MMP-01: Pilot Study Evaluating Activity of the Combination of Anti-PD-1 Antibody with High Dose IL-2 in Metastatic Melanoma

Sponsor Investigator
Ralph Hauke, MD
Nebraska Cancer Specialists
Midwest Cancer Center-Legacy
17201 Wright Street, Suite 200
Omaha, NE 68130
Tel: 402-334-4773
Fax 402-330-7463

Co-Investigator
Arkadiusz Z. Dudek, MD, PhD
HealthPartners Regions Cancer Care and Frauenshuh Cancer Care Centers
Suite 1161 Mail Stop 11101G
640 Jackson Street, St. Paul, MN 55101
Tel: 651 254-3321
Fax: 651 254-3102

Statistician
Menggang Yu PhD

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

Trial Supported by
Prometheus Laboratories

IND exempt per FDA: 12/12/17

Initial Protocol Version Date: 28FEB2018
Protocol Amendment Version Date:

PROTOCOL SIGNATURE PAGE

MMP-01: Pilot Study Evaluating Activity of the Combination of Anti-PD-1 Antibody with High Dose IL-2 in Metastatic Melanoma

VERSION DATE: 28FEB2018

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	MMP-01: Pilot Study Evaluating Activity of the Combination of anti-PD-1 Antibody with High Dose IL-2 in Metastatic Melanoma
SHORT TITLE	Anti-PD-1 Antibody with High Dose IL-2 in Metastatic Melanoma
PHASE	Phase II Pilot
OBJECTIVES	**Primary Objective** Assess the response rate [complete response (CR) + partial response (PR)] of sequential therapy of pembrolizumab followed by high dose IL-2 (HD IL-2) in subjects with stage IV malignant melanoma. Response assessment will be performed using revised RECIST 1.1. **Secondary Objectives** • Characterize safety, tolerability and adverse effects (AE) profile of pembrolizumab followed by HD IL-2 in subjects with stage IV malignant melanoma. • Measure Progression-Free Survival (PFS) using revised RECIST 1.1 after completion of 2 cycles of pembrolizumab and 2 cycles of HD IL-2 in all the subjects enrolled in the study. • Measure overall survival (OS) at 12 months in subjects with stage IV malignant melanoma who showed a response [stable disease (SD), complete response (CR) or partial response (PR)] following completion of 2 cycles of pembrolizumab and 2 cycles of HD IL-2. **Exploratory Objectives** • Correlate PD-L1 expression in archived diagnostic tumor tissue with best clinical response (revised RECIST criteria) and treatment outcome in subjects with stage IV malignant melanoma. • Correlate Th1/Th2 ratio and myeloid-derived suppressor cells in peripheral blood during therapy with best clinical response (revised RECIST criteria) and treatment outcome in subjects with stage IV malignant melanoma. Data will be collected prior to treatment, during treatment after the 2nd dose of pembrolizumab and after each cycle of HD IL-2. • Correlate the change in soluble PD-L1 and arginine levels in serum/plasma during therapy with best clinical response (revised RECIST criteria) and treatment outcome in subjects with stage IV malignant melanoma. Data will be collected prior to treatment, during treatment after the 2nd dose of pembrolizumab and after each cycle of HD IL-2. • Correlate intestinal microbiome at baseline with best clinical response (revised RECIST criteria) and treatment outcome in subjects with stage IV malignant melanoma.

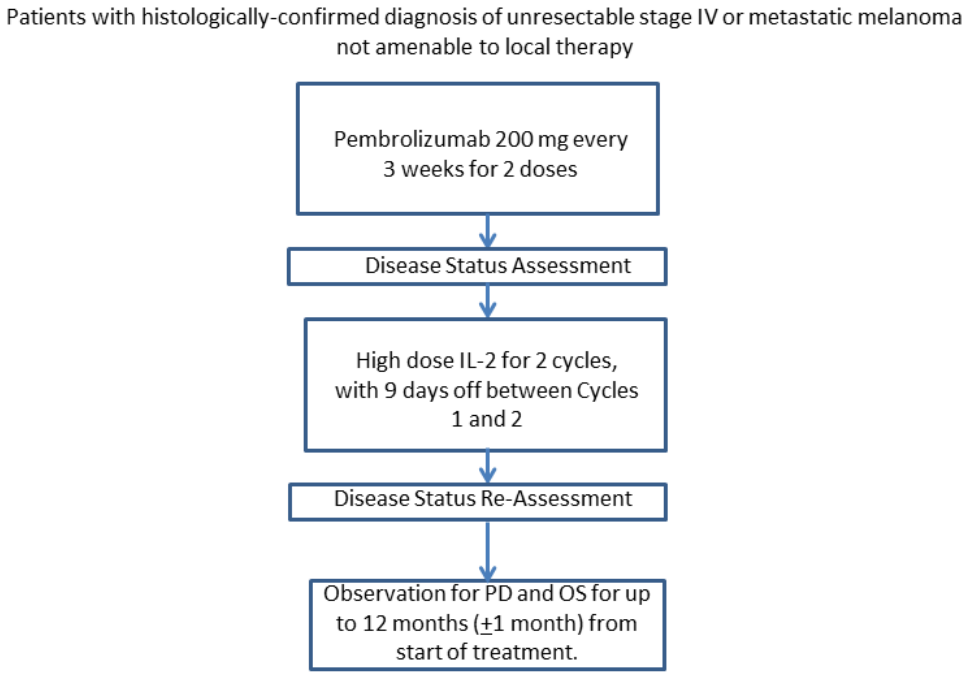
STUDY DESIGN	Single arm 2-stage Phase II Simon design
KEY ELIGIBILITY CRITERIA (See Section 3 for full eligibility criteria)	1. Age ≥ 18 years at the time of consent. 2. Histologically-confirmed diagnosis of unresectable stage IV or metastatic melanoma not amenable to local therapy. 3. Measurable disease, defined as at least 1 tumor that fulfills the criteria for a target lesion according to RECIST 1.1 (Section 9), and obtained by imaging within 28 days prior to registration for protocol therapy. 4. Has an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 within 28 days prior to registration for protocol therapy. 5. Life expectancy of 6 months or greater as determined by the treating physician. 6. Adequate hepatic function within 28 days prior to registration for protocol therapy defined as meeting all of the following criteria: i. total bilirubin ≤ 1.5 mg/dl × upper limit of normal (ULN) **OR** direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 mg/dl x ULN (except in patients with Gilbert's syndrome who must have a total bilirubin less than 3.0 mg/dl.) ii. and aspartate aminotransferase (AST) ≤ 2.5 × ULN or ≤ 5 × ULN for subjects with known hepatic metastases iii. and alanine aminotransferase (ALT) ≤ 2.5 × ULN or ≤ 5 × ULN for subjects with known hepatic metastases 7. Adequate renal function within 28 days prior to registration for protocol therapy defined by either of the following criteria: i. Serum creatinine ≤ 1.5 mg/dL ii. **OR** if serum creatinine > 1.5 mg/dL, estimated glomerular filtration rate (GFR) ≥ 40 mL/min 8. Adequate hematologic function within 28 days prior to registration for protocol therapy defined as meeting all of the following criteria: i. hemoglobin ≥ 9.0 g/dL ii. and absolute neutrophil count (ANC) ≥ 1000/L without the support of filgrastim iii. white blood cells (WBC) ≥ 3000/L iv. and platelet count ≥ 100 × 10^9/L 9. Adequate coagulation functioning within 28 days prior to registration for protocol therapy defined by either of the following criteria: i. INR < 1.5 × ULN ii. **OR** for subjects receiving warfarin or LMWH, the subjects must, in the investigator's opinion, be clinically stable with no evidence of active bleeding while receiving anticoagulant therapy. The INR for these subjects may exceed 1.5 × ULN if that is the goal of anticoagulant therapy.

	10. Adequate baseline pulmonary function test (FEV1 > 2 L or ≥ 75% of predicted for height and age). 11. Because the teratogenicity of pembrolizumab and IL-2 is not known, • Female subject of childbearing potential (WOCP) must not be pregnant confirmed by a negative urine or serum pregnancy test within 72 hours of the study registration. If the urine test cannot be confirmed as negative, a serum pregnancy test will be required. 12. < 2 lines of prior therapy for metastatic melanoma. Cannot have received prior therapy with HD IL-2. May have had one prior line of therapy that included a check point inhibitor. 13. Not received radiation therapy within 21 days of initiation of study treatment, and the measurable disease must have been outside of the radiation port. 14. More than 3 weeks must have elapsed since any prior systemic therapy at the time the subject receives the first dose of pembrolizumab. Subject's toxicities must have recovered to a grade 1 or less (except for toxicities such as alopecia or vitiligo) 15. No second active malignancy. 16. No autoimmune diseases (see full eligibility criteria) 17. Willingness and ability to comply with scheduled visits, treatment plans, laboratory tests, and other study procedures. 18. Provide written informed consent and HIPAA authorization for release of personal health information, approved by an Institutional Review Board/Independent Ethics Committee (IRB/IEC). HIPAA authorization may be included in the informed consent or obtained separately.
STATISTICAL CONSIDERATIONS	The optimal two-stage design to test the null hypothesis that P ≤ 0.260 versus the alternative that P ≥ 0.500 with 80% power and 0.05 level of significance proceeds as follows. After testing the drug on 11 patients in the first stage, the trial will be terminated if 3 or fewer respond. If the trial goes on to the second stage, a total of 29 patients will be studied. If the total number responding is less than or equal to 11, the drug is rejected. The probability of early termination at stage 1 with a total sample size of 11 is 0.685; the probability of going on to stage 2 with a total sample size of 29 is 0.315. The sample size calculation is conducted using the software PASS 13 (NCSS, Kaysville, Utah 2014)
TOTAL NUMBER OF SUBJECTS	N = Up to 29 subjects

ESTIMATED ENROLLMENT PERIOD	Estimated 36 months
ESTIMATED STUDY DURATION	Estimated 40 months

TABLE OF CONTENTS

SCHEMA


Patients with histologically-confirmed diagnosis of unresectable stage IV or metastatic melanoma not amenable to local therapy
Pembrolizumab 200 mg every 3 weeks for 2 doses
Disease Status Assessment
High dose IL-2 for 2 cycles, with 9 days off between Cycles 1 and 2
Disease Status Re-Assessment
Observation for PD and OS for up to 12 months (±1 month) from start of treatment.

1. BACKGROUND AND RATIONALE

1.1 Disease Background

Metastatic melanoma continues to be a challenging disease to treat, with an estimated 9,940 expected deaths in the United States in 2015 [1]. The 5 year survival rate ranges between 5-19% [2] and the 10 year survival is less than 10% [3]. Patients with Stage IV melanoma continue to have a poor prognosis, with a mean survival of 8–10 months in large cohort analysis studies [4]. The National Comprehensive Cancer Network (NCCN) currently recommends the following therapies for treatment of advanced melanoma: 1) immunotherapy with anti-programmed cell death protein 1 (PD-1) monotherapy (Pembrolizumab or Nivolumab (NCCN category 1, which means it is based on high-level evidence, leading to NCCN consensus that the intervention is appropriate), 2) combination immunotherapy with Nivolumab/Ipilimumab, 3) targeted therapy if B-Raf proto-oncogene, serine/threonine kinase (BRAF)- mutated, 4) Dabrafenib/Trametinib (category 1 and preferred by NCCN), 5) Vemurafenib (category 1), 6) Dabrafenib (category 1) or 7) enrollment in a clinical trial. Although no longer recommended as a single agent for metastatic melanoma, Ipilimumab has been associated with long term survival in approximately 20% of patients [5]. The current recommended regimen of ipilimumab/nivolumab, has not provided mature data yet on overall survival (OS). Upon disease progression, a selected number of patients with Eastern Cooperative Oncology Group ECOG performance status (PS) 0-2 are offered the same agents (provided they did not have them before). In this setting (progression after initial treatment) High Dose interleukin-2 (HD IL-2) is one of the therapies recommended by NCCN. Despite these recent advances in increased options for treatment of advanced melanoma the prognosis remains poor for the majority of patients, and improved strategies to increase the number of patients benefited with longer and sustained responses are needed.

We propose to test two drugs in combination in order to determine if response rates can be improved over either agent as single therapy. The drugs which will be testing in combination are pembrolizumab and HD-IL-2. The next two sections will briefly describe the two drugs (their mechanism of action and some seminal clinical trials studies), and then we will conclude with providing the rationale for 1) combining the two drugs to test in this pilot study, and 2) the trial design and treatment sequencing we plan to use.

1.2 Pembrolizumab: Immune Checkpoint Inhibitor

Interrupting the PD-1/programmed death-ligand 1 (PD-L1) interaction has emerged as an important weapon in immunotherapy [6]. Anti-PD-1 antibodies have been shown to induce positive radiological responses and improved survival rates in patients with melanoma. Sustained remissions remain low with average PFS still less than 6 months [7].

Pembrolizumab (formerly known as MK-3475) is a highly selective, humanized monoclonal IgG4–kappa isotype antibody against PD-1 that can disrupt the engagement of PD-1 with its ligands and impede inhibitory signals in T Cells, with resultant tumor recognition by cytotoxic T cells. The anti-tumor activity, side effects and safety of pembrolizumab in patients with melanoma was initially studied as part of the large, international, phase 1 KEYNOTE-001 trial.

In a pooled analysis of 411 patients with advanced melanoma enrolled in KEYNOTE-001, the response rate (RR) was 34% and was maintained in 81% of those patients, the median overall survival (OS) was 25.9 months [8] after a median follow-up duration of 18 months. The KEYNOTE-002 study of pembrolizumab versus chemotherapy confirmed the benefit of pembrolizumab in patients who had disease progression during or after ipilimumab therapy [9].

In this open-label, international expansion cohort of a phase 1 trial, patients (aged >= 18 years) with advanced melanoma whose disease had progressed after at least two ipilimumab doses were randomly assigned to intravenous pembrolizumab at 2 mg/kg every 3 weeks or 10 mg/kg every 3 weeks until disease progression, intolerable toxicity, or consent withdrawal. A total of 173 patients received pembrolizumab 2 mg/kg (n=89) or 10 mg/kg (n=84). Median follow-up duration was 8 months. ORR was 26% at both doses (21 of 81 patients in the 2 mg/kg group and 20 of 76 in the 10 mg/kg group) (difference 0%, 95% CI -14 to 13; p=0.96). Treatment was well tolerated, with similar safety profiles in both groups and no drug-related deaths. The most common drug-related adverse events of any grade in the 2 mg/kg and 10 mg/kg groups were fatigue (29 [33%] vs 31 [37%]), pruritus (23 [26%] vs 16 [19%]), and rash (16 [18%] vs 15 [18%]). Grade 3 fatigue, reported in five (3%) patients in the 2 mg/kg pembrolizumab group, was the only drug-related grade 3 to 4 adverse event reported in more than one patient [8].

Based on these preliminary trials, the landmark KEYNOTE 006 trial [10] was launched and ultimately lead to the FDA approval of pembrolizumab for ipilimumab refractory melanoma. In this randomized controlled, phase 3 study, 834 patients with advanced melanoma in a 1:1:1 ratio were assigned to receive pembrolizumab (at a dose of 10 mg per kilogram of body weight) every 2 weeks or every 3 weeks or four doses of ipilimumab (at 3 mg per kilogram) every 3 weeks. Primary end points were progression-free and overall survival. The estimated 6-month progression-free-survival (PFS) rates were 47.3% for pembrolizumab every 2 weeks, 46.4% for pembrolizumab every 3 weeks, and 26.5% for ipilimumab (hazard ratio [HR] for disease progression, 0.58; P<0.001 for both pembrolizumab regimens versus ipilimumab; 95% confidence intervals [CIs], 0.46 to 0.72 and 0.47 to 0.72, respectively). Estimated 12-month survival rates were 74.1%, 68.4%, and 58.2%, respectively (hazard ratio [HR] for death for pembrolizumab every 2 weeks, 0.63; 95% CI, 0.47 to 0.83; P=0.0005; HR for pembrolizumab every 3 weeks, 0.69; 95% CI, 0.52 to 0.90; P=0.0036). The RR was improved with pembrolizumab administered every 2 weeks (33.7%) and every 3 weeks (32.9%), as compared with ipilimumab (11.9%) (P<0.001 for both comparisons). Responses were ongoing in 89.4%, 96.7%, and 87.9% of patients, respectively, after a median follow-up of 7.9 months. Efficacy was similar in the two pembrolizumab groups. Rates of treatment-related adverse events of grade 3 to 5 severity were lower in the pembrolizumab groups (13.3% and 10.1%) than in the ipilimumab group (19.9%), and the specific adverse events were similar to that observed in the KEYNOTE-002 trial.

1.3 High Dose Interleukin-2 (HD IL-2)

HD IL-2 has been approved since 1998 for the treatment of stage IV malignant melanoma. Interleukin-2 is mostly produced by antigen-stimulated CD4 + T helper cells, and to a lesser extent by CD8 + T cells, natural killer (NK) cells, natural killer T (NKT) cells, and activated Dendritic Cells [11]. IL-2 induces the proliferation of NK cells augmenting their cytolytic capacity, drives proliferation and activation of CD8 + T cells and promotes the proliferation of B

cells and antibody secretion [12]. IL-2 was originally seen as the key factor of augmenting an effector lymphocyte immune response. However, it also serves as a potent immune regulator by expanding immunosuppressive CD4+ FOXP3+ T-regulatory cells (Treg) [13] as well as promoting activation-induced cell death (AICD) of overactivated T cells [14]. Hence, rather than simply enhancing cytotoxic effects it acts as an immune modulator with different activities depending on the target cell and tumor microenvironment.

Studies with high-dose bolus (HDB) IL-2 used doses of 600,000–720,000 U/kg every 8 hours from days 1–5 (cycle 1) and 15–19 (cycle 2) with a maximum of 14 doses per cycle or 28 doses per course (one course = two cycles). IL-2 was also administered either as a single agent or in combination with immunologically active cells: lymphokine-activated killer (LAK) cells and tumor-infiltrating lymphocytes (TILs). A meta-analysis [15,16] of all seven National Cancer Institute-sponsored trials and the one Chiron Corporation sponsored trial conducted between 1985 and 1993 (270 patients) on metastatic melanoma patients revealed an overall objective response of 16 % (95% confidence interval, 12% to 21%); there were 17 complete responses (CRs) (6%) and 26 partial responses (PRs) (10%). Responses occurred with all sites of disease and in patient with large tumor burdens. Nineteen of the 43 responding patients (44%) and 11% of all patients were alive with survivals as long as 150 months. Twelve of these responding patients were continuously disease free. The median response duration was 8.9 months (range, 4–106+months). Twenty-eight percent of responding patients, including 59% of those patients who achieved a complete response remained progression-free at a median follow-up of 62 months. No patient with an ongoing response at 30 months has relapsed, with follow-up extending beyond 20 years in some cases, suggesting that such patients are likely cured [15]. However, the major toxicities associated with this regimen, including hypotension, renal insufficiency and hypoxia, have precluded its widespread application. The use of HD IL-2 is limited to specialized programs with experienced personnel, and it is generally offered only to patients with good performance and excellent organ function [17]. However, in contrast with low dose IL-2, HD IL-2 has shown the ability to provide durable responses in a subset of patients. For this reason, HD IL-2 is selected as the dose to use in this study.

1.4 Study Rationale

One of the several identified difficulties with not obtaining better outcomes to checkpoint blockade is T-cell exhaustion. T cell exhaustion describes a state of T cell dysfunction that was initially observed during chronic lymphocytic choriomeningitis virus (LCMV) infection in mice [18]. Exhausted T cells fail to proliferate and exert effector functions such as cytotoxicity and cytokine secretion in response to antigen stimulation. Further studies identified that exhausted T cells are characterized by sustained expression of the inhibitory molecule PD-1 (programmed cell death 1) and that blockade of PD-1 and PD-L1 interactions can reverse T cell exhaustion and restore antigen-specific T cell responses in LCMV-infected mice [19]. Several lines of evidence also implicate the PD-1–PD-L pathway in T cell exhaustion in cancer [20]. However, targeting the PD-1/PD-L1 pathway does not always result in reversal of T cell exhaustion [21]. IL-2 has the potential to augment memory T-cells [22] in addition to other possible beneficial effects such as increasing NK cells [23]. Administration of IL-2 in combination with anti-PD-L1 blockade in mouse models has been shown to be synergistic in greatly increasing antigen-specific CD8 T cell numbers and increasing their function [24]. For these reasons we are using both pembrolizumab

and IL-2 in the present trial to determine if RR (CR+PR) can be enhanced over either agent alone.

1.5 Rationale for Trial Design and Treatment Sequencing

Despite advances in our understanding of immunology, we lack data on specific responses of cellular subtypes to these different agents and perhaps more pertinent, the interactions of the various immunocellular components. The sequencing of the drugs emerges as an important potential determinant. An ongoing study of ipilimumab with HD IL-2 is exploring sequencing of the agents as well [25] and there is an additional ongoing clinical trial using pembrolizumab, lymphodepletion and TIL effectors in combination with high or low dose IL-2 [26].

Given the limited number of centers and patients that can undertake HD IL-2 therapy, the most practical design would be a single arm study of anti-PD1 with HD IL-2 to determine if response rates can be improved over either agent as single therapy. Indeed, a recent retrospective study [27] using the PROCLAIM registry [28] the largest collection of IL-2 treated patients in the US, revealed that the median overall survival (mOS) for the 236 patients with metastatic melanoma was 18.4 months with a median follow-up of 21.7 months. Patients were stratified into three groups; HD IL-2 only (n=123), HD IL-2 followed by ipilimumab (IL-2→ipi, n=78), and HD IL-2 followed by PD-1 inhibitors (IL-2→aPD-1, n=35). The majority of patients (22 of 35) in the IL-2→aPD-1 group had progressive disease before receiving subsequent treatment with anti-PD-1/PD-L1-containing regimens. Patients in the HD IL-2 only, IL-2→ipi, and IL-2→aPD-1 groups achieved a median OS of 14, 15.8, and 28.7 months, respectively. The estimated 12-month survival rates were 57%, 64%, and 97%, respectively. There were 10/78 (13%) and 2/35 (5.7%) post therapy treatment-related incidences of autoimmune events in the IL-2→ipi and IL-2→aPD-1 groups, respectively. This study was the first report of clinical data relating to HD IL-2 use followed by checkpoint blockade of the PD-1 pathway. Treatment with anti-PD-1 after initial therapy with HD IL-2 had significantly prolonged survival compared to patients treated with ipilimumab (28.7 vs 15.8 months). Moreover, improved survival was not observed in patients treated with follow-on ipilimumab compared to patients treated only with HD IL-2. Anti-PD-1 therapy after HD IL-2 appears to be safe and therapeutically active in this retrospective study [27]. Given that anti PD-1 treatment can result in activation of T-cell cytotoxic effectors by partially reversing T-cell exhaustion, it seems likely that the amplitude of this cytotoxic response can be greatly augmented by adding HD IL-2 resulting in increased tumor destruction and in the generation of a durable T-Cell Memory effector population which may result in a sustained and prolonged remission.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Objectives

2.1.1 Primary Objective

Assess the response rate [complete response (CR) + partial response (PR)] of sequential therapy of pembrolizumab followed by HD IL-2 in subjects with stage IV malignant melanoma. Response assessment will be performed using revised RECIST 1.1 [29].

2.1.2 Secondary Objectives

- Characterize safety, tolerability and adverse effect (AE) profile of pembrolizumab followed by HD IL-2 in subjects with stage IV malignant melanoma.
- Measure Progression-Free Survival (PFS) using revised RECIST guideline (version 1.1) after completion of 2 cycles of pembrolizumab and 2 cycles of HD IL-2 in all the subjects enrolled in the study.
- Measure overall survival (OS) at 12 months in subjects with stage IV malignant melanoma who showed a response [stable disease (SD), complete response (CR) or partial response (PR)] following completion of 2 cycles of pembrolizumab and 2 cycles of HD IL-2.

2.1.3 Correlative/Exploratory Objectives

- Correlate PD-L1 expression in archived diagnostic tumor tissue with best clinical response (revised RECIST 1.1 criteria) and treatment outcome in subjects with stage IV malignant melanoma.
- Correlate Th1/Th2 ratio and myeloid-derived suppressor cells (MDSCs) in peripheral blood during therapy with best clinical response (revised RECIST criteria) and treatment outcome in subjects with stage IV malignant melanoma. Data will be collected prior to treatment, during treatment after the 2nd dose of pembrolizumab and after each cycle of HD IL-2.
- Correlate the change in soluble PD-L1 and arginine level in plasma during therapy with best clinical response (revised RECIST1.1 criteria) and treatment outcome in subjects with stage IV malignant melanoma. Data will be collected prior to treatment, during treatment after the 2nd dose of pembrolizumab and after each cycle of HD IL-2.
- Correlate intestinal microbiome at baseline with best clinical response (revised RECIST 1.1 criteria) and treatment outcome in subjects with stage IV malignant melanoma.

2.2 Endpoints

2.2.1 Primary Endpoint

Assess the response rate [complete response (CR) + partial response (PR)] of sequential therapy of pembrolizumab followed by HD IL-2 in subjects with stage IV malignant melanoma, using revised RECIST 1.1. Response rate will be computed with associated 95% confidence intervals.

2.2.2 Secondary Endpoints

- Proportion of subjects with each grade of adverse events as defined by CTCAE v4.03 will be computed along with 95% confidence intervals, and reported in a tabular and descriptive manner.
- Median PFS times will be computed, and PFS rate at 1 year +/- 3 months will be calculated with associated 95% confidence intervals based on the Kaplan-Meier method. Median OS times will be computed, and OS rate at 1 year +/- 3 months will be calculated with associated 95% confidence intervals based on the Kaplan-Meier method.

3. ELIGIBILITY CRITERIA

3.1 Inclusion Criteria

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

1. Written informed consent and HIPAA authorization for release of personal health information prior to registration. **NOTE:** HIPAA authorization may be included in the informed consent or obtained separately.

2. Age ≥ 18 years at the time of consent.

3. ECOG Performance Status of 0-1 within 28 days prior to registration (Appendix 1).

4. Life expectancy of 6 months or greater as determined by the site investigator.

5. Histologically-confirmed diagnosis of unresectable stage IV or metastatic melanoma not amenable to local therapy.

6. Measurable disease, defined as at least 1 tumor that fulfills the criteria for a target lesion according to RECIST 1.1 (Section 9), and obtained by imaging within 28 days prior to registration for protocol therapy.

7. Archival tissue (from the primary tumor or metastases) is mandatory if available for correlative studies. If archived tumor tissue is not available, the patient does not need to undergo a biopsy to obtain tissue and is still eligible for study.

8. < 2 lines of prior therapy for metastatic melanoma. Cannot have received prior therapy with HD IL-2. May have had one prior line of therapy that included a check point inhibitor.

9. Prior systemic cancer treatment must be completed at least 21 days prior to first dose of study drug and the subject must have recovered from all reversible acute toxic effects of the regimen (other than alopecia or vitiligo) to Grade ≤ 1 or baseline.

10. Not received radiation therapy within 21 days of initiation of study treatment, and the measurable disease must have been outside of the radiation port.

11. Demonstrate adequate organ function as defined in the table below. All screening labs to be obtained within 28 days prior to registration.

System	Laboratory Value
Hematological	
White blood cell (WBC)	≥ 3,000/L
Absolute Neutrophil Count (ANC)	≥ 1,000/L without the support of filgrastim
Hemoglobin (Hgb)	≥ 9 g/dL
Platelet Count	≥ 100×10^9/L
Renal	
Serum creatinine Calculated creatinine clearance[1]	≤ 1.5 mg/dL ≥ 40 mL/min; for subjects with serum creatinine > 1.5 mg/dL
Hepatic	
Total Bilirubin[2] **OR** Direct Bilirubin	≤ 1.5 × upper limit of normal (ULN) ≤ ULN for subjects with total bilirubin levels > 1.5 x ULN
Aspartate aminotransferase (AST)	≤ 2.5 × ULN or ≤ 5 x ULN for subjects with known hepatic metastasis
Alanine aminotransferase (ALT)	≤ 2.5 × ULN or ≤ 5 x ULN for subjects with known hepatic metastasis
Coagulation	
International Normalized Ratio (INR) or	≤ 1.5 × ULN; For subjects receiving warfarin or LMWH, the subjects must, in the site investigator's opinion, be clinically stable with no evidence of active bleeding while receiving anticoagulant therapy. The INR for these subjects may exceed 1.5 × ULN if that is the goal of anticoagulant therapy.

1: Cockcroft-Gault formula will be used to calculate creatinine clearance (See SPM)
2: Except in patients with Gilbert's syndrome who must have a total bilirubin less than 3.0 mg/dl.

12. Adequate baseline pulmonary function test (PFT) (FEV1 > 2 L or ≥ 75% of predicted for height and age).

13. Documented left ventricular ejection fraction (LVEF) of > 45%, testing is required in patients with:
    - Age ≥ 60 years old
    - Clinically significant atrial and or ventricular arrhythmias including but not limited to: atrial fibrillation, ventricular tachycardia, second or third degree heart block
    - History of coronary revascularization or ischemic symptoms

14. Females of childbearing potential must have a negative serum pregnancy test within 3 days prior to registration. **NOTE:** Females are considered of child bearing potential unless they are surgically sterile (have undergone a hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or they are naturally postmenopausal for at least 12 consecutive months.

15. Females of childbearing potential and males must be willing to abstain from heterosexual activity or to use 2 forms of effective methods of contraception from the time of informed consent until 120 days after treatment discontinuation. The two contraception methods can be comprised of two barrier methods, or a barrier method plus a hormonal method.

16. Male subjects who are not surgically sterile (vasectomy) must agree to use an adequate method of contraception. Male subjects with female sexual partners who are pregnant, possibly pregnant or who could become pregnant during the study must agree to use condoms from the first dose of study drug through 120 days after the last dose of study therapy. Total abstinence for the same study period is an acceptable alternative.

17. As determined by the enrolling physician or protocol designee, ability of the subject to understand and comply with study procedures for the entire length of the study

18. Willingness and ability to comply with scheduled visits, treatment plans, laboratory tests, and other study procedures.

3.2 Exclusion Criteria

Subjects meeting any of the criteria below may not participate in the study:

1. Active infection requiring systemic therapy

2. Pregnant or breastfeeding. **NOTE:** breast milk cannot be stored for future use while the mother is being treated on study.

3. Known additional malignancy that is active and/or progressive requiring treatment; exceptions include basal cell or squamous cell skin cancer, in situ cervical or bladder cancer, or other cancer for which the subject has been disease-free for at least five years.

4. Active central nervous system (CNS) metastases. **NOTE:** if prior metastasis but treated and clinically stable for 1 month after treatment are eligible. Subjects with 3 or fewer brain metastases that are less than 1 cm in diameter and asymptomatic are eligible.

5. Surgery within 4 weeks prior to study treatment except for minor procedures. **NOTE:** Hepatic biliary stent placement is allowed.

6. Uncontrolled or poorly-controlled hypertension (> 160 mmHg systolic or > 100 mmHg diastolic for > 4 weeks) despite standard medical management.

7. Serious or non-healing wound, ulcer, or bone fracture within 28 days prior to initiation of study treatment.

8. Any Grade 3-4 GI bleeding within 3 months prior to initiation of study treatment.

9. History of deep vein thrombosis, pulmonary embolism, or any other significant thromboembolism (venous port or catheter thrombosis or superficial venous thrombosis are not considered "significant") during the 3 months prior to initiation of study treatment.

10. Any arterial thromboembolic events, including but not limited to myocardial infarction, transient ischemic attack, cerebrovascular accident, or unstable angina, within 6 months prior to initiation of study treatment.

11. Gross hemoptysis within 2 months of initiation of study treatment.

12. Has any condition that, in the opinion of the investigator, might jeopardize the safety of the patient or interfere with protocol compliance.

13. Has any mental or medical condition that prevents the patient from giving informed consent or participating in the trial.

14. Known hypersensitivity to pembrolizumab or IL-2 or any of their components.

15. Known history of active tuberculosis.

16. Concurrent systemic steroid therapy with doses above physiologic level.

17. History of autoimmune disease, including but not limited to myasthenia gravis, myositis, autoimmune hepatitis, systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, vascular thrombosis associated with anti-phospholipid syndrome, granulomatosis with polyangiitis, Sjögren's syndrome, Guillain-Barré syndrome, multiple sclerosis, vasculitis, or glomerulonephritis. See Study Procedures Manual (SPM) for a detailed list.
    - Subjects with a history of autoimmune-related hypothyroidism on a stable dose of thyroid replacement hormone may be eligible for this study.
    - Subjects with controlled Type I diabetes mellitus on a stable dose of insulin regimen may be eligible for this study.
    - Subjects with a history of celiac disease may be eligible if controlled with diet.

18. Treatment with any investigational agent within 21 days prior to initiation of study treatment and the subject must have recovered from the acute toxic effects of the regimen.

19. Prior organ transplant including allogeneic transplantation.

3.3 Selection of Subjects

Study entry is open to adults regardless of gender or ethnic background. While there will be every effort to seek out and include women and minorities, the patient population is expected to be no different than that of other advanced metastatic melanoma (cutaneous, unresectable) cancer studies at institutions that have experience in administration of HD IL-2.

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.

Subjects must be registered prior to starting protocol therapy. Subjects must begin therapy **within 7 business days** of registration.

5. TREATMENT PLAN

The primary objective of this single arm phase 2 trial is to assess the response rate [complete response (CR) + partial response (PR)] of sequential therapy of pembrolizumab followed by HD IL-2 in subjects with stage IV malignant melanoma. Response assessment will be performed after pembrolizumab therapy, and response reassessment will be performed after HD IL-2 therapy using revised RECIST 1.1.

5.1 Pembrolizumab Administration

Subjects will receive 200 mg pembrolizumab every 3 weeks for two cycles. Cycle length is 21 days (i.e., 3 weeks). On the last day of Cycle 2 (+ 3 days), disease status will be assessed via imaging. Following the pembrolizumab treatment, HD IL-2 treatment will commence.

Correlative research analyses during pembrolizumab administration include examining the relationship between clinical response at the end of Cycle 2 with PD-L1 expression of the archived diagnostic tumor tissue. Research analyses also include examining the relationship between changes in soluble PD-L1 level and Type 1 T helper cell/Type 2 T helper cell (Th1/Th2) ratios during therapy (assessed after the 2nd dose) relative to baseline and characterization of intestinal microbiome to the best clinical response (RECIST 1.1 criteria) and to treatment outcome.

Drug	Dose	Route	Schedule	Cycle Length
Pembrolizumab	200 mg	Intravenously (IV) over 30 minutes	Day 1 of each cycle	3 weeks (21 days)
[1] A window of - 1 or + 2 days may be applied to all study visits to accommodate observed holidays, inclement weather, scheduling conflicts etc. Date and time of each drug administration should be clearly documented in subject's chart and electronic case report forms (eCRFs).				

5.1.1 Pre-Medication for Pembrolizumab Administration

Premedication will be given at the discretion of the site investigator. For patients who experience a Grade 1 or Grade 2 infusion reaction, acetaminophen 650 mg PO and/or 50 mg IV ranitidine (or equivalent H2 antagonist) may be considered prior to subsequent infusions.

5.1.2 Supportive Care for Pembrolizumab

Subjects should receive full supportive care during the study, including transfusion of blood and blood products, treatment with antibiotics, analgesics, erythropoietin, or bisphosphonates, when appropriate.

Although acetaminophen at doses of ≤ 2 grams/day is permitted, it should be used with caution in subjects with impaired liver function.

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events that are related to pembrolizumab 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 investigator determines the events to be related to pembrolizumab.

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.
- For guidelines on treatment holds, timing for restarting treatment or discontinuing treatment permanently, see Section 6.1.3, Table 2.

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.
- For guidelines on treatment holds, timing for restarting treatment or discontinuing treatment permanently, see Section 6.1.3, Table 2.

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

- 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.
- For guidelines on treatment holds, timing for restarting treatment or discontinuing treatment permanently, see Section 6.1.3, Table 2.

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 liothyroinine, is indicated per standard of care.
- **Grade 3-4** hyperthyroidism
  - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
  - For guidelines on treatment holds, timing for restarting treatment or discontinuing treatment permanently, see Section 6.1.3, Table 2.

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.
  - For guidelines on treatment holds, timing for restarting treatment or discontinuing treatment permanently, see Section 6.1.3, Table 2.

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.
  - For guidelines on treatment holds, timing for restarting treatment or discontinuing treatment permanently, see Section 6.1.3, Table 2.

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.
- See Table 1 in Section 6.1.2, Management of Allergic Reaction/Hypersensitivity, for detailed information on how Grades 1-4 reactions are treated.

5.2 HD IL-2 Administration

After completing the 2 cycles of pembrolizumab, High Dose Interleukin-2 (HD IL-2) will be administered. This will require a hospital stay of at least 5 days for each treatment cycle. Established guidelines will be followed for safely administering HD-IL-2 and managing toxicities from this treatment [31,32]. Two treatment cycles will be administered, Cycle 2 being separated from Cycle 1 by approximately 9 days after completion (assuming subject has recovered from Cycle 1 adequately to proceed with Cycle 2). Four weeks after completion of the 2 cycles (called a course of HD IL-2 therapy), disease status will be monitored via CT of the chest and abdomen/pelvis using revised RECIST guidelines (version v1.1, Section 9 of protocol).

Correlative research analyses in this phase include examining the relationship between changes in soluble PD-L1 level and Type 1 T helper cell/Type 2 T helper cell (Th1/Th2) ratios assessed after each of the two HD IL-2 cycles relative to baseline (i.e., pre-dose on Day 1 of Cycle 1 of pembrolizumab treatment), to the best clinical response (modified RECIST criteria) and to treatment outcome.

Drug	Dose	Route	Schedule	Cycle Length
IL-2	600,000 IU/kg[2]	Intravenously (IV) over 15 minutes	Every 8 hours for up to 14 doses over 5 days	Days 1-5 = Cycle 1 9 days of rest in between Days 15-19 = Cycle 2
[1] A window of - 1 or + 2 days may be applied to all study visits to accommodate observed holidays, inclement weather, scheduling conflicts etc. Date and time of each drug administration should be clearly documented in subject's chart and electronic case report forms (eCRFs). [2]Actual weight will be used unless the patient is 30% over ideal body weight in which case adjusted body weight will be used (Males: IBW = 50 kg + 2.3 kg for each inch over 5 feet. Females: IBW = 45.5 kg + 2.3 kg for each inch over 5 feet); ABW = IBW + 0.4(actual weight - IBW)				

5.2.1 Supportive Care specific to HD IL-2

Subjects should receive appropriate supportive care measures as deemed necessary by the site investigator. Several treatment-related toxicities have been uniquely associated with the administration of HD IL-2. Suggested management are listed in the Study Procedures Manual (SPM).

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

5.3.1 Allowed Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medications will be recorded on the electronic case report form (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 beyond 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs.

Myeloid growth factors to treat subjects with neutropenia according to the American Society of Clinical Oncology (ASCO) Guidelines are permitted. Myeloid growth factors should be avoided (if medically appropriate) in Cycle 1 until subjects have developed a dose-limiting Grade 4 neutropenia.

Antiemetic agents may be administered at the discretion of the site investigator but are not commonly required as a prophylactic agent. All other manifestations of the subject's malignancy should be treated at the discretion of the investigator.

Medications with potential central nervous system (CNS) effects are not prohibited in this study, but it is recommended that their use be minimized to avoid confusion in the interpretation of CNS effects should they occur during the course of treatment with pembrolizumab.

In appropriate settings, such as combinations with agents known to produce frequent thrombocytopenia, restricted uses of anticoagulants should be considered.

All other medical conditions should be treated at the discretion of the site investigator in accordance with local community standards of medical care.

5.3.2 Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the Screening and Pembrolizumab Treatment Phase of this trial:

- Anti-cancer systemic chemotherapy or biological therapy not specified in this protocol
- Immunotherapy not specified in this protocol
- Concomitant use of chemotherapy
- Investigational agents
- Radiation therapy
  - Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed after consultation with study chair.
- 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, chicken pox, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (e.g. Flu-Mist®) are live attenuated vaccines, and are not allowed.

- 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-investigator.

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.

5.4 Contraception

It is not known if pembrolizumab has adverse effects on a fetus *in utero* nor if there are transient adverse effects on the composition of sperm. Non-pregnant, non-breast-feeding women may be enrolled if they are willing to use 2 methods of birth control or are considered highly unlikely to conceive. Highly unlikely to conceive is defined as 1) surgically sterilized, or 2) postmenopausal (a woman who is ≥45 years of age and has not had menses for greater than 1 year will be considered postmenopausal), or 3) not heterosexually active for the duration of the study. The two birth control methods can be either two barrier methods or a barrier method plus a hormonal method to prevent pregnancy. Subjects should start using birth control from time of informed consent throughout the study period and up to 120 days after the last dose of study drug.

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

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. To participate in the study, they must adhere to the contraception requirements described above for the duration of the study and 120 days following the last dose of study drug. If there is any question that a subject will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

Male subjects who are not surgically sterile (vasectomy) must agree to use an adequate method of contraception. Male subjects with female sexual partners who are pregnant, possibly pregnant or who could become pregnant during the study must agree to use condoms from the time of informed consent through 120 days after the last dose of study therapy. Total abstinence for the same study period is an acceptable alternative.

5.5 Use in Pregnancy

If a subject inadvertently becomes pregnant while on study treatment, 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 Hoosier Cancer Research Network (HCRN) **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). HCRN will report this information **within 1 business day** to Prometheus. 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, he must immediately inform the site study personnel and the pregnancy reported and followed as described above.

5.6 Breastfeeding

It is unknown whether pembrolizumab or IL-2 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) v4.03 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 Delays for Pembrolizumab Administration

6.1.1 Start of a New Cycle

A new treatment cycle will only be initiated when all of the following conditions are met:

- ANC $\geq 1{,}500\times 10^9$/L
- platelets $\geq 100 \times 10^9$/L
- Non-hematologic treatment related toxicities have improved to $\leq$ Grade 1 or to the subject's baseline values (except alopecia)

If blood counts (first two bullet points above) are below this threshold, blood work is to be repeated weekly until counts are at an acceptable level. If treatment is unable to restart within 12 weeks of the planned treatment date, the subject will be permanently discontinued from study therapy.

6.1.2 Management of Hypersensitivity Reaction

Table 1: Management of allergic reaction/hypersensitivity based on CTCAE Grade

Description	Action
Grade I Allergic Reaction/Hypersensitivity: (Transient flushing or rash, drug fever < 38°C):	Supervise without further treatment for allergic reaction/hypersensitivity.
Grade 2 Hypersensitivity Reaction: (Rash, flushing, dyspnea, urticaria, drug fever greater than or equal to 38°C) and/or asymptomatic bronchospasm:	Interrupt the infusion. If symptoms abate, attempt re-infusion at a slower rate. If the symptoms recur, discontinue infusion and follow for recurrent allergic reaction/hypersensitivity in the next paragraph.
Recurrent Grade 2 or Grade 3 or 4 Hypersensitivity Reaction:	Stop the infusion. Administer additional doses of H1 and H2 blockers intravenously. Administer IV steroids and consider epinephrine and bronchodilators as clinically indicated.
Grade 3 or 4 Hypersensitivity Reaction:	Will be permanently discontinued from study treatment.

Prior to re-challenge of Grade 2 allergic reaction/HSR and if there is a subsequent cycle, the following prophylactic premedications are recommended prior to the pembrolizumab infusion: give both an H1 and H2 blocker intravenously within 30 to 60 minutes before pembrolizumab infusion. If treatment delay necessitates a period longer than 12 weeks, treatment is stopped and the subject is discontinued from the study.

6.1.3 Dose Modification for Toxicity

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 2 below.

Table 2: Dose Modification and Toxicity Management Guidelines 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		

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		
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 Delays for HD IL-2

6.2.1 Start of New Cycle

A new treatment cycle will only be initiated when all of the following conditions are met:

- ANC ≥ 1,500× 10^9/L
- Platelets ≥ 100 × 10^9/L
- Non-hematologic treatment related toxicities have improved to ≤ Grade 1 or to the subject's baseline values (except alopecia)

If blood counts are below this threshold (first two bullet points), blood work is to be repeated weekly until counts are at an acceptable level. If treatment is unable to restart within 12 weeks of the planned treatment date, the subject will be permanently discontinued from study therapy.

6.2 Protocol Therapy Discontinuation

In addition to discontinuation from therapy related to toxicities as outlined above, a subject will also be discontinued from protocol therapy and followed per protocol under the following circumstances outlined below. The reason for discontinuation of protocol therapy will be documented on the electronic case report form (eCRF)

- Documented disease progression
- Site investigator determines a change of therapy would be in the best interest of the subject
- Subject requests to discontinue protocol therapy, whether due to unacceptable toxicity or for other reasons
    - In a subject decides to prematurely discontinue protocol therapy ("refuses treatment"), the subject should be asked if he or she may still be contacted for further scheduled study assessments. The outcome of that discussion should be documented in both the medical records and in the eCRF.
- Female subject becomes pregnant
- HD IL-2 will be discontinued if there is a ≥ 12-week delay between cycles
- Pembrolizumab will be discontinued if there is a ≥ 12-week delay between cycles

6.3 Protocol Discontinuation

If a subject decides to discontinue from the protocol (and not just from protocol therapy) all efforts should be made to complete and report study assessments as thoroughly as possible. A complete final evaluation at the time of the subject's protocol withdrawal should be made with an explanation of why the subject is withdrawing from the protocol. If the reason for removal of a subject from the study is an adverse event, it will be recorded on the eCRF.

7. STUDY CALENDAR & EVALUATIONS

Study Evaluations Cycle = 21 days for Pembrolizumab Cycle = 5 days for HD IL-2	Screening	Cycles 1-2 Pembrolizumab (P)	End of Cycle 2 Pembro	Cycle 1 HD IL2	Cycle 2 HD IL2	Safety follow up visit[11]	Long-term Follow up[12]
	-28 days	Day 1 (± 3 days)	Day 21 (± 3 days)	Days 1-5	Days 15-19	28 days post last dose (± 7 days)	Every 3 months (±14 days)
REQUIRED ASSESSMENTS							
Informed Consent/HIPPA authorization	X						
Medical History[1]	X						
Diagnosis and Staging[2]							
Physical Exam	X	X	X	X	X	X	
Vital signs and ECOG Performance Status[3]	X	X	X	X	X	X	
AEs & concomitant medications	X	X	X	X	X	X	
LABORATORY ASSESSMENTS							
Complete Blood Cell Count with diff (CBC)	X	X[10]	X	X	X	X	
Comprehensive Metabolic Profile (CMP)	X	X[10]	X	X	X	X	
PT/INR and aPTT	X	X[10]					
Thyroid Function (TSH, T4, free T3)	X		X				
Vitamin D level	X						
Pregnancy test (serum or urine) (WOCBP)[4]	X	X[4]					
Urinalysis	X	X[10]	X		X		
DISEASE ASSESSMENT							
Chest X-ray				X			
CT of chest[5]	X		X			X	
CT or MRI of abdomen and pelvis[5]	X		X			X	
CT or MRI Brain[5]	X		X			X	
TREATMENT EXPOSURE							
Pembrolizumab		X					
HD IL-2				X	X		
SPECIMEN COLLECTION							
Archival Tumor Tissue[6]	X						
Blood Samples[7]		X[7]	X	X	X[7]		
Stool sample[8]		X					

Study Evaluations Cycle = 21 days for Pembrolizumab Cycle = 5 days for HD IL-2	Screening	Cycles 1-2 Pembrolizumab (P)	End of Cycle 2 Pembro	Cycle 1 HD IL2	Cycle 2 HD IL2	Safety follow up visit[11]	Long-term Follow up[12]
	-28 days	Day 1 (± 3 days)	Day 21 (± 3 days)	Days 1-5	Days 15-19	28 days post last dose (± 7 days)	Every 3 months (±14 days)
BANKING SAMPLES							
Whole blood, Plasma and PBMCs[9]		X[9]	X			X	
FOLLOW-UP							
Survival Status, Subsequent Therapy							X

Key to Footnotes
CBC with differential and platelet to include: Hgb, Hct, WBC, ANC, lymphocyte count, platelets. CMP to include sodium, potassium, chloride, creatinine, blood urea nitrogen; liver function tests (LFTs) to include AST, ALT, total bilirubin, alkaline phosphatase

1: Medical History to include documentation of prior anti-cancer treatment including medications (chemotherapy, checkpoint inhibitors, etc.), radiation, or surgery. A question of how the subject learned about the study will be asked.
2: Diagnosis and staging to include pathology report and Tumor Node Metastasis (TNM) staging.
3: Vital signs to include blood pressure, weight, and height (screening only) and ECOG performance status.
4: For women of childbearing potential (WOCBP): urine or serum βhCG if clinically appropriate will be done during screening and within 72 hours of initiation of study treatment. If a urine test is done and it is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
5: Appropriate scans including CT chest, abdomen/pelvis to assess disease status will be obtained within 4 weeks before initiation of study treatment (screening). Post treatment scans will be performed on Cycle 2 Day 21 (end of Cycle 2 pembrolizumab; +3 days) of pembrolizumab and Cycle 2 of HD IL-2; +9 days) of HD IL-2 including CT chest, abdomen/pelvis. CT or MRI of the brain should be done if clinically indicated at site investigator's discretion.
6: Required if available: submission of unstained slides from archived tumor tissue for PD-L1 expression are to be identified during screening and submitted after the patient is registered to the trial prior to Cycle 2 of pembrolizumab. If archived tumor tissue is not available, the patient does not need to undergo a biopsy to obtain tissue and is still eligible for study.
7: Required: submission of blood for serum (soluble PD-L1, Th1/Th2 ratio and arginine levels) will be collected pre-dose Cycle 1 Day 1 of pembrolizumab, and on Cycle 2 Day 21 (+3 days) of pembrolizumab. Blood samples for these levels will also be collected at the end of each of the HD IL-2 cycles; End of Cycle 1 sample will be drawn (+3 days) prior to dosing Cycle 1 Day 5 (after completion of HD IL-2). The end of Cycle 2 sample will be drawn (+ 9 days) at the end of Cycle 2 Day 19 (after completion of HD IL-2.

8: Required: submission of a stool specimen (for microbiome characterization) will be collected at baseline (pre-dose Cycle 1 Day1 of pembrolizumab). Stool for microbiome analysis will be performed prior to treatment. Subjects will be provided a kit with detailed instructions regarding collection of the sample prior to the timepoint it is due. Please see the CLM for additional details.
9: Optional: submission of whole blood, plasma and PBMCs for banking to be collected at baseline pre-dose Cycle 1 Day 1 of pembrolizumab and on Cycle 2 Day 21 (+3 days) of pembrolizumab. This sample will also be collected about 4 weeks after the last dose of HD IL-2 (safety visit)
10: For Cycle 1 Day of pembrolizumab only: labs do not need to be repeated if done within 7 days of Day 1.
11: The safety follow-up visit should occur when subjects permanently stop study treatment for whatever reasons (toxicity, progression, or at discretion of site investigator) and should be performed 30 days (±7 days) after the last dose of treatment. Subjects who have an ongoing Grade ≥ 2 or serious AE (SAE) at this visit will continue to be followed until the AE resolves to ≤ Grade 1 or baseline, deemed clinically insignificant, and/or until a new anti-cancer treatment starts, whichever is earlier.
12: Long-term follow up will occur in all subjects after the safety follow up visit. Subjects who discontinue treatment for any reason will be followed every 3 months for 12 months. Follow up may be accomplished via clinic visit, phone call, or other avenues as appropriate.

8. BIOSPECIMEN STUDIES AND PROCEDURES

8.1 Correlate PD-L1 expression in archived diagnostic tumor tissue with best clinical response (revised RECIST criteria) and treatment outcome in subjects with stage IV malignant melanoma

Expression of PD-L1 in archived diagnostic tumor tissue will be determined by IHC by referring laboratory using anti-PD-L1 antibody clone 22C3, and will be correlated with clinical response assessed by imaging. Unstained slides are to be submitted; a tumor block is not acceptable.

8.2 Correlate the change in soluble PD-L1 and arginine levels in plasma during therapy with best clinical response (revised RECIST criteria) and treatment outcome in subjects with stage IV malignant melanoma

Whole blood for serum submission will be collected pre-dose Cycle 1 Day 1 of pembrolizumab, and on Cycle 2 Day 21 (+3 days) of pembrolizumab. Blood samples for these levels will also be collected at the end of each of the HD IL-2 cycles; End of Cycle 1 sample will be drawn prior to dosing Cycle 1 Day 5 (+3 days). The end of Cycle 2 sample will be drawn (+ 9 days) at the end of Cycle 2 Day 19 for analysis of soluble PD-L1 and arginine levels. Change in soluble PD-L1 and arginine levels as a result of treatment will be examined in relation to the best clinical response (RECIST, v1.1) and treatment outcome.

8.3 Correlate Th1/Th2 ratio and MDSCs in peripheral blood during therapy with best clinical response (revised RECIST criteria) and treatment outcome in subjects with stage IV malignant melanoma

Whole blood for serum submission will be collected pre-dose Cycle 1 Day 1 of pembrolizumab, and on Cycle 2 Day 21 (+3 days) of pembrolizumab. Blood samples for these levels will also be collected at the end of each of the HD IL-2 cycles; End of Cycle 1 sample will be drawn prior to dosing Cycle 1 Day 5 (+3 days). The end of Cycle 2 sample will be drawn (+ 9 days) at the end of Cycle 2 Day 19 for analysis of Th1/Th2 ratio levels and MDSC quantification. Change in Th1/Th2 ratio and MDSCs levels as a result of treatment will be examined in relation to the best clinical response (RECIST, v1.1) and treatment outcome.

8.4 Correlate intestinal microbiome at baseline with best clinical response (revised RECIST criteria) and treatment outcome in subjects with stage IV malignant melanoma

Samples of stool will be collected prior to pembrolizumab treatment in a stool collection tube per manufacturer's guidelines. Microbial DNA will be isolated using the Invitek PSP® Spin Stool DNA Plus Kit (with lysis enhancer) as described by the manufacturer [29]. Subsequent microbiome characterization will be performed after study complete accrual using Illumina 16S Metagenomic sequencing protocol.

8.5 Correlative samples for future unspecified cancer related studies

Subject consent will be obtained for additional samples (whole blood, plasma and PBMCs) to be collected and banked for future unspecified cancer related research. Samples will be collected at baseline (pre-dose Cycle 1 Day 1 of pembrolizumab) and on Cycle 2 Day 21 (+3 days) of pembrolizumab. This sample will also be collected about 4 weeks after the last dose of HD IL-2 (safety visit). These samples will be banked at HCRN.

8.5.1 Storage of Biospecimens
Remaining specimens once protocol described biospecimen-based studies are complete will be stored for future unspecified cancer related research. Permission for this will be obtained from subjects in the informed consent.

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

9. CRITERIA FOR DISEASE EVALUATION
Response assessments will be made using RECIST v1.1 [30], allowing additional comparisons among these criteria for disease response assessment. The same measurable and non-measurable lesions will be followed by RECIST v1.1. Disease progression will be determined using RECIST v1.1 criteria.

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

9.2 Measurable Lesions
Measurable lesions are 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).

9.2.1 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.

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

NOTE: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts. 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same subject, these are preferred for selection as target lesions.

9.4 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.

9.5 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.

9.6 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
Progressive Disease (PD)	At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).
Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study

9.7 Evaluation of Non-Target Lesions

Complete Response (CR)	Disappearance of all non-target lesions and normalization of tumor marker level.
Incomplete Response/Stable Disease	Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits
Progressive Disease (PD)	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions.

Although a clear progression of "non-target" lesions only is exceptional, 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.

9.8 Evaluation of Best Overall Response

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/ Non-PD	No	PR
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.

9.9 Definitions for Response Evaluation – RECIST 1.1

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

9.9.2 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.

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

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

9.9.5 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).

9.9.6 Clinical Benefit Rate
The proportion of patients with a confirmed complete response, a partial response or stable disease based on RECIST v1.1.

9.9.7 Progression-Free Survival
A measurement from the date of treatment initiation 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.

9.9.8 Overall Survival
Overall survival is defined by the date of treatment initiation to date of death from any cause.

10 DRUG INFORMATION

10.1 Keytruda® (Pembrolizumab; MK-3475 [Anti-PD-1 Antibody MK-3475]

Please refer to the current version of the Investigator's Brochure (IB) for additional information regarding this drug.

10.1.1 Chemical name and properties
Humanized X PD-1_mAb (H409A11) IgG4

10.1.2 Availability
Pembrolizumab is commercially available and will be ordered through outpatient pharmacy where the subject is being treatment.

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

10.1.4 Dosage and Administration
Pembrolizumab is 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.

10.1.5 Side Effects
Please refer to the current version of the 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, thyroid disorders (hypothryoidism/hyperthyroidism), hepatitis, hypophysitis, Type I diabetes mellitis, uveitis, nephritis, pancreatitis, myositis, and severe skin reaction. After a recent review of data, events newly characterized as identified risks also include vitiligo, Guillan-Barre Syndrome, and adrenal insufficiency. The majority of immune-mediated adverse events were mild to moderate in severity, were manageable with appropriate care, and rarely required discontinuation of therapy.

Further details around frequency, reporting, and management of immune-related adverse events (irAEs) can be found in the current version of the Investigator's Brochure. 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 Proleukin (Aldesleukin; Interleukin-2)

10.2.1 Chemical name and properties

Desalanyl-1, serine-125 human recombinant interleukin-2
MW: 15.3 KD
This IL-2 differs from native interleukin-2 in the following ways:

- Not glycosylated
- No N-terminal alanine
- C125S mutation
- Aggregation state

Biological potency: 1.1 mg = 18 x 10^6 IU

10.2.2 Availability

Proleukin is commercially available and will be ordered through outpatient pharmacy where the subject is being treatment.

10.2.3 Package

Sterile single-use vials intended for intravenous administration. Each vial contains 22 x 106 IU of lyophilized Proleukin.

10.2.4 Storage

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label. Store vials of lyophilized Proleukin in a refrigerator at 2° to 8°C. Protect from light. Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site. Clinical supplies may not be used for any purpose other than that stated in the protocol.

10.2.5 Dosage and Administration

Please refer to the Pharmacy Manual for a comprehensive description of Proleukin preparation. Reconstituted or diluted Proleukin is stable for up to 48 hours at 2° to 8° C.

Prometheus Laboratories will supply directly to sites at no cost to patients in this clinical trial. Proleukin is supplied as a sterile, white to off-white, lyophilized cake in single-use vials intended for intravenous administration. When reconstituted with 1.2 mL sterile water for injection, USP, each mL contains 18 x 10^6 IU (1.1 mg) Proleukin, 50 mg mannitol, and 0.18 mg sodium dodecyl sulfate, buffered with approximately 0.17 mg monobasic and 0.89 mg dibasic sodium phosphate to a pH of 7.5 (range 7.2 to 7.8). The manufacturing process for Proleukin involves fermentation in a defined medium containing tetercycline hydrochloride. The presence of the antibiotic is not detectable in the final product. Proleukin contains no preservatives in the final product.

The recommended Proleukin treatment regimen is administered in the inpatient setting on a monitored unit. Each course of treatment consists of two 5-day treatment cycles, the cycles separated by rest period for 9 days. In each cycle, 600,000 IU/kg (0.037 mg/kg) will be administered as a 15-minute intravenous infusion every 8 hours for up to 14 consecutive doses over 5 days.

10.2.6 Side Effects

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

The following adverse events (Grades 1-4) were seen in ≥30% of 525 patients (255 with metastatic renal cell cancer and 270 with metastatic melanoma) treated with Proleukin®: hypotension (71%), diarrhea (67%), oliguria (63%), chills (52%), vomiting (50%), dyspnea (43%), rash (42%), bilirubinemia (40%), thrombocytopenia (37%), nausea (35%), confusion (34%), and creatinine increase (33%). Due to the potential severe adverse events that might accompany Proleukin therapy at the recommended dosages, thorough clinical evaluation will be performed to exclude subjects that are contraindicated to Proleukin therapy and patients that have significant cardiac, pulmonary, renal, hepatic, or CNS impairment. Adverse events are frequent, often serious, and sometimes fatal. Further details around frequency, reporting, and management of immune-related adverse events (irAEs) can be found in the current version of the Investigator's Brochure. 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.

11 ADVERSE EVENTS

The descriptions and grading scales found in the NCI CTCAE v4.03 will be utilized for AE assessment. A copy of the CTCAE v4.03 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 HD IL-2.
- AEs will be recorded regardless of whether or not they are considered related to the pembrolizumab or HD IL-2.
- All AEs will be recorded in the subject's medical record and on the appropriate study specific eCRF form within the EDC system.
- Asymptomatic laboratory abnormalities that do not require treatment will not be collected as adverse events.

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 30 days after discontinuation of HD IL-2.
- 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 pembrolizumab or HD IL-2.
- All SAEs will be recorded in the subject's medical record and on the appropriate study specific eCRF form within the EDC system.

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 above), 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 Prometheus

HCRN will report all SAEs to Prometheus as stipulated by each company (i.e., all SAEs, only those related etc.) **within 1 business day** of receipt of the SAE Submission Form from a site. Follow-up information will be provided to Prometheus as it is received from site.

Contact information for Prometheus
Drug Safety Email: drugsafety@prometheuslabs.com
Drug Safety Fax: (858) 754-3046

11.3 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.

11.4 HCRN Responsibilities to FDA

HCRN will continue to facilitate compliance of applicable requirements for the sponsor-investigator in relation to this study. This includes but is not limited to 21 CFR 50.20 informed consent, 21 CFR Part 56 IRB, and pertinent sections of the Public Health Service Act and FDAAA.

11.5 IND Safety Reports Unrelated to this Trial

Prometheus 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.

12 STATISTICAL METHODS

12.1 Study Design

The primary objective of this single arm Phase II trial is to assess the response rate [complete response (CR) + partial response (PR)] of sequential therapy of pembrolizumab followed by HD IL-2 in subjects with stage IV malignant melanoma. Response assessment will be performed using revised RECIST guideline (version 1.1). The trial will consist of two phases. In the first phase of this trial, therapy will be initiated with pembrolizumab followed by HD IL-2 therapy. Pembrolizumab 200 mg will be given every 3 weeks for 2 doses (cycles). Staging will occur approximately 20 days after the last treatment dose (i.e., Day 21 [+3 days] of Cycle 2). Then subjects will receive HD IL-2 for 2 cycles, Cycle 2 separated from Cycle 1 by 9 days (one course). Subjects are hospitalized during each cycle which consists of up to 14 infusions over a 5 day period (3 infusions/day spaced 8 hours apart). Four weeks after the last HD IL-2 administration of Cycle 2, the subjects will be restaged (i.e., their disease status reassessed). For subjects with progressive disease as revealed by the restaging, they will be followed for OS up to one year from initiation of the first treatment (pembrolizumab) of the study. For subjects with CR, PR, and SD, scans will be performed at 3-month intervals until disease progression or up to a year after initiation of the first treatment (with pembrolizumab) on study. For the subjects who progress, although they will be taken off the study they will be followed for OS up to one year from initiation of the first treatment (pembrolizumab) of the study.

12.2 Endpoints and Analyses

12.2.1 Primary Endpoint

The primary endpoint of this single arm Phase II trial is to assess the response rate [complete response (CR) + partial response (PR)] of sequential therapy of pembrolizumab followed by HD IL-2 in subjects with stage IV malignant melanoma, using revised RECIST guideline (version 1.1). Response rate will be computed with associated 95% confidence intervals constructed from the exact Binomial method.

12.2.2 Secondary Endpoints

To characterize safety, tolerability and adverse effects (AE) profile of pembrolizumab followed by HD IL-2 in subjects with stage IV malignant melanoma. Proportion of subjects with each grade of adverse events as defined by CTCAE v4.03 will be computed along with 95% confidence intervals, and reported in a tabular and descriptive manner.

To measure Progression-Free Survival (PFS) using revised RECIST 1.1 after completion of 2 cycles of pembrolizumab and 2 cycles of HD IL-2 in all the subjects enrolled in the study. Median PFS times will be computed if reached, and PFS rates at 1 year +/- 3 months will be calculated with associated 95% confidence intervals based on the Kaplan-Meier method.

To measure overall survival (OS) at 12 months in subjects with stage IV malignant melanoma who showed a response [stable disease (SD), complete response (CR) or partial response (PR)] following completion of 2 cycles of pembrolizumab and 2 cycles of HD IL-2. Median OS times will be computed, and OS rate at 1 year +/- 3 months will be calculated with associated 95% confidence intervals based on the Kaplan-Meier method.

12.2.3 Analysis for Correlative Objectives

Correlative research analyses include examining the relationship between best clinical response/treatment outcome and PD-L1 expression in archived diagnostic tumor tissue, as well as examining the relationship between best clinical response/treatment outcome and change in soluble PD-L1 level and Th1/Th2 ratio level in serum during treatment after the 2nd dose of pembrolizumab, after each cycle of HD IL-2, relative to baseline (i.e., pre-pembrolizumab dose Day 1 of Cycle 1) and baseline microbiome diversity. PD-L1 expression can be discretized into different levels and tests such as Fisher's exact test and log-rank test will be used for its related analysis depending on the outcome types. Similar type of analyses will also be conducted to examine the change in soluble PD-L1 level, change in Th1/Th2 ratio level, arginine levels and type of intestinal microbiome at baseline.

12.3 Sample Size and Accrual

Response rates for anti-PD-1 treatment ranges from 20%-40% [9,33-35]. Response for IL-2 in the pivotal study was 16% for melanoma [15,16]. We based our sample size calculation on the premise that response rate must be 38% or higher – otherwise the drug treatment regimen described in this protocol will be rejected (i.e., not considered further for clinical trials).

The optimal two-stage design to test the null hypothesis that $P \leq 0.260$ versus the alternative that $P \geq 0.500$ with 80% power and 0.05 level of significance proceeds as follows. After testing the drug on 11 patients, the trial will be terminated if 3 or fewer respond. If the trial goes on to the second stage, a total of 29 patients will be studied. If the total number responding is less than or equal to 11, the drug is rejected. The probability of early termination at stage 1 with a total sample size of 11 is 0.685; the probability of going on to stage 2 with a total sample size of 29 is 0.315. The sample size calculation is conducted using the software PASS 13 (NCSS, Kaysville, Utah 2014).

12.4 Analysis Datasets

Population	Definition
Enrolled	This will comprise all subjects who meet the eligibility criteria and are registered onto the study.
Evaluable	This will comprise all subjects who receive at least one dose of trial drug and either undergo at least one post-baseline assessment or die before any evaluation. Unevaluable patients will be replaced.
Safety	This will comprise all subjects that receive at least one dose of study drug.

12.5 Interim Analysis/Criteria for Stopping Study

A Two-Stage Phase II Simon design will be used in this study. After testing the study drug on 11 patients in the first stage, the trial will be terminated if 3 or fewer respond. During this analysis, accrual to the trial will be halted.

13 TRIAL MANAGEMENT

13.1 Data and Safety Monitoring Plan (DSMP)

The study will be conducted with guidance from the Health Partner'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 DSMB for review according to DSMB Charter

13.2 Data Safety Monitoring Board

This study will have a Data and Safety Monitoring Board (DSMB). The DSMB is chaired by an independent medical oncologist external to this trial. The DSMB will provide a recommendation to the sponsor-investigator after all information is reviewed. This information will also be provided to HCRN who will distribute to the site investigator/participating sites for submission to their respective IRB according to the local IRB's policies and procedures.

The DSMB review will include but is not limited to:

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

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.

Participating sites may also be subject to quality assurance audits by Prometheus 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 Prometheus 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, 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. Select 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, Prometheus, 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. SEER database (http://seer.cancer.gov/statfacts/html/melan.html)
2. Sandru A, Voinea S, Panaitescu E, Blidaru A. Survival rates of patients with metastatic malignant melanoma. J Med Life 2014; 7(4):572–576.
3. Barth A, Wanek LA, Morton DL. Prognostic factors in 1,521 melanoma patients with distant metastases. J Am Coll Surg. 1995;181(3):193-201.
4. Balch CM, Gershenwald JE, Soong SJ, et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol 2009;27:6199–6206.
5. Delyon J, Maio M, Lebbé C. The ipilimumab lesson in melanoma: achieving long-term survival. Semin Oncol. 2015;42(3):387-401.
6. Mahoney KM., Freeman GJ., McDermott DF. The Next Immune-Checkpoint Inhibitors: PD-1/PD-L1 Blockade in Melanoma. Clin Ther. 2015;37(4):764-782
7. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. N Engl J Med. 2015;373(1):23-34.
8. Ribas A, Wolchok JD, Robert C, Kefford K, Hamid O, et al. Updated clinical efficacy of the anti-PD-1 monoclonal antibody pembrolizumab in 411 patients with melanoma. Pigment Cell Melanoma Res 2014;27:1223-1223.
9. Robert C, Ribas A, Wolchok JD, et al. Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. Lancet 2014;384(9948):1109-1117
10. Robert C and all KEYNOTE-006 investigators. Pembrolizumab versus Ipilimumab in Advanced Melanoma. N Engl J Med. 2015;372(26):2521-2532.
11. Granucci F, Vizzardelli C, Pavelka N, Feau S, Persico M, Virzi E, Rescigno M, Moro G, Ricciardi-Castagnoli P. Inducible IL-2 production by dendritic cells revealed by global gene expression analysis. Nat Immunol 2001;2(9):882-888
12. Gaffen SL, Liu KD. Overview of interleukin-2 function, production and clinical applications. Cytokine 2004;28:109-123.
13. Ahmadzadeh M, Rosenberg SA. IL-2 administration increases CD4+ CD25(hi) Foxp3+ regulatory T cells in cancer patients.. Blood 2006;107:2409-2414.
14. Refaeli Y, Van Parijs L, London CA, Tschopp J, Abbas AK. Biochemical mechanisms of IL-2-regulated Fas-mediated T cell apoptosis. Immunity 1998;8:615-623.
15. Atkins MB, Lotze MT, Dutcher JP, Fisher RI, Weiss G, Margolin K, Abrams J, Sznol M, Parkinson D, Hawkins M, Paradise C, Kunkel L, Rosenberg SA. High-Dose Recombinant Interleukin 2 Therapy for patients with metastatic melanoma: Analysis of 270 patients treated between 1985-1993. J Clin Oncol 1999;17(7):2105-2116.
16. Atkins MB, Kunkel L, Sznol M, Rosenberg SA. High-Dose Recombinant Interleukin-2 therapy in patients with metastatic melanoma: Long term survival update. Cancer J Sci Am 2000;6 Supp 1:S11-S14.
17. Schwartzentruber DJ. High-dose interleukin-2 is an intensive treatment regardless of the venue of administration. Cancer J 2001;7:103-104.
18. Zajac AJ, Blattman JN, Murali-Krishna K, Sourdive DJ, Suresh M, Altman JD, Ahmed R. Viral immune evasion due to persistence of activated T cells without effector function. J. Exp Med 1998;188:2205–2213.

19. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, Freeman GJ, Ahmed R. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 2006;439:682–687.
20. Ahmadzadeh M, Johnson LA, Heemskerk B, Wunderlich JR, Dudley ME, White DE, Rosenberg SA. Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. Blood 2009;114:1537–1544.
21. Blackburn SD, Shin H, Freeman GJ, Wherry EJ. Selective expansion of a subset of exhausted CD8 T cells by alpha PD-L1 blockade. Proc Natl Acad Sci USA 2008;105:15016–15021.
22. Boyman O, Cho JH, Sprent J. The role of interleukin-2 in memory CD8 cell differentiation. Adv Exp Med Biol 2010;684:28-41.
23. Floros T, Tarhini AA. Anticancer Cytokines: Biology and Clinical Effects of Interferon-α2, Interleukin (IL)-2, IL-15, IL-21, and IL-12. Semin Oncol 2015;42(4):539-548.
24. West EE, Jin HT, Rasheed AU, Penaloza-Macmaster P, Ha SJ, Tan WG, Youngblood B, Freeman GJ, Smith KA, Ahmed R. PD-L1 blockade synergizes with IL-2 therapy in reinvigorating exhausted T cells. J Clin Invest. 2013;123(6):2604-2615.
25. Kaufman H, Wang J, Curti BD, Clark J, Ernstoff MS, Silk A, Mehner JM, Zloza A, Shih J, David F. McDermott DF. A Phase II multicenter trial to evaluate combination ipilimumab and high-dose IL-2 in patients with unresectable stage III and IV melanoma. J Clin Oncol 2015;33: suppl; abstr TPS3095
26. MK-3475 With Lymphodepletion, TIL and High or Low Dose Interleukin-2 (IL-2) ClinicalTrials.gov Identifier: NCT02500576
27. Wong MK, Morse MA, McDermott DF, Clark JI, Kaufman HL, Daniels GA, Perritt JC, Hua H, Aung S. Overall survival of metastatic melanoma patients treated with HD IL-2 followed by immune checkpoint blockade of the CTLA-4 or the PD-1 pathways: analysis of data on the current use of HD IL-2. J Immunother Cancer 2015;**3**(Suppl 2):P359.
28. http://www.proclaimregistry.com/
29. Mitra S, Förster-Fromme K, Damms-Machado A, Scheurenbrand T, Biskup S, Huson DH, Bischoff SC. Analysis of the intestinal microbiota using SOLiD 16S rRNA gene sequencing and SOLiD shotgun sequencing. BMC Genomics. 2013;14 Suppl 5:S16.
30. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228-247.
31. Schwartzentruber DJ. Guidelines for the safe administration of high-dose interleukin-2. J Immunother. 2001;24(4):287-293.
32. Pousta JC, Woolery JE, Green MR. Management of toxicities associated with high-dose interleukin-2 and biochemotherapy. Anti-Cancer Drugs 2013;24:1-13.
33. Robert C, Long GV, Brady B, et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med 2015;372(4):320-330.
34. Wolchok JD, Kluger H, Callahan MK, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med. 2013;369(2):122-133.
35. Hamid O, Robert C, Daud A, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. N Engl J Med. 2013;369(2):134-144.

APPENDIX 1: ECOG Performance Status

ECOG Score	Performance Status
0	Asymptomatic
1	Symptomatic, fully ambulatory
2	Symptomatic, in bed < 50% of the day
3	Symptomatic, in bed > 50% of the day but not bedridden
4	Bedridden
5	Dead