discontinuation of the treatment, death.

12.2.3 Definition of Exploratory Endpoints

- The density of CD3 and CD8 in the tumor interior and invasive margin of the tumor, and the clonality of the T-cell repertoire.
- Proliferation of CD8+ T cells, Granzyme B+ cells and proliferation of tumor cells.

12.3 Sample Size and Accrual

The primary objective of the study is to test that addition of Sylatron will improved the response rate of pembrolizumab from 17% to 35% in patients with advanced cholangiocarcinoma. The sample size is based on the number of patients that are anticipated to complete the primary objective.

We propose that the addition of Sylatron to a pembrolizumab regimen will improve the response rate of pembrolizumab from 17% to 35%. Simon's two-stage optimum design will be used to test the null hypothesis of P <= 0.17 versus the alternative hypothesis of P >= 0.350. At the first stage, 16 patients will be treated with the combination of Sylatron and pembrolizumab. The trial will be terminated if 3 or fewer respond. If there are at least 4 responders, the trial will go on to the second stage and 28 patients will be enrolled to a total of 44 patients. If the total number responding among the 44 patients is less than or equal to 11, the null hypothesis will not be rejected and the combination of Sylatron plus pembrolizumab will not be investigated further. Using this statistical method, there is a 0.046 (4.6%) probability of erroneously concluding that this drug combination is effective when it actually is not (targeted value of 0.05). If the drug is actually effective, there is a 0.191 (19.1%) probability of concluding that it is not (targeted value of 0.2). The power of the design is 80% with a one-sided significance level of 0.05.

We anticipate that 2 patients will be recruited per month and total time for study recruitment will be 24 months, with each patient followed for a minimum of 6 months, the anticipated time to complete the study will be 24 – 30 months. Allowing for a 4% drop out rate, a total of 46 patients will be enrolled for this trial.

12.4 Analysis Datasets

Population	Definition
Enrolled	This will comprise all subjects who meet the eligibility criteria and are registered onto the study.

Population	Definition
Evaluable for Efficacy	This will comprise all subjects who receive at least one dose of sylatron and pembrolizumab and either undergo at least one post-baseline assessment or die before any evaluation. Non evaluable patients will be replaced.
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.
Per Protocol Set (Valid Cases, Efficacy Sample, Evaluable Subjects Sample)	This will comprise all subjects who complied with the protocol sufficiently to ensure that these data would be likely to exhibit the effects of treatment, according to the underlying scientific model. Compliance covers such considerations as exposure to treatment, availability of measurements and absence of major protocol violations. This population should be specifically defined in the protocol.
Safety	This will comprise all subjects who receive at least one dose of sylatron and pembrolizumab
Treated	This will comprise all subjects who have been exposed to the planned course of treatment to any extent.

12.4.1 Analysis Plans for Primary Objective

The overall response rate will be estimated with its 95% exact confidence interval.

12.4.2 Analysis Plans for Secondary Objectives

Progression free survival (PFS) and overall survival (OS) will be evaluated using the methods of Kaplan and Meier (1958). 95% confidence interval will be constructed for the estimated median PFS and OS. The exploratory objective response rate will be estimated with its 95% exact confidence interval. Safety summaries will be presented by the severity of the adverse event and by relationship to study drug. Descriptive statistics (minimum, maximum, mean median, standard deviation for continuous variables; counts, percentages for categorical variables) will be used in summarizing of all laboratory and safety parameters (adverse events, serious adverse events, adverse events leading to discontinuation, deaths) for all treated subjects.

12.4.3 Analysis Plans for Exploratory Objectives

Density of CD3+ T cells and CD8+ T cells, proliferation of CD8+ T cells, Granzyme B+ cells and proliferation of tumor cells will be listed and summarized using the descriptive statistics (minimum, maximum, mean median, standard deviation). The CD8 + Tcells, Granzyme B+

cells, and proliferation of tumor cells will be correlated with the response by comparing the means of different groups using either 2-sample t-tests or non-parametric Wilcoxon rank-sum test, or the Chi-square test based on the distribution and structure of the data.

13 TRIAL MANAGEMENT

13.1 Data and Safety Monitoring Plan (DSMP)

The study will be conducted with guidance from the Georgetown Lombardi Comprehensive Cancer Center Cancer Center's DSMP.

HCRN oversight activities include:

- Review all adverse events requiring expedited reporting as defined in the protocol
- Provide trial accrual progress, safety information and data summary reports to the sponsor-investigator
- Submit data summary reports to the lead institution Data Safety Monitoring Committee according to Georgetown Lombardi Comprehensive Cancer Center DSMP.

13.2 Georgetown Lombardi Comprehensive Cancer Center Data Safety Monitoring Committee

HCRN will provide the following for the Georgetown Lombardi Comprehensive Cancer Center DSMC to review:

- Adverse event summary report
- Audit results if applicable
- Data related to stopping/decision rules described in study design
- Study accrual patterns
- Protocol deviations

The Georgetown Lombardi Comprehensive Cancer Center DSMC will review study data every quarter. Documentation of DSMC reviews will be provided to sponsor-investigator and HCRN. Issues of immediate concern by the DSMC will be brought to the attention of the sponsor-investigator and other regulatory bodies as appropriate. The sponsor-investigator will work with HCRN to address the DSMC's concerns.

13.3 Data Quality Oversight Activities

Remote validation of the EDC system data will be completed on a continual basis throughout the life cycle of the study. Automated edit check listings will be used to generate queries in the EDC system and transmitted to the site to address in a timely fashion. Corrections will be made by the study site personnel.

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

13.3.1 Onsite Monitoring

There will be at least one routine visit per site per year for sites that have accrued. Additional for cause visits may occur as necessary. Selected source documents will be reviewed for verification of agreement with data entered into the EDC system. It is important for the site investigator and

their relevant personnel to be available for a sufficient amount of time during the monitoring visits or audit, if applicable. The site investigator and institution guarantee access to source documents by HCRN or its designee.

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

13.4 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the sponsor-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. All results of primary and secondary objectives must be posted to CT.gov within a year of completion. The sponsor-investigator has delegated responsibility to HCRN for registering the trial and posting the results on 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 study site contact information.

14 DATA HANDLING AND RECORD KEEPING

14.1 Data Management

HCRN will serve as the Clinical Research Organization for this trial. Data will be collected through a web based clinical research platform, the EDC system, a system compliant with Good Clinical Practices and Federal Rules and Regulations. HCRN personnel will coordinate and manage data for quality control assurance and integrity. All data will be collected and entered into the EDC system by study site personnel from participating institutions.

14.2 Case Report Forms and Submission

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(s). 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.

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

14.3 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 file and retained by the site investigator in compliance with the site contract with HCRN. No records will be destroyed until HCRN confirms destruction is permitted.

14.4 Confidentiality

There is a slight risk of loss of confidentiality of subject information. All records identifying the subjects 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 site personnel.

Subjects will be informed in writing that some organizations including the sponsor-investigator and his/her research associates, HCRN, Merck, 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.

15 ETHICS

15.1 Institutional Review Board (IRB) Approval

The final study protocol and the final version of the informed consent form must be approved in writing by an IRB. The site investigator must submit written approval by the IRB to HCRN before he or she can enroll subjects 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, as local regulations require.

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

15.2 Ethical Conduct of the Study

The study will be performed in accordance with ethical principles originating from the Declaration of Helsinki. Conduct of the study will be in compliance with ICH Good Clinical Practice, and with all applicable federal (including 21 CFR parts 56 & 50), state, or local laws.

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 informed consent form. A copy of the signed informed consent form must be given to the subject.

16 REFERENCES

1. Topalian SL, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. New England Journal of Medicine. 2012; 366:2443–2454. [PubMed: 22658127]
2. Brahmer JR, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. New England Journal of Medicine. 2012; 366:2455–2465. [PubMed: 22658128]
3. Hamid O, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. New England Journal of Medicine. 2013; 369:134–144.10.1056/NEJMoa1305133 [PubMed: 23724846]
4. Wolchok JD, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med. 2013; 369:122–133.10.1056/NEJMoa1302369 [PubMed: 23724867]
5. Topalian SL, et al. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. J Clin Oncol. 2014; 32:1020–1030.10.1200/JCO.2013.53.0105 [PubMed: 24590637]
6. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nature Reviews Cancer. 2012; 12:252–264.
7. Spranger S, et al. Up-regulation of PD-L1, IDO, and T(regs) in the melanoma tumor microenvironment is driven by CD8(+) T cells. Sci Transl Med. 2013; 5:200ra116.10.1126/scitranslmed.3006504
8. PC Tumeh PC, et al.PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014 November 27; 515(7528): 568–571. doi:10.1038/nature13954.
9. Shaib YH, Davila JA, McGlynn K, El-Serag HB. Rising incidence of intrahepatic cholangiocarcinoma in the United States: a true increase? J Hepatol. 2004; 40:472–477.
10. Weber SM, Jarnagin WR, Klimstra D, DeMatteo RP, Fong Y, Blumgart LH. Intrahepatic cholangiocarcinoma: resectability, recurrence pattern, and outcomes. J Am Coll Surg. 2001; 193:384–391.
11. Endo I, Gonen M, Yopp AC, Dalal KM, Zhou Q, Klimstra D, D'Angelica M, et al. Intrahepatic cholangiocarcinoma: rising frequency, improved survival, and determinants of outcome after resection. Ann Surg. 2008; 248:84–96.
12. Konstadoulakis MM, Roayaie S, Gomatos IP, Labow D, Fiel MI, Miller CM, Schwartz ME. Fifteenyear, single-center experience with the surgical management of intrahepatic cholangiocarcinoma: operative results and long-term outcome. Surgery. 2008; 143:366–374.
13. Ercolani G, Vetrone G, Grazi GL, Aramaki O, Cescon M, Ravaioli M, Serra C, et al. Intrahepatic cholangiocarcinoma: primary liver resection and aggressive multimodal treatment of recurrence significantly prolong survival. Ann Surg. 252:107–114.
14. DeOliveira ML, Cunningham SC, Cameron JL, Kamangar F, Winter JM, Lillemoe KD, Choti MA, et al. Cholangiocarcinoma: thirty-one-year experience with 564 patients at a single institution. Ann Surg. 2007; 245:755–762.
15. Valle J, Wasan H, Palmer DH, Cunningham D, Anthoney A, Maraveyas A, et al. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. New Engl J Med. 2010; 362:1273–81.

16. Oh SY, Jeong CY, Hong SC, Kim TH, Ha CY, Kim HJ, Lee GW, Hwang IG, Jang JS, Kwon HC, Kang JH. Phase II study of second line gemcitabine single chemotherapy for biliary tract cancer patients with 5-fluorouracil refractoriness. Invest New Drugs. 2011 Oct;29(5):1066-72. doi: 10.1007/s10637-010-9417-3. Epub 2010 Apr 1.
18. Leal JL, Roa JC, Jarufe N, Madrid J, Ibanez C, Herrera ME, Garrido M, Nervi B.Second-line FOLFOX chemotherapy in patients with metastatic gallbladder cancer and cholangiocarcinoma. J Clin Oncol 32, 2014 (suppl 3; abstr 322).
19. Goeppert B, Frauenschuh L, Zucknick M, Stenzinger A, Andrulis M, Klauschen F, Joehrens K, Warth A, Renner M, Mehrabi A, Hafezi M, Thelen A, Schirmacher P and Weichert W. Prognostic impact of tumour-infiltrating immune cells on biliary tract cancer. British Journal of Cancer (2013) 109, 2665–2674 | doi: 10.1038/bjc.2013.610.
20. Buechner, Stanislaw A. "Intralesional interferon alfa-2b in the treatment of basal cell carcinoma: immunohistochemical study on cellular immune reaction leading to tumor regression." Journal of the American Academy of Dermatology 24.5 (1991): 731-734.
21. Håkansson A, Gustafsson B, Krysander L, Håkansson L. Tumor-infiltrating lymphocytes in metastatic malignant melanoma and response to interferon alpha treatment. Br J Cancer. 1996 Sep;74(5):670-6.
22. Dunn GP, Bruce AT, Ikeda H, et al: Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 3:991-8, 2002.
23. Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, Wolchok JD, Hersey P, Joseph RW, Weber JS, Dronca R, Gangadhar TC, Patnaik A, Zarour H, Joshua AM, Gergich K, Elassaiss-Schaap J, Algazi A, Mateus C, Boasberg P, Tumeh PC, Chmielowski B, Ebbinghaus SW, Li XN, Kang SP, Ribas A.Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. N Engl J Med. 2013 Jul 11;369(2):134-44. doi: 10.1056/NEJMoa1305133. Epub 2013 Jun 2.
24. Ye Y, Zhou L, Xie X, Jiang G, Xie H, Zheng S. Interaction of B7-H1 on intrahepatic cholangiocarcinoma cells with PD-1 on tumor-infiltrating T cells as a mechanism of immune evasion. J Surg Oncol. 2009 Nov 1;100(6):500-4. doi: 10.1002/jso.21376.
25. Patt YZ, Jones DV, Jr., Hoque A, Lozano R, Markowitz A, Raijman I, et al. Phase II trial of intravenous flourouracil and subcutaneous interferon alfa-2b for biliary tract cancer. J ClinOncol. 1996;14:2311–5.
26. Bald T, Landsberg J, Lopez-Ramos D, Renn M1, Glodde N, Jansen P, Gaffal E, Steitz J, Tolba R, Kalinke U, Limmer A, Jönsson G, Hölzel M1, Tüting T. Immune cell-poor melanomas benefit from PD-1 blockade after targeted type I IFN activation. Cancer Discov. 2014 Jun;4(6):674-87. doi: 10.1158/2159-8290.CD-13-0458. Epub 2014 Mar 3.
27. Hassane M. Zarour, Hussein Tawbi, Ahmad A. Tarhini, Hong Wang, Cindy Sander, Amy Rose, Julien J. Fourcade, Joe-Marc Chauvin, Zhaojun Sun, Ornella Pagliano and John M. Kirkwood. Study of anti-PD-1 antibody pembrolizumab and pegylated-interferon alfa-2b (Peg-IFN) for advanced melanoma. Journal of Clinical Oncology, 2015 ASCO Annual Meeting (May 29 - June 2, 2015), Vol 33, No 15_suppl (May 20 Supplement), 2015: e20018

28. Yaman Suleiman, Domenico Coppola, Sherma Zibadi, Samir Dalia, TzuHua Juan, Jae K Lee, Mokenge Peter Malafa, Hatem Hussein Soliman, and Richard D. Kim. Prognostic value of tumor-infiltrating lymphocytes (TILs) and expression of PD-L1 in cholangiocarcinoma. J Clin Oncol 33, 2015 (suppl 3; abstr 294)
29. Robins HS, et al. Comprehensive assessment of T-cell receptor beta-chain diversity in alphabeta T cells. Blood. 2009; 114:4099–4107.10.1182/blood-2009-04-217604 [PubMed: 19706884]
30. Carlson CS, et al. Using synthetic templates to design an unbiased multiplex PCR assay. Nature communications. 2013; 4:2680.10.1038/ncomms3680