A Phase II Study Assessing the Effect of Pembrolizumab Induced Changes to the NK Cell Exhaustion Phenotype on the Efficacy of PD-1 Targeted Treatment in Patients With Unresectable Stage III or Stage IV Melanoma

PI: Dr. Nina Bhardwaj

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TITLE: A phase II Study Assessing the Effect of Pembrolizumab Induced Changes to the NK Cell Exhaustion Phenotype on the Efficacy of PD-1 Targeted Treatment in Patients with Unresectable Stage III or Stage IV Melanoma

Principal Investigator: Nina Bhardwaj
Co-Principal Investigator: Philip Friedlander

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1.0 TRIAL SUMMARY

Abbreviated Title	Pembrolizumab effects on NK cell exhaustion in melanoma
Trial Phase	II
Clinical Indication	Unresectable stage III and stage IV
Trial Type	Treatment
Type of control	No control arm
Route of administration	IV
Trial Blinding	Not blinded
Treatment Groups	Pembrolizumab 200 mg every 3 weeks
Number of trial subjects	125, 100 melanoma patients and 25 healthy donors
Estimated enrollment period	3 yr
Estimated duration of trial	5 yr
Duration of Participation	5 yr (includes OS follow up)

2.0 TRIAL DESIGN

2.1 Trial Design

100 patients with unresectable stage III or stage IV melanoma will receive intravenous treatment with pembrolizumab at a dose of 200 mg every 3 weeks. This study consists of several treatment cycles, where each treatment cycle is defined as 21 days (3 weeks). Therefore treatment cycles 1,2,3,4, 5, 6,7,8, 9 are defined as starting respectively at week 1, 4,7,10, 13, 16, 19, 22, 25. Treatment will be continued every 3 weeks unless there is a reason to discontinue. Once the drug is discontinued there will be follow up visits. There will be one visit at the time the drug is discontinued (Discontinuation visit), one Safety Follow up Visit, and subsequent Follow up visits every 12 weeks until progression or if patient withdraws from the study or up to 3 years after enrollment. Patient will be called about every 12 weeks to monitor their survival. Blood samples for cell profiling , plasma measurements and transcriptome analysis will be collected at baseline (2 values; visit 2 and prior to cycle 1) and prior every cycle, to assess for: (a) NK cell exhaustion phenotype; (b) plasma levels of MICA, HMGB1 and CEACAM-1; (c) ALC; (d) MDSC frequency and (e) frequency of ICOS+CD4+ and EOMES+ CD8+ T cells.

Clinical responses will be assessed according to Immune-Related Response Criteria. Patients who fail to receive the first 4 cycles of treatment due to toxicity of treatment will be replaced. **All patients will be followed until progression or up to 3 years of follow up.**

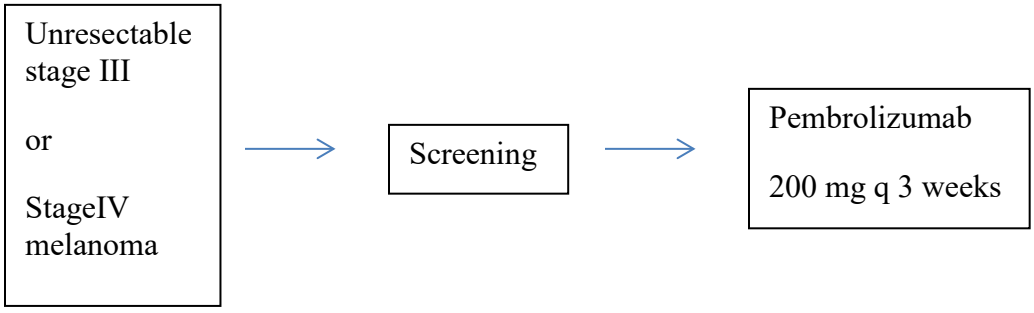
Changes in the NK cell exhaustion phenotype will be correlated with best overall response as a primary endpoint and with 24 week progression free survival and 1-year overall survival as secondary endpoints.

Analysis of NK cells will comprise assessment of phenotypic and functional parameters. Phenotypic characterization includes expression of exhaustion markers Tim-3, CEACAM-1, IL-2R subunits, activating and inhibitory NK cell receptors (NKR). Functional analysis includes cytokine production, cytotoxicity and proliferation. These studies will be supplemented by CyTOF analysis to validate pathways associated with the exhaustion state. Biomarkers associated with the NK cell exhausted state will be monitored in plasma by ELISA (MICA, HMGB1 and CEACAM-1).

We expect to see a clinical response rate of 27% when measured by immune related response criteria [1]. An inverse correlation of clinical response with pre-treatment plasma CEACAM-1, HMGB-1 and MICA levels is projected. We anticipate that responding patients will display previously established biomarkers of response (higher ALC, ICOS+ CD4+ T cells, EOMES+ CD8+ T cells, and lower levels of MDSC).

25 healthy donors will be recruited into the study to donate blood leukocytes and plasma in order to provide baseline "normal" ranges and parameters of NK cell phenotype and function, biomarker distribution, ALC levels, MDSC levels and frequency of ICOS+CD4+ and EOMES+ CD8+ T cells.

2.2 Trial Diagram



- Research blood samples for cell profiling and plasma measurements will be obtained at baseline (2 values; visit 2 and prior to cycle 1), week 4 (prior to cycle 2), week 7 (prior to cycle 3), week 10 (prior to cycle 4), week 13 (prior to cycle 5), week 16 (prior to cycle 6), week 19 (prior to cycle 7), week 22 (prior to cycle 8), week 25 (prior to cycle 9), week 34 (prior to infusion 10), week 52, week 78, week 104, and at end of treatment and 30 days post discontinuation. Imaging studies to assess efficacy of treatment will be performed during week 3 of every 4th treatment cycle while receiving pembrolizumab infusion.

3.0 OBJECTIVES & HYPOTHESES

3.1 Primary Objective & Hypothesis

(1) Objective: To correlate changes in the NK cell exhaustion phenotype with the clinical efficacy (best overall response) of pembrolizumab treatment in patients with **Unresectable** stage III or IV melanoma using Immune-Related Response Criteria.

Hypothesis: Pharmacodynamic reversal of NK cell exhaustion is associated with clinical responsiveness to pembrolizumab induced PD-1 checkpoint blockade.

3.2 Secondary Objectives & Hypotheses

(1) **Objective**: To identify biomarkers associated with a reversal of NK cell exhaustion *in vivo* following treatment with pembrolizumab.

Hypothesis: Reversal of NK cell exhaustion is correlated with biomarkers such as HMGB-1, CEACAM-1 and MICA.

(2) **Objective**: To determine how treatment with pembrolizumab modulates NK cell exhaustion in patients with stage IV melanoma.

Hypothesis: The metastatic melanoma microenvironment induces the development of exhausted NK cells.

(3) **Objective**: To identify Tim-3 associated and non-associated molecular or protein targets that potentiate this phenotype.

Hypothesis: The development of exhausted NK cells in the microenvironment of a melanoma metastasis is potentiated by protein targets, including Tim-3 associated and non-associated targets.

(4) **Objective:** To correlate changes in the NK cell exhaustion phenotype with one year survival following pembrolizumab treatment in patients with **Unresectable** stage III or IV melanoma using Immune-Related Response Criteria.

Hypothesis: Pharmacodynamic reversal of NK cell exhaustion is associated with improved survival following pembrolizumab induced PD-1 checkpoint blockade.

(5) **Objective:** To correlate changes in the NK cell exhaustion phenotype with 6 month progression free survival (PFS) in pembrolizumab treated patients with **Unresectable** stage III or IV melanoma using Immune-Related Response Criteria.

Hypothesis: Pharmacodynamic reversal of NK cell exhaustion is associated with improved PFS in response to pembrolizumab induced PD-1 checkpoint blockade.

(6) Objective: To corroborate the presence of exhausted NK cells within the tumor and its reversal after treatment.

Hypothesis: NK cells in the tumor microenvironment will be exhausted but function and frequency can be restored with pembrolizumab treatment.

As a control for these exploratory studies, NK cells and plasma will be obtained from peripheral blood of 25 healthy donors (HD).

4.0 BACKGROUND & RATIONALE

4.1 Background

4.1.1 Melanoma

It is estimated that in 2014 approximately 76,100 people in the United States will be diagnosed with melanoma and 9710 will die because of the malignancy. The median life expectancy for stage IV melanoma (distant metastases) is under one year. Prior to 2011 only two agents, dacarbazine and high dose interleukin-2 (HD-IL2), were Food and Drug Administration (FDA) approved for the management of stage IV melanoma. The rate of response to dacarbazine chemotherapy is less than 20% and the responses are largely partial and treatment does not confer an overall survival benefit. HD-IL2 is a cytokine based treatment requiring in-patient administration and confers a 16% response rate with a 5% durable complete response rate.

Approximately 50% of melanomas select for an activating mutation at position 600 of BRAF (V600) leading to constitutive activation of the Mitogen Activated Protein Kinase signaling pathway (MAPK pathway). Targeted inhibition of BRAF or the combination of BRAF plus MEK confers survival benefits in patients with melanoma expressing a V600 BRAF mutation. The rate of response to dual BRAF and MEK inhibition using dabrafenib and trametinib is approximately 67% with median duration approximately 10 months.

In 2011, ipilimumab, which is a monoclonal antibody which targets and blocks CTLA-4, was FDA approved for the management of stage IV melanoma. CTLA4 is a checkpoint molecule that down regulates the activation of T-cells. In a phase III trial, treatment of stage IV melanoma patients with ipilimumab resulted in a statistically significant overall survival benefit relative to treatment with a peptide vaccine. The median overall survival increased from 6.4 months to 10.1 months and 2-year survival increased from 13.7% to 23.5% of patients.

In 2014, two immunotherapies targeting PD-1, nivolumab and pembrolizumab, were FDA approved for the treatment of stage IV melanoma. Additional background information is provided below.

In 2015, pembrolizumab was approved as initial treatment of patients with unresectable or metastatic melanoma.

4.1.2 NK Cells

NK cells are key participants in the initial immune response to tumorigenesis, equipped to respond rapidly through induction of cytokines and deployment of cytotoxic activity[2]. A dynamic balance of positive and negative signals regulates NK cell target cell recognition and lysis, through engagement of a variety of activation and inhibitory receptors (NK cell receptors; NKR). NK cells detect a state of 'missing-self' by MHC class I-specific inhibitory receptors and they recognize cell stress-induced self-ligands on cancer cells via their activating receptors. NK cells contribute to containing tumor growth as NK cell infiltration in tumor tissue correlates with better prognosis, whereas reduced activity of peripheral blood NK cells is associated with an increased risk of cancer[3]. **We have now obtained clear evidence that NK cells progressively undergo a process of "exhaustion" that coincides with disease progression from early to late stage melanoma. Freshly purified NK cells from 113 patients with untreated melanoma (melanoma donors; stages I, II and III/IV) were analyzed for this study.** Similar to exhausted T cells, melanoma associated NK cells are characterized by a failure to proliferate, to produce interferon gamma (IFNγ) or to kill target cells. They (i) down regulate activation receptors (CD16, NKG2D, NKp46, and DNAM-1), IL-2R subunits and NK cell regulatory transcription factors (T-bet, Eomes), (ii) upregulate inhibitory receptors (KIR3DL1, KIR2DL3) and (iii) express high levels of the checkpoint molecule Tim-3, a phenotype that is consistent with "NK cell exhaustion". **Significantly, blockade of Tim-3 reverses this phenotype and state of exhaustion in vitro, restoring 30-65% of the NK cell dysfunction[4]**. Most strikingly however, we find that NK cell function is spontaneously restored in patients who have a clinical response to ipilimumab treatment despite the fact that these cells express little or no CTLA-4 (or PD-1 or PDL-1), possibly through reversal of tumor-associated systemic immune suppression. Characterizing NK cell dysfunction in melanoma, will be critical to understanding the modulation of NK cell biology in the tumor microenvironment (TME), and, ultimately, developing approaches that restore both innate and adaptive immunity, *in vivo*.

PRELIMINARY DATA:

Defining the spectrum of NK cell exhaustion in melanoma and identification of molecular or protein targets that potentiate NK cell exhaustion.

NK cell exhaustion in melanoma: Blood NK cells from melanoma patients develop a phenotypic and functional profile consistent with progressive exhaustion from stage I to stage IV (Figure 1). This is characterized by reductions in:

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(i) cytotoxicity (expression of LAMP-1/CD107a in response to co-culture with K562 cells),
(ii) IFNγ production in response to IL-12, and
(iii) proliferation in response to IL-2, consistent with the concept that advanced disease is progressively associated with systemic immune suppression (Figure 1).
The exhaustion profile is global as it is distributed through both CD56hi and CD16 hi NK cell subsets (data not shown) [4].

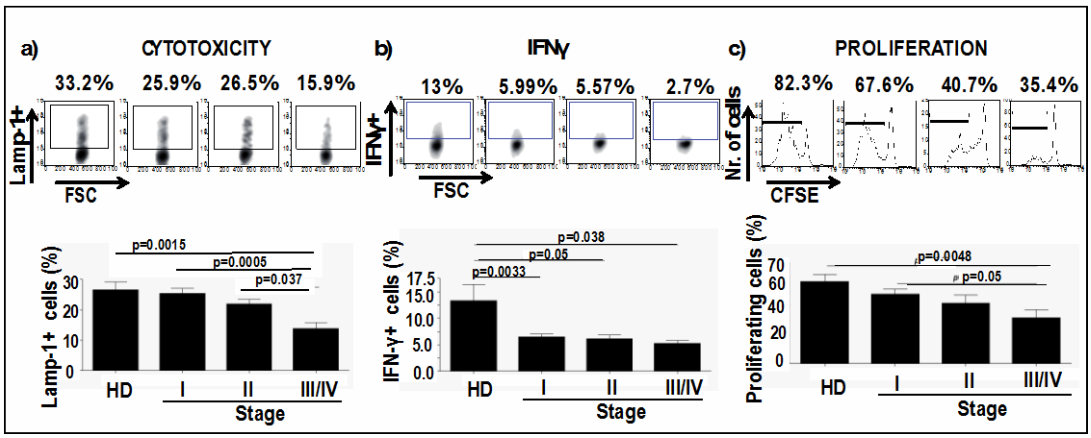

Figure 1 a-c: NK cells progressively develop a phenotypic and functional profile consistent with exhaustion. Represented by a) cytotoxicity b) IFNγ production c) proliferation. Patient numbers: Stage I (n=56) Stage II (n=21) Stage III/IV (n=23).

NK cell exhaustion and Tim-3: In an analysis of stage III/IV melanoma patients, NK cell exhaustion was found to be directly associated with up regulation of checkpoint molecules (Tim-3), reduced NK cell cytotoxic activity, IFNγ production, and proliferative capacity, and lower levels of T-bet (Supplementary Table 1). Elevated Tim-3 was also significantly associated with expression of the inhibitory receptor KIRB1 and poor prognostic factors, such as, tumor thickness >1mm, mitotic rate >/= 1/mm^2, ulceration and metastases (Supplementary Table 2).

Strikingly, Tim-3 blockade substantially reversed exhaustion, improving NK cell cytotoxicity, IFNγ production and IL-2 dependent proliferation (Figure 2). Reversal was associated with up regulation of the activating CD16 receptor and α and γ chains of IL-2R[4]. These data denote a major role for Tim-3 in modulating NK cell function in melanoma.

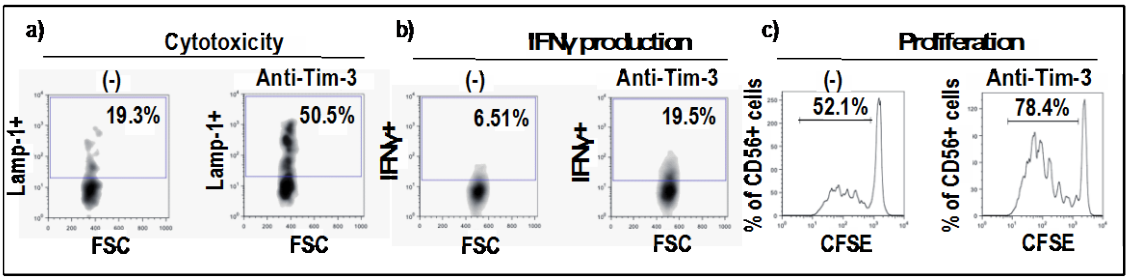

Figure 2: Tim-3 blockade partially reverses NK cell exhaustion. Anti-Tim-3 (20µg/ml) reverses blockade of (a) cytotoxicity, (b) cytokine production and (c) proliferation of melanoma NK cells.

Tim-3 ligation inhibits NK cell function: Tim-3 ligands include galectin-9[5], HMGB-1 (high mobility group box-1, released by necrotic tumor cells)[6], phosphatidylserine and CEACAM-1 (carcinoembryonic antigen cell adhesion molecule 1), which functions in cis and trans configurations as a heterophilic ligand to promote the tolerance-inducing functions of TIM-3[7]. We have confirmed that CEACAM-1 is expressed on normal NK cells and its expression level increases on IL-2 and/or IL-15 activated $CD56^{hi}$ NK cells as previously described[8] (Figure 3).

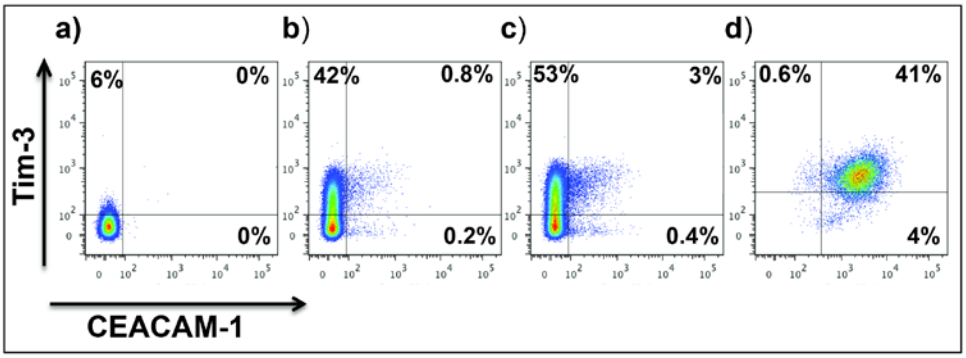

Figure 3: Detection of Tim-3 and CEACAM-1 in human NK cells and 721.221 cell line by Flow Cytometry. NK cells were freshly isolated from a healthy donor's blood (a) then stimulated O/N with (b) rhIL-2 (1000U/ml) or (c) rhIL-15 (100ng/ml). (d) CEACAM-1 expression in the 721.221 cell line that stably over-expresses CEACAM-1 is shown as a positive control.

We found NK cell mediated cytotoxicity was significantly reduced in Gal-9+ but not Gal9- Gmel melanoma cells, supporting a role for Tim-3-galectin 9 engagement in the potentiation of NK cell exhaustion.[4] To address the role of other NKR ligands, we evaluated plasma levels of HMGB1 and MICA, a known NKG2D ligand produced by melanoma cells and associated with dysfunctional NK cells in melanoma[9] (Figure 4). Levels of both proteins rose progressively with advancing stage of disease, and an independent cohort of patients (n=204) validated the association of higher levels of MICA (>500 pg/ml) with worse prognosis (data not shown). Altogether these data warrant a closer examination of the role of Tim-3 and NKR ligands in mediating exhaustion and as possible prognostic biomarkers.

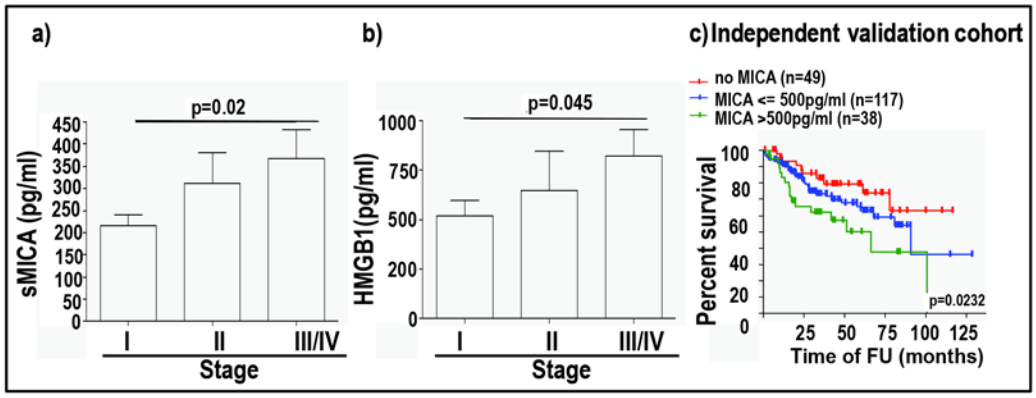

Figure 4: MICA and HMGB1 levels in plasma from melanoma patients. Levels were measured by ELISA. The numbers of patients evaluated in each cohort are: (a) Stage I (n=72), Stage II (n=24), Stage III/IV (n=15); (b) Stage I (n=36), Stage II (n=11), Stage III/IV (n=14); (c) an independent validation cohort shows that MICA >500 pg/ml predicts OS.

Biomarkers associated with reversal of NK cell exhaustion through check-point blockade.

Preliminary studies indicate that ipilimumab, an anti-CTLA-4 monoclonal antibody, can reverse blood NK cell exhaustion in melanoma patients. Blood NK cells from stage IV donors recovered their natural response to IL-2 stimulation (500U/ml) over 7 days after 3 cycles of ipilimumab (Figure 5). Recovered responses included up regulation of IL-2 R α and γ chains and activating NKR (NKG2D and NKp46), cytotoxicity (Lamp-1 expression) and IFNγ production. In contrast, NK cells from patients who failed to respond to ipilimumab did not restore IL-2R α chain expression in response to IL-2 (not shown). As ipilimumab resistant patients can respond to subsequent anti-PD-1 therapy [1,10], we propose that clinical responders to anti-PD-1 treatment will have effectively reversed NK cell exhaustion.

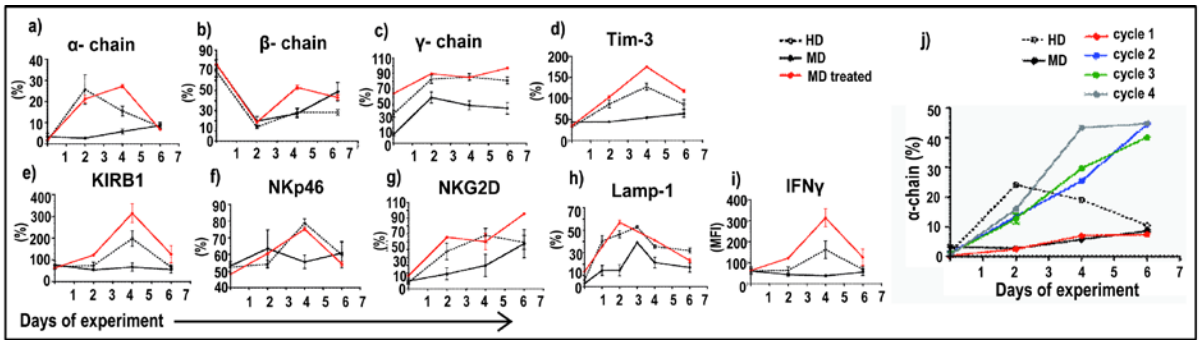

Figure 5 (a-i). NK-cell exhaustion can be reversed following treatment with 3 cycles of ipilimumab. NK cells were cultured with IL-2 and phenotype and function monitored over time. Black solid line (HD=healthy donor); Black dashed line (MD= Melanoma donor); Red solid line (MD treated=Melanoma donor). **(j) NK-cell exhaustion is rapidly reversed with ipilimumab.** NK cells from patients pre/post 1, 2, 3 or 4 cycles of Ipilimumab at 3 week intervals were cultured with IL-2. Levels of IL-2Rα chain were monitored over 6d.

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Our studies indicate that MD (melanoma donor) NK cells exhibit a progressively exhausted phenotype characterized by up-regulation of inhibitory receptors (TIM-3, KIRB1 and KIRNKAT2), down-regulation of activating receptors (NKG2D and NKp46), IL-2R subunits and transcription factors (T-bet and Eomes), and loss of IFNγ production, proliferation and cytotoxicity. Tim-3 expression, which is critical to conferring this exhausted NK cell phenotype, is associated with poor prognostic markers of overall survival (OS). Systemic elevation of NKR ligands such as MICA (NKG2D ligand) and HMGB1 (Tim-3 ligand) may portend worse outcome by potentiating NK cell exhaustion. Checkpoint blockade can reverse this phenotype, even in the absence of CTLA-4 or PD-1 on NK cells, warranting further investigation of parameters that may predict this outcome.

The primary objective of this proposal, therefore, is to correlate changes in the NK cell exhaustion phenotype with the clinical efficacy (best overall response) of pembrolizumab treatment in patients with Unresectable stage III or IV melanoma using Immune-Related Response Criteria. Secondary objectives include identifying biomarkers associated with a reversal of NK cell exhaustion *in vivo* following treatment, identifying the associated proteins that potentiate this phenotype, and evaluating tumor associated NK cells, if feasible.

A cohort of 25 healthy donors will be included to determine the normal ranges for the biomarkers and proteins associated with the NK exhaustion phenotype.

4.1.3 Pharmaceutical and Therapeutic Background (pembrolizumab)

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

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1), an Ig superfamily member related to CD28 and CTLA-4, has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and its family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine

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

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. On December 18, 2015, the U. S. Food and Drug Administration (FDA) expanded the label to include the approval of pembrolizumab for the treatment of patients with unresectable or metastatic melanoma. This expansion now includes the initial treatment of patients with unresectable or metastatic melanoma with pembrolizumab.

Refer to the Investigator's Brochure (IB)/approved labeling for detailed background information on MK-3475 (pembrolizumab).

4.1.4 Preclinical and Clinical Trial Data

Refer to the Investigator's Brochure for Preclinical and Clinical data.

4.2 Rationale

Antibodies targeting the checkpoint molecules CTLA-4 and PD-1 have dramatically improved the treatment of metastatic melanoma, although there are subsets of patients who

fail to respond. Identifying parameters that predict a durable response are urgently needed. We observed rapid reversal of NK cell exhaustion in patients with a clinical response following treatment with anti-CTLA-4, even though NK cells do not express this molecule. However, patients who failed to respond clinically also failed to reverse this exhaustion profile. We propose that early reversal of function may predict clinical response to anti-PD-1 therapy in patients who have either failed ipilimumab or other therapies, or who are ineligible for these therapies. Progressive melanoma is characterized by elevated levels of plasma CEACAM-1, MICA and HMGB1, all known ligands for various NKR, and which have been associated with worse OS. NKR ligand levels, therefore, may function as pretreatment biomarkers for checkpoint blockade inhibition.

4.2.1 Rationale for the Trial and Selected Subject Population

Melanoma is an immune-modulated malignancy and immune checkpoint modulators which inhibit PD-1 function (pembrolizumab, nivolumab) have demonstrated clinical efficacy as treatment for patients with stage IV melanoma. Pembrolizumab across a range of doses in phase I investigation has demonstrated clinical efficacy with RR approximately 27%. By better understanding how NK cell function and exhaustion interplays with PD1 function and activity, potentially more efficacious combination therapies can be developed. The pharmacodynamic studies to be performed as part of this trial will provide such information.

4.2.2 Rationale for Dose Selection/Regimen/Modification

An open-label Phase I trial (Protocol 001, PN001) is being conducted to evaluate the safety and clinical activity of single agent MK-3475. The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of MK-3475 showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No MTD has been identified to date. 10.0 mg/kg Q2W, the highest dose tested in PN001, was chosen as the dose and schedule utilized in Cohorts A, B, C and D of that protocol to test for initial tumor activity. Recent data from other clinical studies within the MK-3475 program has shown that a lower dose of MK-3475 and a less frequent schedule may be sufficient for target engagement and clinical activity.

PK data analysis of MK-3475 administered both Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life (refer to IB). Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days). This early PK and pharmacodynamic data provided the scientific rationale for testing a Q2W and Q3W dosing schedule.

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of MK-3475 were found to be dependent on body weight. The relationship

between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. MK-3475 has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg Q3W body weight based regimen are anticipated to remain well within the established exposure margins of 0.5 – 5.0 for MK-3475 in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W vs. the dose regimen of 2 mg/kg Q3W (i.e. 5-fold higher dose and exposure). The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

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

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

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

5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1 Melanoma Subject Inclusion Criteria

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

1. Be willing and able to provide written informed consent/assent for the trial.
2. Have Unresectable stage III or stage IV melanoma
3. Be ≥ 18 years of age on day of signing informed consent.
4. Have measurable disease based on RECIST 1.1 and be able to be followed over time by Immune related response criteria (irRC) for treatment decisions.
5. Have a performance status of 0, 1, or 2 on the ECOG Performance Scale.
6. Demonstrate adequate organ function as defined in Table 1, all screening labs should be performed within 10 days of treatment initiation.

Table 1 - Adequate Organ Function Laboratory Values

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

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

8. Female subjects of childbearing potential should be willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication (Reference Section 5.6.2). Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.

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

5.1.2 Melanoma Subject Exclusion Criteria

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

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

2. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of systemic immunosuppressive therapy within 7 days prior to the first dose of trial treatment. Topical, inhaled, ocular, and intra-articular steroids are not exclusionary.

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

4. Hypersensitivity to pembrolizumab or any of its excipients.

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

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

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

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

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

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

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

10. Has a history of (non-infectious) pneumonitis that required steroids or current pneumonitis.

11. Has an active infection requiring systemic therapy.

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

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

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

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

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

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

18. Has received a live vaccine within 30 days of planned start of study therapy.

    Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines and are not allowed.

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5.1.3 Healthy subject inclusion and exclusion criteria.

25 healthy volunteers will serve as the "baseline" healthy population for comparison purposes to assess NK cell function and protein biomarkers in the blood. They will not received trial treatment.

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

1. Be willing and able to provide written informed consent/assent for the trial.
2. Be ≥ 18 years of age on day of signing informed consent.
3. Be free of chronic illness, without known heart, lung, kidney, bleeding disorders, infectious disease (HIV, HBV or HCV infection).
4. Not taking regularly prescribed medication such as steroids, hormone therapy or immunosuppressive agents.
5. Not pregnant or breastfeeding.
6. Have adequate organ function (see Table 1).

5.2 Trial Treatments

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

Table 2 - Trial Treatment

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
Pembrolizumab	200 mg	Q3W	IV infusion	Day 1 of each 3 week cycle	Experimental

All enrolled melanoma subjects will receive treatment with pembrolizumab at the dose of 200mg intravenous every 3 weeks.

5.2.1 Dose Selection/Modification

5.2.1.1 Dose Selection

The rationale for selection of doses to be used in this trial is provided in Section 4.0 – Background and Rationale.

Details on preparation and administration of pembrolizumab (MK-3475) are provided in the Pharmacy Manual.

5.2.1.2 Dose Modification

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per Table 3 below. See Section 5.5.1 and the Investigator Brochure for MK-3475 for supportive care guidelines, including use of corticosteroids.

Table 3 - Dose Modification Guidelines for Drug-Related Adverse Events

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

Note: Permanently discontinue for any severe or Grade 3 drug-related AE that recurs or any life-threatening event.

[1] For patients with liver metastasis who begin treatment with Grade 2 AST or ALT, if AST or ALT increases by greater than or equal to 50% relative to baseline and lasts for at least 1 week then patients should be discontinued.

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Discontinue Subject
[2] Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 12 weeks of the last dose.			

Dosing interruptions are permitted in the case of medical / surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). Subjects should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the patient's study record.

5.2.2 Timing of Dose Administration

Trial treatment should be administered on Day 1 of each cycle after all procedures/assessments have been completed as detailed on the Trial Flow Chart (Section 6.0). Trial treatment may be administered up to 3 days before or after the scheduled Day 1 of each cycle due to administrative reasons.

All trial treatments will be administered on an outpatient basis.

Pembrolizumab 200 mg will be administered as a 30 minute IV infusion every 3 weeks. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

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

5.2.3 Trial Blinding/Masking

This is a single agent, open-label trial; therefore, the Sponsor, investigator and subject will know the treatment administered.

5.3 Randomization or Treatment Allocation

There is no randomization. All enrolled melanoma subjects will receive treatment with pembrolizumab at the dose of 200 mg intravenous every 3 weeks.

5.4 Concomitant Medications/Vaccinations (allowed & prohibited)

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this

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

5.4.1 Acceptable Concomitant Medications

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

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs as defined in Section 7.2.

5.4.2 Prohibited Concomitant Medications

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

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy
    - Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed at the investigator's discretion.
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor.

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

The Exclusion Criteria describes other medications which are prohibited in this trial.

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

5.5 Rescue Medications & Supportive Care

5.5.1 Supportive Care Guidelines

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined below and in greater detail in the ECI guidance document. 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.

Note: if after the evaluation the event is determined not to be related, the investigator is instructed to follow the ECI reporting guidance but does not need to follow the treatment guidance (as outlined in the ECI guidance document). Refer to Section 5.2.1 for dose modification.

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event. Suggested conditional procedures, as appropriate, can be found in the ECI guidance document.

- **Pneumonitis**:
  - For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
  - For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
  - Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.

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

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

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

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

- **Hypophysitis**:

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

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

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

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

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

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

Table

~~**Table**~~ 4 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab (MK-3475).

Table 4 Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
indicated		
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab (MK-3475) with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

5.6 Diet/Activity/Other Considerations

5.6.1 Diet

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

5.6.2 Contraception

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

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

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

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study they must adhere to the contraception requirement (described above) for the duration of the study and during the follow-up period defined in section 7.2.2-Reporting of Pregnancy and Lactation to the Sponsor and to Merck. If there is any question that a subject will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

5.6.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab, the subject will immediately be removed from the study. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor and to Merck without delay and within 24 hours to the Sponsor and within 2 working days to Merck if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn).

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the Sponsor and to Merck and followed as described above and in Section 7.2.2.

5.6.4 Use in Nursing Women

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

5.7 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator or the Sponsor if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding discontinuation or withdrawal are provided in Section 7.1.3.1 – Other Procedures.

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

- The subject or legal representative (such as a parent or legal guardian) withdraws consent.
- Confirmed radiographic disease progression as described in Section 5.7.1
- Unacceptable adverse experiences as described in Section 7.2.3
- Intercurrent illness that prevents further administration of treatment
- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up
- Completed 24 months of uninterrupted treatment with pembrolizumab or 35 administrations of study medication, whichever is later.

  Note: 24 months of study medication is calculated from the date of first dose.
  Administrative reasons

The End of Treatment and Follow-up visit procedures are listed in Section 6 (Protocol Flow Chart) and Section 7.1.4 (Visit Requirements). After the end of treatment, each subject will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for 90 days after the end of treatment as described in Section 7.2.3. Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent or becoming lost to follow-up. After documented disease progression each subject will be followed by telephone for overall survival until death, withdrawal of consent, or the end of the study, whichever occurs first.

5.7.1 Discontinuation of Study Therapy after CR

Discontinuation of treatment may be considered for subjects who have attained a confirmed CR that have been treated for at least 24 weeks with pembrolizumab and had at least two treatments with pembrolizumab beyond the date when the initial CR was declared.

5.8 Subject Replacement Strategy

Subjects who do not receive the first 4 cycles of treatment due to toxicity will be replaced.

5.9 Clinical Criteria for Early Trial Termination

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

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

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

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6.0 TRIAL FLOW CHART

6.1 Study Flow Chart

Trial Period:	**Screening Phase**		**Treatment Cycles[a]**								**End of Treatment**	**Post-Treatment**		
							To be repeated beyond 8 cycles							
Treatment Cycle/Title:	Pre-screening (Visit 1)	Main Study Screening (Visit 2)	1	2	3	4	5	6	7	8	Discon	Safety Follow-up	Follow Up Visits[b]	Survival Follow-Up
Scheduling Window (Days):		-28 to -1		± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discon	30 days post discon	Every 12 weeks post discon	Every 12 weeks
Administrative Procedures														
Informed Consent	X	X												
Inclusion/Exclusion Criteria		X												
Demographics and Medical History		X												
Prior and Concomitant Medication Review		X	X	X	X	X	X	X	X	X	X	X	X	
Trial Treatment Administration			X	X	X	X	X	X	X	X				
Post-study anticancer therapy status														X
Survival Status														X
Clinical Procedures/Assessments														
Review Adverse Events		X	X	X	X	X	X	X	X	X	X	X		
Full Physical Examination		X	X	X	X	X	X	X	X	X	X	X		
Directed Physical Examination													X	
Vital Signs and Weight		X	X	X	X	X	X	X	X	X	X	X	X	
ECOG Performance Status		X	X	X	X	X	X	X	X	X	X	X	X	
Laboratory Procedures/Assessments: analysis performed by LOCAL laboratory														
Pregnancy Test – Urine or Serum β-HCG		X	X											

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Trial Period:	**Screening Phase**		**Treatment Cycles[a]**								**End of Treatment**	**Post-Treatment**		
Treatment Cycle/Title:	Pre-screening (Visit 1)	Main Study Screening (Visit 2)	1	2	3	4	To be repeated beyond 8 cycles 5	6	7	8	Discon	Safety Follow-up	Follow Up Visits[b]	Survival Follow-Up
Scheduling Window (Days):		-28 to -1		± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discon	30 days post discon	Every 12 weeks post discon	Every 12 weeks
PT/INR and aPTT		X												
CBC with Differential with platelet count		X	X	X	X	X	X	X	X	X	X	X		
Comprehensive Serum Chemistry Panel		X	X	X	X	X	X	X	X	X	X	X		
Urinalysis[c]		X	X	X	X	X	X	X	X	X	X	X	X	
T3, FT4 and TSH and cortisol [c]		X	X	X	X	X	X	X	X	X	X	X		
Autoimmunity panel[d]		X												
HIV, Hepatitis B and C, Influenza[e]		X												
HLA class I (A, B, C) and II (DR, DQ, DP) Testing		X												
Efficacy Measurements														
Tumor Imaging[f]		X				X				X				
Tumor Biopsies/Archival Tissue Collection/Correlative Studies Blood														
Archival or Newly Obtained Tissue Collection[g]		X				X[h]								
Correlative Studies Blood Collection[i]		X	X	X	X	X	X	X	X	X	X	X		

a: treatment cycle is defined as 21 days (3 weeks). Therefore treatment cycles 1, 2, 3, 4, 5, 6, 7, 8, and 9 are defined as starting respectively at week 1, 4, 7, 10, 13, 16, 19, 22, and 25. Treatment will be continued every 3 weeks unless there is a reason to discontinue. Treatment can be given +/- 3 days of the onset of each treatment cycle.

b: follow up every 12 weeks (+/-1 week) following completion or discontinuation of pembrolizumab infusions until progression or patient consent withdrawal or 3 years post-enrollment. The safety follow-up visit will be 30 days post discontinuation of study agent +/- 3 days)

c: results of the endocrine studies are not required to be back to initiate treatment each cycle. In cases where urinalysis not able to be provided treatment could still be administered following discussion with the PI.

d: autoimmunity panel includes: RF (rheumatoid factor), ANA (antinuclear antibody) and anti-DNA.

e: include HCV, RNA (qualitative) or Hepatitis C antibody, HBsAg, and HIV type I and type 2 (e.g., HIV-1/-2 antibody screening test and evaluation of HIV viral load as needed).

f: imaging should be performed during week 3 of every 4th treatment cycle while receiving pembrolizumab, i.e. every 12 weeks. For example week 12 and 24. If imaging raises concern for pseudo- progression, imaging should be repeated 4-6 weeks later to help differentiate between true versus pseudo- progression.

g: archival if available, or optional new biopsy by incisional or excisional or core needle biopsy if easily accessible metastasis (epidermal, dermal, or subcutaneous or palpable lymph node) prior to cycle 1 day 1. Optional incisional or excisional or core needle biopsy if easily accessible metastasis (epidermal, dermal, or subcutaneous or palpable lymph node) during week 11 or 12 of the study.

h: optional tissue biopsy.

i: correlative blood collection obtained at baseline (2 values), week 4, week 7, week 10, week 13, week 16, week 19, week 22, week 25, week 34, week 52, week 78, week 104 and/or at end of treatment (EOT), and 30 days post discontinuation.. Samples should be obtained prior to pembrolizumab infusion, ideally on the day of the planned infusion. Samples can be obtained +/- 3 days of the start of the treatment cycle. Two baseline values will be obtained, one to be drawn the day of first planned pembrolizumab infusion but prior to the infusion and the other within 7 days prior to cycle1 day1.

Blood volumes for melanoma patients: Screening, week 1, week 16 and 30 days post discontinuation up to 80ml; all other visits up to 40ml.

Blood volumes for healthy donors: Healthy donors will provide up to 60 ml of blood at 2 time points at least one month apart.

7.0 TRIAL PROCEDURES

7.1 Trial Procedures

The Trial Flow Chart - Section 6.0 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

Furthermore, additional evaluations/testing may be deemed necessary by the Sponsor and/or Merck for reasons related to subject safety. In some cases, such evaluation/testing may be potentially sensitive in nature (e.g., HIV, Hepatitis C, etc.), and thus local regulations may require that additional informed consent be obtained from the subject. In these cases, such evaluations/testing will be performed in accordance with those regulations.

7.1.1 Administrative Procedures

7.1.1.1 Informed Consent

The Investigator must obtain documented consent from each potential subject prior to participating in a clinical trial.

Consent must be documented by the subject's dated signature or by the subject's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion.

A copy of the signed and dated consent form should be given to the subject before participation in the trial.

The initial informed consent form, any subsequent revised written informed consent form and any written information provided to the subject must receive the IRB/ERC's approval/favorable opinion in advance of use. The subject or his/her legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the subject's dated signature or by the subject's legally acceptable representative's dated signature.

Specifics about a trial and the trial population will be added to the consent form template at the protocol level.

The informed consent will adhere to IRB/ERC requirements, applicable laws and regulations and Sponsor requirements.

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7.1.1.2 Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the trial.

7.1.1.3 Medical History

A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the Investigator. Details regarding the disease for which the subject has enrolled in this study will be recorded separately and not listed as medical history.

7.1.1.4 Prior and Concomitant Medications Review

7.1.1.4.1 Prior Medications

The investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the subject within 28 days before starting the trial. Treatment for the disease for which the subject has enrolled in this study will be recorded separately and not listed as a prior medication.

7.1.1.4.2 Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the subject during the trial. All medications related to reportable SAEs and ECIs should be recorded as defined in Section 7.2.

7.1.1.5 Disease Details and Treatments

7.1.1.5.1 Disease Details

The investigator or qualified designee will obtain prior and current details regarding disease status.

7.1.1.5.2 Prior Treatment Details

The investigator or qualified designee will review all prior cancer treatments including systemic treatments, radiation and surgeries.

7.1.1.5.3 Subsequent Anti-Cancer Therapy Status

The investigator or qualified designee will review all new anti-neoplastic therapy initiated after the last dose of trial treatment. If a subject initiates a new anti-cancer therapy within 30 days after the last dose of trial treatment, the 30 day Safety Follow-up visit must occur before

the first dose of the new therapy. Once new anti-cancer therapy has been initiated, the subject will move into the survival follow-up phase.

7.1.2 Clinical Procedures/Assessments

7.1.2.1 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 4.0 (see Section 11.3). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

For subjects receiving treatment with pembrolizumab all AEs of unknown etiology associated with pembrolizumab exposure should be evaluated to determine if it is possibly an event of clinical interest (ECI) of a potentially immunologic etiology (termed immune-related adverse events, or irAEs); see the separate ECI guidance document in Appendix 4 regarding the identification, evaluation and management of potential irAEs.

Please refer to section 7.2 for detailed information regarding the assessment and recording of AEs.

7.1.2.2 Full Physical Exam

The investigator or qualified designee will perform a complete physical exam during the screening period. Clinically significant abnormal findings should be recorded as medical history. A full physical exam should be performed during screening,

7.1.2.3 Directed Physical Exam

For cycles that do not require a full physical exam per the Trial Flow Chart, the investigator or qualified designee will perform a directed physical exam as clinically indicated prior to trial treatment administration.

7.1.2.4 Vital Signs

The investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment and at treatment discontinuation as specified in the Trial Flow Chart (Section 6.0). Vital signs should include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening only.

7.1.2.5 Eastern Cooperative Oncology Group (ECOG) Performance Scale

The investigator or qualified designee will assess ECOG status (see Section 11.1) at screening, prior to the administration of each dose of trial treatment, and at discontinuation of trial treatment as specified in the Trial Flow Chart.

7.1.2.6 Tumor Imaging and Assessment of Disease

Contrast enhanced CT imaging is preferred and to be obtained of chest, abdomen and pelvis and should encompass other known areas of metastases. MRI imaging or fusion PET/CT imaging can be performed if not able to tolerate CT contrast. This imaging will be performed for baseline assessment between day -28 and day -1.

A brain MRI will be obtained (contrast enhanced preferred) or if cannot tolerate MRI a CT head (contrast enhanced preferred) as baseline assessment between day -28 and day -1.

Imaging will be repeated during week 3 of every 4th treatment cycle (approximately every 12th week) while receiving pembrolizumab. The extracranial imaging will be performed every 8 weeks in patients with response or stable disease no longer taking pembrolizumab due to toxicity for up to 2 years.

If patient has delay in treatment timing because of toxicity or another reason unrelated to study drug such as unrelated medical or personal family event imaging will remain as per original schedule with dates based on timing of cycle #1 day#1 (approx. every 12 weeks).

7.1.2.7 Tumor Tissue Collection and Correlative Studies Blood Sampling

Samples will be stored and processed in the laboratory of Dr. Nina Bhardwaj, Hess Center for Science and Medicine, 1470 Madison Avenue 5th floor. Investigators performing these assays will be blinded to clinical response data.

Blood specimens. Blood samples for cell profiling and plasma measurements will be collected at baseline (2 values; visit 2 and cycle 1 prior to cycle 1), week 4 (prior to cycle 2), week 7 (prior to cycle 3), week 10 (prior to cycle 4), week 13 (prior to cycle 5), week 16 (prior to cycle 6), week 19 (prior to cycle 7), week 22 (prior to cycle 8), week 25 (prior to cycle 9), week 34 (prior to infusion10), week 52, week 78, week 104, at time of discontinuation (EOT= End of treatment) and 30 days post discontinuation to assess for (a) NK cell exhaustion phenotype; (b) plasma levels of MICA, HMGB1 and CEACAM-1; (c) ALC; (d) MDSC frequency and (e) frequency of ICOS+CD4+ and EOMES+ CD8+ T cells.

NK cells will be analyzed for a set of phenotypic and functional parameters. Their phenotype will be assessed by flow cytometry for expression of Tim-3, CEACAM-1, in addition to other selected exhaustion markers, IL-2R subunits (alpha, beta, gamma), and a panel of

activating and inhibitory NKR (NK cell receptors). To assess functional activity, NK cells will be analyzed for cytokine production, cytotoxicity and proliferation.

An exhausted NK cell phenotype will be characterized by up-regulation of inhibitory receptors (Tim-3, CEACAM1 and others), down-regulation of activating receptors (NKG2D, NKp46 and others), IL-2R and transcription factors (T-bet and Eomes), and/or loss of IFNγ production, proliferation and cytotoxicity. Significant reduction (>30%), compared with a mean of healthy donor values, obtained from healthy subjects in this trial and as previously reported[4], in any one or more of three functional parameters, namely, proliferation, CTL, IFNγ production and/or IL-2Receptor expression will constitute the phenotype of "NK cell exhaustion". Our prior data indicate that these parameters generally go hand in hand. These studies will be supplemented by CyTOF analysis to validate pathways we find to be associated with the exhaustion state Plasma MICA, HMGB1 and CEACAM-1 will be monitored by ELISA.

a) NK cell exhaustion phenotype

NK cell purification: NK cells isolated from peripheral blood mononuclear cells (PBMCs) purified from healthy donor blood (HD; obtained as part of an IRB approved protocol or from melanoma donor blood (MD) by Ficoll-Paque Plus centrifugation. NK-cell enrichment will be performed by negative selection using Easy-Sep Human NK cell Enrichment Kit (StemCell Technologies) according to the manufacturer's recommendations, obtaining more than 95% $CD3^{-}CD56^{+}$ populations.

NK Cell Staining and Flow Cytometry Analysis: NK cells from HD and MD will be assessed by Flow Cytometry for the activating receptors CD16, NKp30, NKp44, and DNAX accessory molecule-1 (DNAM-1), and inhibitory receptors that recognize MHC class I molecules and block NK cell-mediated cytotoxicity. They include the Killer cell Ig-like Receptor (KIR) family, the Leukocyte immunoglobulin-like Receptor (LILR, CD85) family and the family of CD94/NKG2A lectin-like receptors. Expression of checkpoint molecules such as Tim-3, CEACAM-1, B7-H3, VISTA, LAG-3, CD96, PD-1, CTLA-4 and TIGIT will also be monitored. Expression patterns of receptors for cytokines (IL-2, IL-12, IL-15 and IL-18) that mediate NK cell growth, activation and function will be analyzed. Finally, NK cells will be assessed by intracellular analysis for expression of the transcription factors T-bet and Eomes.

Flow cytometry analysis will be undertaken at two different points; i) cells freshly isolated from blood, and ii) after in vitro O/N stimulation with hrIL-2 (1000U/ml).

IFNγ production assay: Purified NK cells will be cultured O/N with recombinant human IL-2 (rhIL-2, 1000U/ml) and after overnight culture, stimulated for 4h in the presence of 1μg/ml of rhIL-12 and brefeldin A (10μg/ml, Sigma). Cells are then fixed (1% paraformaldehyde,

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Electron Microscopy Sciences) and permeabilized (0.1% saponin, Sigma)). Permeabilized cells will be stained for intracellular IFNγ and analyzed by flow cytometry.

Cytotoxicity Assay: Purified NK cells will be cultured O/N with rhIL-2 (1000U/ml) and then stimulated with target cells (K562 cell) at a 5:1 effector/target ratio in combination with anti-CD107 antibody (0.5µg/ml) and monensin 1000X (BioLegend). After a 4h incubation, CD107 expression on CD56+ cells is quantified by flow cytometry. CD107 is expressed on the cell surface as a result of degranulation.

Proliferation assay: Purified NK cells will be loaded with 2µM CFSE (Life Technologies) and cultured in complete media supplemented with 200U/ml of rhIL-2. After 6 days, CFSE dilution is analyzed by flow cytometry as a measure of cell proliferation.

b) Identification of inflammatory pathways that are modulated in exhausted NK cells

Mass cytometry measurement (CyTOF):

Isolated PBMCs will be cultured overnight in the presence of 1000U/ml of rhIL-2 and complete media. After removal and replacement of media, cells are stimulated with rhIL-2 (1000U/ml), rhIL-15 (100 ng/ml), rhIL-12 (1ug/ml) and/or anti-CD16 (10ug/ml) for 0, 15, 30 min-1h. A commercial viability marker, Cisplatin, is added to the culture 5min before treatment is completed. Cells will be washed and fixed with 1% PFA for 20min at RT and subsequently stained with the appropriate barcode antibody, CD45-115, CD45-141, CD45-159 and CD45-169 (each barcode will designate a specific time point). Cells are washed again and incubated for 10 min at 4C with a cocktail of metal conjugated antibodies containing i) anti-CD56, -CD3, -CD4, -CD16 to identify cellular subsets and distinguish NK cells; ii) anti-Tim-3, -CEACAM-1, -NKG2D, -NKp46, -DNAM-1, -IL-2R, -KIR3DL1,-KIR2DL3 to identify cellular subsets of exhausted NK cells.

Identification of signaling mechanisms that promote exhaustion will be performed by intracellular staining. NK cells will be evaluated for intracellular mediators that signal activation pathways including STAT and Lck proteins. STAT4 and STAT5 are involved in IFNγ production, critically important for early control of tumors, and Lck, which is modulated by inhibitory signals mediated by Tim-3 via Bat3[11].

Cells will be permeabilized with 100% cold Methanol for 20min at 4C, washed and stained with anti-phospho STAT-4, STAT-5 and anti-phospho-Lck antibodies. Acquisition and detection of samples will be done using a CyTOF mass cytometer device in our Immune Monitoring Core facility. Data will be analyzed using Cytobank software. NK cells will be analyzed by CyTOF prior to beginning treatment, and at two additional time points; week 16 (prior to cycle 6) and 30 days post discontinuation.

Transcriptome analysis

Global changes in gene expression through RNA seq (RNA poly A library and small RNA sequencing to target miRNA) using the Illumina platform will be applied to observe and

confirm cellular pathway alterations and gene expression level changes from HD and MD NK cells during progression of the treatment. RNA from HD and MD enrolled in this trial will be isolated from collected whole blood.

Immune multiplex gene expression

A multiplex gene expression analysis with 770 genes from 24 different immune cell types covering both the adaptive and innate immune response will be performed on RNA isolated from HD and MD enrolled in this trial. RNA will be isolated from collected whole blood. The analysis and detection of immune-related genes will be done by nCounter PanCancer Immune Profiling Panel (Nanostring Technologies).

cc) Plasma levels of MICA, HMGB1 and CEACAM-1

ELISA assays: Expression levels of MICA, HMGB1 and CEACAM1 on HD and patients' plasma will be performed using commercially available kits (R&D systems and antibodies-online, and SIGMA respectively).

Luminex or equivalent technology: Expression levels of human cytokines on HD and patient's plasma will be analyzed using standard multiplexed Luminex technology (Cytokine Human Magnetic 35-Plex Panel for Luminex^{TM}) and/or Olink or equivalent methodology.

d) Frequency of MDSC; ICOS+CD4+ and EOMES+ CD8+ T cells

Flow cytometry analysis: Myeloid-derived suppressor cells (MDSC) will be detected by flow cytometry and are characterized by $CD3^+$ HLA-$DR^{-/lo}$, $CD11b^+$, $CD33^+$, $CD34^+$, arginase-I^+ and ROS^+. Circulating $ICOS^+CD4^+$ T cells and $EOMES^+CD8^+$ T cells will also be assessed by Flow Cytometry.

e) Tumor-infiltrating NK cells in tissue samples

Archived tumor (at least 10 unstained slides or tissue block) will be requested at baseline, if available. Subjects may undergo optional tumor biopsies of easily accessible metastatic deposits, if present (epidermal, dermal, subcutaneous, palpable lymph node), by incisional, excisional, or core needle biopsy prior to cycle 1 day 1. Optional incisional, excisional, or core needle biopsy of easily accessible metastasis (epidermal, dermal, or subcutaneous or palpable lymph node) may be performed during week 11 or 12 of the study.

Depending on tumor tissue availability, tumor biopsy specimens will be subdivided for paraffin embedding and for snap freezing for immuno-fluorescence analysis. If sufficient material is available, fresh tumor will be digested to obtain cell suspensions for flow cytometry analysis, and immune functional assays.

Immunohistochemistry (IHC): The following monoclonal antibodies will be used for detecting NK cells in tissue specimens: CD56, CD16 and CD57. Expression of NK cell

receptors and analysis of exhaustion will be performed by analyzing NKp30, NKp46, Tim-3 and CEACAM1 expression and other relevant exhaustion markers. A multiplex immunofluorescence assay designed at Mount Sinai by Dr. Sacha Gnjatic, co-Director of the Immune Monitoring Core will be used for these assessments.

Single-cell isolation from tissue samples: Similar assays as described in a) will be performed on NK cells isolated from fresh tissue, where possible. Single-cell isolation will be performed on collagenase treated tissue and NK cells selected by sorting.

7.1.3 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments (Hematology, Chemistry and Urinalysis) to be performed in this trial are provided in Table 5.

Table 5 - Laboratory Tests

Hematology	Chemistry	Urinalysis	Other
Hematocrit	Albumin	Blood	Serum β-human chorionic gonadotropin†
Hemoglobin	Alkaline phosphatase	Glucose	(β-hCG)†
Platelet count	Alanine aminotransferase (ALT)	Protein	PT (INR)
WBC (total and differential)	Aspartate aminotransferase (AST)	Specific gravity	aPTT
Red Blood Cell Count	Lactate dehydrogenase (LDH)	Microscopic exam *(If abnormal)* results are noted	T3
Absolute Neutrophil Count	Carbon Dioxide	Urine pregnancy test †	Free tyroxine (T4)
Absolute Lymphocyte Count	*(CO_2 or biocarbonate)*		Thyroid stimulating hormone (TSH)
	Uric Acid		Cortisol
	Calcium		
	Chloride		Blood for correlative studies
	Glucose		Hepatitis HBsAg
	Phosphorus		Hepatitis C (HCV RNA) or Hepatitis C antibody
	Potassium		HIV
	Sodium		Antinuclear antibody (ANA)
	Magnesium		Rheumatoid factor (RF)
	Total Bilirubin		Anti-DNA
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*		
	Total protein		
	Blood Urea Nitrogen		

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

Laboratory tests for screening should be performed within 10 days prior to the first dose of treatment. After Cycle 1, pre-dose laboratory procedures can be conducted up to 72 hours prior to dosing. Results must be reviewed by the investigator or qualified designee and found to be acceptable prior to each dose of trial treatment.

7.1.3.1 Withdrawal/Discontinuation

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 7.2 - Assessing and Recording Adverse Events. Subjects who a) attain a CR or b) complete 24 months of treatment with pembrolizumab may discontinue treatment. After discontinuing treatment following assessment of CR, these subjects should return to the site for a Safety Follow-up Visit (described in Section 7.1.4) and then proceed to the Follow-Up Period of the study (described in Section 7.1.4).

7.1.4 Visit Requirements

Visit requirements are outlined in Section 6.0 - Trial Flow Chart. Specific procedure-related details are provided above in Section 7.1 - Trial Procedures.

Screening Period: from day-28 to day-1. See Section 6 (Study Flow Chart) for details of screening items

Treatment Period: every 3 weeks starting cycle1 day 1 with window +/- 3 days.

Post-Treatment Visits: follow up every 12 weeks (+/-1 week) following completion or discontinuation of pembrolizumab infusions until progression, patient consent withdrawal, or 3 years post-enrollment.

Safety Follow-Up Visit

The mandatory Safety Follow-Up Visit should be conducted approximately 30 days after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever comes first. All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Subjects with an AE of Grade > 1 will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-neoplastic therapy, whichever occurs first. SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded.

Follow-up Visits

Subjects who discontinue trial treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed every 8 weeks (± 7 days) by radiologic imaging to monitor disease status. Every effort should be made to collect information regarding disease status until the start of new anti-neoplastic therapy, disease progression, death, end of the study. Information regarding post-study anti-neoplastic treatment will be collected if new treatment is initiated.

Survival Follow-up

Once a subject experiences confirmed disease progression or starts a new anti-cancer therapy, the subject moves into the survival follow-up phase and should be contacted by telephone every 12 weeks to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.

7.2 Assessing and Recording Adverse Events

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of Pembrolizumab, is also an adverse event.

Changes resulting from normal development that do not vary significantly in frequency or severity from expected levels are not to be considered adverse events. Examples of this may include, but are not limited to, onset of menopause occurring at a physiologically appropriate time.

Adverse events may occur during the course of the use of Pembrolizumab in clinical trials or within the follow-up period specified by the protocol, or prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

Adverse events may also occur in screened subjects during any pre-allocation baseline period as a result of a protocol-specified intervention, including washout or discontinuation of usual therapy or a procedure.

Progression of the cancer under study is not considered an adverse event unless it is considered to be drug related by the investigator.

All adverse events will be recorded from the time the consent form is signed through 30 days following cessation of treatment and at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in section 7.2.3.1.

7.2.1 Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor and to Merck

For purposes of this trial, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater (≥5 times the indicated dose). No specific information is available on the treatment of overdoses of pembrolizumab. Appropriate supportive treatment should be provided if clinically indicated. In the event of overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

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

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

All overdoses with and without an adverse event must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

7.2.2 Reporting of Pregnancy and Lactation to the Sponsor and to Merck

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

Such events must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

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

7.2.3.1 Serious Adverse Events

A serious adverse event is any adverse event occurring at any dose or during any use of pembrolizumab that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose;
- Is an other important medical event

Refer to Table 6 for additional details regarding each of the above criteria.

Any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any subject from the time the consent is signed through 90 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to pembrolizumab, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety.

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

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

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

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

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

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7.2.3.2 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be recorded as such on the Adverse Event case report forms/worksheets and reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety (Attn: Worldwide Product Safety; FAX 215 993-1220). Events of clinical interest for this trial include:

1. an overdose of pembrolizumab, as defined in Section 7.2.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.

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

   *Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology. The trial site guidance for assessment and follow up of these criteria can be found in the MK-3475 Investigator Brochure.

3. Additional adverse events:

   A separate guidance document has been provided entitled “Investigator’s Brochure. This document is provided with this protocol and provides guidance regarding identification, evaluation and management of ECIs and irAEs.

   ECIs (both non-serious and serious adverse events) identified in this guidance document from the date of first dose through 90 days following cessation of treatment, or 30 days after the initiation of a new anticancer therapy, whichever is earlier, need to be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety (Attn: Worldwide Product Safety; FAX 215 993-1220), regardless of attribution to study treatment, consistent with standard SAE reporting guidelines.

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

7.2.4 Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 4.0. Any adverse event which changes CTCAE grade over the course of a given episode will have each change of grade recorded on the adverse event case report forms/worksheets.

All adverse events regardless of CTCAE grade must also be evaluated for seriousness.

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Table 6 - Evaluating Adverse Events

An investigator who is a qualified physician, will evaluate all adverse events as to:

V4.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of pembrolizumab that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization [including hospitalization for an elective procedure] for a preexisting condition which has not worsened does not constitute a serious adverse event.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	Is a new **cancer;** (that is not a condition of the study) **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours.	
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause pembrolizumab to be discontinued?	
Relationship to Pembrolizumab	Did pembolizumab cause the adverse event? The determination of the likelihood that pembrolizumab caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between pembrolizumab and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely pembrolizumab caused the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to pembrolizumab such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of pembrolizumab? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

Relationship to Pembrolizumab (continued)	**The following components are to be used to assess the relationship between the test drug and the AE: (continued)**	
	Dechallenge	Was pembrolizumab discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of pembrolizumab; or (3) the trial is a single-dose drug trial); or (4) pembrolizumab(s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to pembrolizumab in this study? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial); or (3) pembrolizumab(s) is/are used only one time). NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY PEMBROLIZUMAB, OR IF REEXPOSURE TO PEMBROLIZUMAB POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE U.S. CLINICAL MONITOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding pembrolizumab or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following	**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of a pembrolizumab relationship).**	
Yes, there is a reasonable possibility of Pembrolizumab relationship.	There is evidence of exposure to pembrolizumab. The temporal sequence of the AE onset relative to the administration of pembrolizumab is reasonable. The AE is more likely explained by pembrolizumab than by another cause.	
No, there is not a reasonable possibility of Pembrolizumab relationship	Subject did not receive pembrolizumab OR temporal sequence of the AE onset relative to administration of pembrolizumab is not reasonable OR there is another obvious cause of the AE. (Also entered for a subject with overdose without an associated AE.)	

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7.2.5 Sponsor Responsibility for Reporting Adverse Events

All Adverse Events will be reported to regulatory authorities, IRB/IECs and the principal investigator in accordance with all applicable global laws and regulations.

Each serious adverse event must be reported to the Principal Investigator within 2 business days of learning of the occurrence by Fax or email. In the event that the study team does not become aware of the serious adverse event immediately (e.g., participant sought treatment elsewhere), the study team is to report the event within 2 business days after learning of it and document the time of his or her first awareness of the adverse event. Report serious adverse events by email or facsimile to:

Nina Bhardwaj MD PhD fax: 646-537-9571
email: nina.bhardwaj@mssm.edu
(Telephone: 212-824-8427)

Within the following 7 business days, the study team must provide follow-up information on the serious adverse event. Follow-up information should describe whether the event has resolved or continues, if and how the event was treated, and whether the participant will continue or discontinue study participation.

Also, all AEs must be reported the IRB according to ISMMS policies.

8.0 STATISTICAL ANALYSIS PLAN

8.1 Primary Objective

The primary endpoint is to correlate changes in NK cell exhaustion phenotype with the clinical efficacy (best overall response) of pembrolizumab treatment in patients with unresectable stage III or IV melanoma. We define NK cell exhaustion being present based on reductions of three functional parameters, namely, cytotoxicity, IFNγ production, and proliferation. Blood NK cells from melanoma patients develop a functional profile with progressive exhaustion from stages I to IV. The exhaustion profile for stages I, II, and III/IV melanoma based on mean cytotoxicity is 25%, 22%, and 14%, respectively. The exhaustion profile for stage III/IV melanoma shows a mean cytotoxicity of only 14% and a standard deviation of 9% (Supplementary Table 1). We expect to see a clinical response rate of 27% for pembrolizumab. We therefore estimate that 25-30 patients will respond on pembrolizumab and 70-75 patients will not respond. In order to determine if clinical response correlates with an improved NK cell function, the % cytotoxicity will be compared between responding and non-responding stage III/IV patients. A total of 100 patients (25 responding on pembrolizumab and 75 non-responding) would allow detection of a difference of 6% between the mean cytotoxity percentages for responding and non-responding patients with 0.80 power for a two-sided test at the 0.05 level. This difference of 6% (20% for responding

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patients and 14% for non-responders) corresponds to having a 20% mean cytoxicity for responding stage III/IV patients that is close to the expected cytotoxicity for stage II patients (mean cytotoxity 22%). This analysis will also be undertaken for IFNγ production and proliferation.

8.2 Secondary Endpoints

The first secondary objective is to identify biomarkers associated with a reversal of NK cell exhaustion in vivo following treatment with pembrolizumab. A reversal of NK cell exhaustion will be defined as reversal of at least one parameter of exhaustion (responsiveness to IL-2, IFNg production, and CTL activity). Patients with a reversal of NK exhaustion will be compared to patients without a reversal with respect to biomarkers such as HMGB-1, CEACAM-1, and MICA. The two groups of patients will be compared over all time points of measured plasma levels of HMGB-1, CEACAM-1, and MICA by a repeated measure mixed model analysis. This analysis takes into account the correlation between repeated measurements obtained on the same patient. A goal here would be to determine whether patients with and without a reversal of NK cell exhaustion differ in the pattern of variation over time in MICA, HMGB-1, and CEACAM-1 plasma levels. All assumptions to statistical procedures will be thoroughly evaluated and transformation of the data will be implemented if appropriate. Several approaches will be considered to address the issue of missing data. A non-ignorable pattern-mixture model for dropouts will be first proposed. A dropout indicator will be included as a covariate in the model. This pattern-mixture model incorporates reason for missingness and is most appropriate in this clinical trial setting.

Other secondary objectives include correlation of changes in NK cell exhaustion phenotype with one year survival (and 6 month PFS) following pembrolizumab treatment. Survival (and PFS) will be compared for patients with reversal of NK exhaustion and patients without reversal by the logrank test.

8.3 Stopping Rules

Stopping rules are not applicable as Pembrolizumab is routinely used as standard of care treatment for stage IV melanoma patients. Toxicities are largely autoimmune and well defined from the clinical trials. We are using a flat dose here instead of the FDA approved 2mg/kg but the flat dose falls within the same dosing range and much higher dosing was used in the phase I studies.

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9.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

9.1 Investigational Product

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

Clinical Supplies will be provided by Merck in one of two forms, as summarized in Table 7.

Table 7 - Product Descriptions

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

9.2 Packaging and Labeling Information

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

9.3 Clinical Supplies Disclosure

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

9.4 Storage and Handling Requirements

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

Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site.

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

9.5 Returns and Reconciliation

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

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

10.0 ADMINISTRATIVE AND REGULATORY DETAILS

10.1 Compliance

Protocol Review and Amendments:
This protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) and any other necessary documents must be submitted, reviewed and approved by a properly constituted IRB.
Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB. The ISMMS Principal Investigator or delegate will disseminate protocol amendment information to all participating investigators.
All decisions of the IRB concerning the conduct of the study must be made in writing.

Informed Consent:
All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and dated by the participant or the participant's legally authorized representative, and by the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

Ethics and Good Clinical Practice (GCP):
This study is to be conducted according to the following considerations, which represent good and sound research practice:

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E6 Good Clinical Practice: Consolidated Guidance
www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM129515.pdf

US Code of Federal Regulations (CFR) governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki

Title 21 Part 11 – Electronic Records; Electronic Signatures
www.access.gpo.gov/nara/cfr/waisidx_02/21cfr11_02.html

Title 21 Part 50 – Protection of Human Subjects
www.access.gpo.gov/nara/cfr/waisidx_02/21cfr50_02.html

Title 21 Part 54 – Financial Disclosure by Clinical Investigators
www.access.gpo.gov/nara/cfr/waisidx_02/21cfr54_02.html

Title 21 Part 56 – Institutional Review Boards
www.access.gpo.gov/nara/cfr/waisidx_02/21cfr56_02.html

Title 21 Part 312 – Investigational New Drug Application
www.access.gpo.gov/nara/cfr/waisidx_02/21cfr312_02.html

State laws

ISMMS research policies and procedures

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. In such case, the deviation must be reported to the IRB according to the local reporting policy.

Study Documentation

The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research participant. This information enables the study to be fully documented and the study data to be subsequently verified.
Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinical charts, laboratory and pharmacy records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

Records Retention

They will be retained for a minimum of three years following withdrawal of all subjects from the study. All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.

10.2 Compliance with Trial Registration and Results Posting Requirements

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

10.3 Quality Management System

Data Management:
The Study team and the Mount Sinai Clinical Trial Office (CCTO) will collect, manage, and monitor data for this study. The principal investigator will assess response rate data.

ERAP will be used for case report forms and CRFs reviewed by study investigator for accuracy. Adverse events will be monitored and if unexpected but related SAEs or unexpected but related high grade (grade 3 or higher) toxicities felt at least possibly related to study drug are observed, the investigators will convene in person or by telephone or email to discuss.

Monitoring:
This study will be monitored by an independent monitor. The purpose of these audits or inspections is to examine study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol, institutional policy, Good Clinical Practice (GCP), and any applicable regulatory requirements.
All data will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of participant registration and will continue throughout protocol performance and completion.

11.0 APPENDICES

11.1 ECOG Performance Status

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

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

11.2 RECIST 1.1 Evaluation criteria

Measurable lesions must have a longest diameter of ≥ 10 mm on CT with a slice thickness of ≤5mm (oralongestdiameterof≥20mmon nonhelical CT with a slice thickness of > 10 mm) or a longest diameter of ≥ 20 mm on chest radiography.

Nonmeasurable lesions include other lesions that do not meet the criteria as measurable lesions, such as small lesions with a longest diameter of < 10 mm, skeletal metastases without a soft-tissue component, ascites, pleural effusion, lymphangitic spread of tumor, leptomeningeal disease, inflammatory breast disease, cystic or necrotic lesions, lesions in an irradiated area, and an abdominal mass not confirmed by imaging.

The number of target lesions to be assessed is two per organ and a maximum of five target lesions total.

Lymph nodes with a short axis of ≥ 15 mm are considered measurable and assessable as target lesions, and the short-axis measurement should be included in the sum of target lesion measurements in the calculation of tumor response as opposed to the longest axis used for measurements of other target lesions. Lymph nodes with a short axis of < 10 mm are de-

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fined as “nonpathologic”. All other pathologic nodes-that is, those with a short axis of ≥ 10 mm but < 15 mm—should be considered nontarget lesions.

11.3 Common Terminology Criteria for Adverse Events V4.0 (CTCAE)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for adverse event reporting. (http://ctep.cancer.gov/reporting/ctc.html)

11.4 Response Evaluation Criteria according to Immune-Related Response Criteria – iRC

Guidelines for the Evaluation of Immune Therapy Activity in Solid Tumors: Immune-Related Response Criteria

At the baseline tumor assessment, the sum of the products of the two largest perpendicular diameters (SPD) of all index lesions (five lesions per organ, up to 10 visceral lesions and five cutaneous index lesions) is calculated. At each subsequent tumor assessment, the SPD of the index lesions **and** of new, measurable lesions (≥5 x 5 mm; up to 5 new lesions per organ: 5 new cutaneous lesions and 10 visceral lesions) are added together to provide the total tumor burden:

Tumor Burden = SPD index lesions + SPD new, measurable lesions

At each tumor assessment, the response in index and new, measurable lesions is defined based on the change in tumor burden (after ruling out irPD). Decreases in tumor burden must be assessed relative to baseline measurements (i.e., the SPD of all index lesions at screening).

Overall Response using the irRC:
The overall response according to irRC is derived from time-point response assessments (based on tumor burden) as follows:

irCR:
Complete disappearance of all lesions (whether measurable or not, and no new lesions) confirmed by a repeat, consecutive assessment done no less than 4 weeks from the date first documented.

irPR:
Decrease in tumor burden ≥50%relative to baseline confirmed by a consecutive assessment done at least 4 weeks after first documentation

irSD,
Not meeting criteria for irCR or irPR, in absence of irPD

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irPD
Increase in tumor burden ≥25% relative to nadir (minimum recorded tumor burden). Confirmation by a repeat, consecutive assessment done no less than 4 weeks from the date first documented.

Reference:

Guidelines for the Evaluation of Immune Therapy Activity in Solid Tumors: Immune-Related Response Criteria. Wolchok, JD, Hoos, A, O'Day, S, Weber, JS, Hamid, O, Lebbe ', C, Maio, M, Binder, M, Bohnsack, O, Nichol, G, Humphrey, R, and Hodi, FS, Clin Cancer Res 2009;15:7412-7420

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Measurable Response	Non-measurable Response	Overall Response

Index and new, measurable lesions (tumor burden) [1]%	Non-index lesions	New, nonmeasurable lesions	Using irRC
Decrease 100%	Absent	absent	irCR[2]
Decrease 100%	Stable	Any	irPR[2]
Decrease 100%	Unequivocal progression	Any	irPR[2]
Decrease ≥ 50%	Absent/stable	Any	irPR[2]
Decrease ≥ 50%	Unequivocal progression	Any	irPR[2]
Decrease< 50% to < 25% increase	Absent/stable	Any	irSD
Decrease< 50% to < 25% increase	Unequivocal progression	Any	irSD
Increase ≥ 25%	Any	Any	irPD

1. Decreases assessed relative to baseline, including measurable lesions only (>5x5 mm)
2. Assuming response (irCR) and progression (ir PD) are confirmed by a second, consecutive assessment done at least 4 weeks apart

12.0 REFERENCES

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7. Huang Yu-Hwa, Z.C., et al. CEACAM1 regulates TIM-3-mediated tolerance and exhaustion. *Nature* (2014).
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13.0 SUPPLEMENTARY TABLES

Demographic parameters	Number of patients (N=100(%))	Tim-3+ cells(MFI) Mean (SD)	P value	KIRB1+ cells(%) Mean (SD)	P value	Cytotoxicity(%) Mean (SD)	P value	IFN-γ (%) Mean (SD)	P value	Proliferation(%) Mean (SD)	P value	T-bet+ cells(%) Mean (SD)	P value
Age Groups[2]			**0,2231**		**0,1648**		**0,041**		**0,1648**		**0,5496**		**0,08**
<=45y	26 (26)	33,62(8,790)		13,51(10,20)		23,90(10,66)		7,675(5,847)		40,68(24,56)		79,70(21,20)	
46-70y	38(38)	31,41(7,356)		16,29(12,59)		25,58(14,75)		6,545(3,931)		41,26(27,67)		81,81(18,45)	
>=71y	36(36)	30,16(7,283)		20,48(17,60)		18,39(8,440)		6,572(3,035)		48,23(30,16)		69,37(25,62)	
Gender2			**0,5189**		**0,4541**		**0,804**		**0,4519**		**0,771**		**0,445**
Female	36(36)	30,86(7,410)		16,88(15,18)		22,40(12,23)		6,594(3,893)		44,06(28,36)		75,25(24,86)	
Male	64(64)	31,91(7,993)		14,66(11,24)		23,06(12,14)		7,284(4,802)		42,35(25,44)		79,24(17,80)	
Stage[2]			**0,0008**		**0,0044**		**0,002**		**0,0016**		**0,0223**		**1E-04**
I	56(56)	29,44(5,372)		13,35(10,93)		25,31(12,48)		6,459(4,187)		35,35(20,01)		83,10(13,21)	
II	21(21)	32,72(7,275)		16,01(14,66)		21,81(7,153)		6,082(3,617)		25,23(18,56)		68,37(31,13)	
III/IV	23(23)	33,52(9,798)		25,60(16,86)		13,72(8,603)		5,189(2,824)		11,07(9,332)		63,59(29,22)	

Supplementary Table 1: Higher expression of Tim-3 and KIRB1, and lower cytotoxicity, cytokine production, proliferation and T-bet expression are associated with higher stages. Values have been evaluated by flow cytometry in a total number of 100 patients. Values inside a red square have been validated in an independent cohort (n=50).

[1]One way analysis of variance (ANOVA)

[2] Unpaired t test

Demographic parameters	Number of patients (N=88(%))	Tim-3+ cells(MFI) Mean (SD)	P value	KIRB1+ cells(%) Mean (SD)	P value	Cytotoxicity(%) Mean (SD)	P value	IFN-γ (%) Mean (SD)	P value	Proliferation(%) Mean (SD)	P value	T-bet+ cells(%) Mean (SD)	P value
Thickness[2]			**0,0406**		**0,0153**		**0,027**		**0,0295**		**0,2602**		**5E-04**
<=1mm	58(66)	29,36(6,297)		12,83(10,58)		25,17(12,28)		7,591(4,584)		45,69(27,71)		83,10(13,21)	
>1mm	30(34)	32,24(6,464)		19,30(13,57)		19,44(8,184)		5,531(3,292)		39,00(25,39)		64,91(29,85)	
Mitotic Index[2]			**0,0176**		**0,5935**		**0,17**		**0,6104**		**0,6579**		**0,616**
<1/mm²	43(49)	29,05(6,246)		14,46(12,34)		25,45(13,12)		7,214(4,556)		43,77(26,95)		77,75(20,68)	
>=1/mm²	40(45)	32,24(6,464)		15,89(12,02)		21,78(10,94)		6,720(4,230)		46,44(27,07)		75,07(24,33)	
Unclassified	5(6)												
Ulceration[2]			**0,0277**		**0,9155**		**0,934**		**0,282**		**0,5553**		**0,792**
Absent	65(74)	29,45(5,721)		14,58(11,07)		23,77(12,26)		7,020(4,620)		43,57(27,20)		78,19(19,14)	
Present	15(17)	33,61(5,843)		14,23(13,96)		23,45(12,62)		5,586(3,670)		38,87(24,20)		80,00(27,25)	
Unclassified	8(9)												
	(N=100(%))												
Metastasis[2]			**0,0042**		**0,0009**		**4E-04**		**0,0625**		**0,0904**		**0,016**
Absent	77(77)	30,33(6,744)		14,06(11,99)		24,83(11,91)		7,243(4,402)		45,39(27,52)		79,38(19,88)	
Present	23(23)	35,55(9,614)		25,60(16,86)		13,72(8,603)		5,189(2,824)		31,62(21,71)		63,59(29,22)	

Supplementary Table 2: Higher expression of Tim-3 and a lower cytotoxicity and cytokine production ability are associated with thicker melanomas. Also h**igher expression of Tim-3 and KIRB1, and lower cytotoxicity and T-bet expression are associated with local or distant metastases**. Values have been evaluated by flow cytometry in a total number of 88 (upper panel) and 100 patients(lower panel). Values inside a red square have been validated by an independent cohort (n=50).

[1]One way analysis of variance (ANOVA)