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Phase Ib/II Study of Autologous Dendritic Cell Therapy Delivered Intratumorally After Cryoablation in Combination With Pembrolizumab for Patients With Metastatic or Unresectable Melanoma

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Mayo Clinic Cancer Center

MC1771: Phase Ib/II Study of Autologous Dendritic Cell Therapy Delivered Intratumorally after Cryoablation in Combination with Pembrolizumab for Patients with Metastatic or Unresectable Melanoma

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Drug Availability
Supplied Investigational Agents:
Mayo Clinic: Autologous dendritic cells (IND#17522)
Commercial Agent: Pembrolizumab (Anti-PD-1 antibody, MK-3475)
Supplied Commercial Agent: Prevnar13®

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Activation	15Nov2017
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MCCC Amendment 2	05Feb2019
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Protocol Resources

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*No waivers of eligibility

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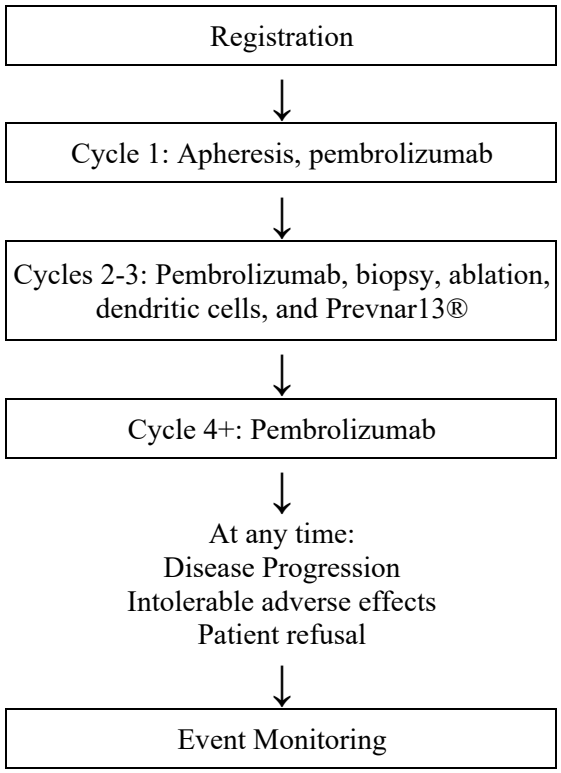
Schema

Prior to discussing protocol entry with the patient, call the Mayo Clinic Cancer Center Registration Office (507-284-2753) to confirm study status and insure that a place on the protocol is currently available to the patient



Cycle length = 21 days

Generic name: Dendritic cells Brand name: NA Mayo abbreviation: mDC Availability: Provided through Mayo Clinic Immune, Progenitor, and Cell Therapeutics (IMPACT) Lab	Generic name: Pneumococcal 13-valent Conjugate Vaccine (Diphtheria CRM197 Protein) Brand name: Prevnar13® Mayo abbreviation: PREVNAR13 Availability: Provided through Mayo Clinic Pharmacy	Generic name: Pembrolizumab, MK-3475 (anti-PD-1 antibody) Brand name: Keytruda® Mayo abbreviation: MK-3475 Availability: Commercial supply

1.0 Background

1.1 Immune checkpoint blockade responses in advanced melanoma

Melanoma is the most malignant form of skin cancer, and the fifth most common cancer in men and sixth in women in the United States with its highest incidence in the Caucasian population.[1-3] New cases of melanoma in the USA would reach 73,870 in 2015 and that year, 9,940 patients were expected to die from the disease.[4] Over the last decade, the understanding of the immune checkpoint pathway has paved the way for immunotherapy in metastatic melanoma.

Immune checkpoints are crucial for maintenance of self-tolerance under normal physiological conditions. [5-8] However, such maintenance can be dysregulated in tumors as an important mechanism of tumor resistance to immune control.[9,10] Accumulating evidence shows that antitumor immune responses can be unleashed by immune checkpoint blockade, and blockade of cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed death (PD-1) receptor have shown durable anti-melanoma effects.[11,12]

The PD-1 receptor-ligand interaction is a major pathway used by tumors to suppress immune control. PD-1 receptor (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4. It is expressed on the cell surface of activated T-cells under normal conditions.[6] By binding to its ligand (PD-L1 and PD-L2), PD-1 down-regulates T-cell activation and therefore dampens unwarranted and excessive immune responses, including autoimmunity. The interaction between PD-L1 expressed on tumor and stromal cells and PD-1 on T cells can trigger inhibitory signaling pathways that reduce effector cell functions and T-cell-killing capacity. Blocking the PD-1/PD-L1 interaction has been shown to potentiate tumor-specific CD8+ T-cell infiltration and effector T-cell activation that promote tumor rejection.[13,14]

Anti-PD1/PD-L1 therapy is, to date, one of the most effective single-agent therapies used in the treatment of melanoma, however, it has been shown that as many as 60 % of patients who receive it display primary resistance, and, approximately 25 % of melanoma patients who had objective response to anti-PD1 therapy developed acquired resistance, as characterized by disease progression at a median follow-up of 21 months.[15-17]

Several studies of advanced melanoma patients suggest that combining therapies that target tumor mechanisms of immune evasion (e.g., removing suppressive cell types via PD1/PD-L1 neutralization) with activation of normal immune cell functionality (i.e. T cell activation) may provide optimal benefits for patients. [18,19]

1.2 Pembrolizumab for advanced melanoma

Pembrolizumab is a human IgG4 PD-1 blocking antibody that is recently approved by the FDA for the treatment of patients with unresectable or metastatic melanoma and disease, including progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor. Pembrolizumab showed an unprecedented rate of durable clinical responses, with an overall response rate of 26% and median progression-free survival of 22 weeks (95% CI: 12-36 weeks).[12] Treatment has generally been well-tolerated, and the most common drug-related adverse events (AEs) of any grade were fatigue (33%), pruritus (26%), and rash (18%). Patients responding to treatment showed proliferation of CD8 T cells in their tumors.[14]

Despite the promising data associated with pembrolizumab treatment, the majority of melanoma patients do not respond to pembrolizumab. Several possible mechanisms for pembrolizumab (and other PD-1 blockade) resistance exist, including additional mechanisms of immunosuppression in the tumor microenvironment and derangements in systemic immune competence.[20,21] The evaluation of melanoma gene expression revealed that tumors infiltrated with CD8+ T cells also

showed expression of a type I interferon transcriptional signature. [22,23] Interferon gamma (IFNγ) has the capacity to increase MHC-dependent antigen presentation in a variety of cell populations, including transformed melanocytes both in vitro and in vivo[24], and is produced in response to T cell recognition of tumor-associated antigens[25]. IFNγ also causes many tumor cells to upregulate PD-L1, suggesting that an ongoing T cell response in which IFNγ is produced may prime tumors to benefit from anti-PD-1 therapy. Conversely, tumors with few tumor-related T cells have minimal IFNγ and are unlikely to benefit from PD-1-based therapies.

The failure of the immune system to recognize and eradicate cancer cells may partly be a result of insufficient immunological activation. One potential hurdle to benefit from anti-PD-1 therapy is a lack expanded tumor-related T cells. As such, there is strong rationale for combining a strategy to expand melanoma-specific tumor-infiltrating lymphocytes (TILs) with pembrolizumab.

1.3 Evidence for dendritic cell vaccine immunotherapy in cancer.

Dendritic cells (DCs) are the professional antigen-presenting cells that induce and regulate both adaptive and innate immune responses.[26,27] DCs have been used in the past by exposing these cells to some form of tumor antigen *in vitro*, and then returning antigen-loaded DCs to the patient to stimulate anti-tumor immunity.[28] Clinical trials of DC immunotherapy have suggested that this approach can result in significant stimulation of the immune response against many different forms of cancer.[29]

The use of DCs as vaccines showed beneficial effects in an autologous setting and led to the first FDA approved immunotherapy.[30,31] DC immunotherapy consists of exposing dendritic cells to tumor antigen and using the antigen-loaded DCs as a vaccine to stimulate anti-tumor response.[32,33]

Since 1996, there have been several clinical studies investigating tumor antigen-loaded DC-based vaccines, mainly in metastatic melanoma patients. [34,35] Over the years, many parameters in DC vaccination have been optimized in clinical studies; DC vaccination has only minimal side effects and thus provides a well-tolerable treatment. [36]

1.31 Dendritic Cell generation for vaccine therapy

Results of this immunotherapy method against many different forms of cancer have been encouraging in animal models but have had mixed results in clinical trials [37] Available evidence suggests that the quality of the DC preparation used in a vaccination protocol has a substantial impact on the immune response elicited. [38 35 39] Specifically, the functional status of DCs depends on their state of activation at the time they are delivered, completely activated DC (mature DCs or mDCs) are able to migrate to lymph nodes and initiate stimulation of the immune system *in vivo,* while immature DCs can inhibit effector T cell function and induce tolerance.[40] Unfortunately, many clinical trials were initiated before this distinction came to light and patients received immature DCs; inhibiting the immune response instead of stimulating it. [37] Thus, the lack of potent immune stimulation and unsatisfactory clinical response demonstrated in earlier trials of DC immunotherapy may at least in part be attributable to the functional state of the DCs employed. [40]

1.32 Ex vivo-generated DC vaccines.

DCs that are generated *ex vivo* by culturing hematopoietic progenitor cells or monocytes with cytokine combinations have been tested as therapeutic vaccines in cancer patients for more than a

decade. [29] [41] Treatment of metastatic prostate cancer with sipuleucel-T (also known as APC 8015), approved by the US Food and Drug Administration (FDA) for the treatment of metastatic prostate cancer, consists of a cellular product based on enriched blood antigen-presenting cells (APCs) that are briefly cultured with a fusion protein of prostatic acid phosphatase (PAP) and GM-CSF; this resulted in an approximately 4-month-prolonged median survival in Phase III trials. [42] [29]

A Phase III trial in metastatic melanoma that tested peptide vaccine in combination with high-dose IL-2 versus IL-2 alone showed significant improvement in overall response rate and progression-free survival in patients who received the vaccine.[43] Also, a Phase III trial in patients with follicular lymphoma (FL) showed that an idiotype vaccine therapy significantly prolongs the duration of chemotherapy-induced remission [44] (Table 1).

Table 1 Examples of clinical trials testing vaccination with ex vivo DCs [65]

Vaccine and antigen	Indication	Key observations	Refs
GM-CSF–IL-4 DCs with or without HLA-A*0201-restricted peptides or peptides alone	Metastatic prostate cancer	One of the first studies that tested the immunogenicity of DCs	45
GM-CSF–IL-4 DCs with peptides, tumour lysates or autologous tumour-eluted peptides	Stage IV melanoma, renal cell carcinoma and malignant glioma	• Loading DCs with complex antigen preparations • Objective clinical responses	46-48
Blood DCs and idiotype antigens	Multiple myeloma	• Immunogenicity of DCs • Tumour regression	49,50
Mature GM-CSF–IL-4 DCs and peptides	Stage IV melanoma	• Well-controlled and validated vaccine manufacture process • Testing mature DCs • Immunogenicity • Objective clinical responses	51
$CD34^+$ HPC-derived DCs and peptides	Stage IV melanoma	• One of the first studies to test $CD34^+$ HPC-derived DCs • Loading vaccines with a mixture of well-defined peptides • Durable immune responses in long-term survivors • Objective clinical responses	52,53
FLT3 ligand-expanded blood DCs and altered peptides	Advanced CEA^+ cancer	• Immunogenicity • Objective clinical responses	54
Immature GM-CSF–IL-4 DCs	Healthy volunteers	Antigen-specific inhibition of effector T cell function after injection of immature DCs	40
GM-CSF–IL-4 DCs and tumour lysates	Refractory pediatric solid tumors	• Immunogenicity • Objective clinical responses	55
Mature cryopreserved GM-CSF–IL-4 DCs	Stage IV melanoma	Immunogenicity	56
DCs loaded with autologous tumour RNA	Colon cancer	• Feasibility • Immunogenicity	57
DCs loaded with killed allogeneic tumour cells	Stage IV melanoma	• Immunogenicity • Durable objective clinical responses • Long-term survival	58,59
Monocyte-derived DCs loaded with the NK T cell ligand α-galactosylceramide	Advanced cancer	Adjuvant effect of NK cell activation on $CD8^+$ T cell-mediated immune response	60
Monocyte-derived DCs	Melanoma	*In vivo* identification of antigen-specific immune response by PET imaging in patients	61
		Route of DC administration affects T cell activation, with intra-dermal administration showing better responses than intra-nodal administration	62

Vaccine and antigen	Indication	Key observations	Refs
Comparative study of $CD34^+$ HPC-derived Langerhans cells versus monocyte-derived DCs	Melanoma	Langerhans cell-based vaccines stimulated significantly greater tyrosinase-HLA-A^*0201 tetramer reactivity than the monocyte-derived DC vaccines	63
Type 1-polarized monocyte-derived DCs	Glioma	Combination of DC vaccination with polyICLC to trigger systemic inflammation driven by type I interferon family members	64
CEA, carcinoembryonic antigen; DC, dendritic cell; IL-4, interleukin-4; GM-CSF, granulocyte–macrophage colony-stimulating factor; HLA, human leukocyte antigen; HPC, haematopoietic progenitor cell; NK cell, natural killer cell; PET, positron emission tomography; polyICLC, polyinosinic–polycytidylic acid stabilized with poly-L-lysine and carboxymethylcellulose.			

1.33 *In situ* DC tumor antigen loading for vaccine therapy

Differences in loading of DC with antigen *in vitro* may induce immune tolerance rather than stimulation.[27,39] The optimal method for preparation of antigen and antigen loading to DC remains to be identified. [35] Optimal tumor antigen delivery is one of the most important factors for DC-based immunotherapy success.[66] For that reason, autologous tumor cell lysate, whole tumor cells, and mRNA have been tested as antigen providers for DCs. [67-69] Furthermore, allogeneic melanoma cell lysates also constitute a valuable alternative (Table 1).[58,66]

Route of DC delivery also critically impacts efficacy of DC to stimulate an anti- tumor response. Inconsistent generation of systemic anti-tumor immune responses with intravenous and subcutaneous administration has prompted evaluation of novel delivery approach. [70] Intratumor DC delivery was shown to generate anti-tumor immunity and tumor regression in animal models.[71] Also, clinical trials to date have shown this approach to be safe with promising response[72]

Antigen can be generated *in vivo* using conventional therapies including chemical, radiation or even cryoablation.[73-75] Recently Kolstad et al reported promising results in FL patients treated with radiation, and intra- tumor injection of rituximab and DC.[75] Direct injection *in vivo* offers two advantages: delivery of mature DCs into a milieu of dying tumor cells, resulting in maximum exposure of tumor cell antigens; and avoidance of the suppressive effects of blood monocytes.[76] This combined approach was found to be feasible and safe in prior studies performed by our group in Lymphoma (Lin, et. al., unpublished observations). We will evaluate this method in addition to pembrolizumab treatment to enhance clinical efficacy against melanoma.

1.34 Safety considerations from DC therapy

DC vaccine therapy clinical trials in melanoma and other types of have thus far demonstrated no significant adverse events or autoimmunity; DC immunotherapy has been used for many different tumors types where adverse events (such as induced autoimmunity) were monitored. Importantly, very few significant adverse effects of DC vaccination have been reported and DC immunotherapy clinical trials performed to date have shown no evidence of resultant generation of auto-immunity. (Table 1). To date, 15 patients with lymphoma, 19 patients with ovarian cancer, and 30 patients with glioblastoma have been treated with DC therapy at Mayo Clinic without any serious drug related toxicity reported. The most common adverse event reported was injection site pain, more often at the time of cryoablation, that usually resolved within 2 days of treatments.

1.4 Rationale for pneumococcal conjugate vaccine (PCV) therapy

PCV (Prevnar®) is approved by the US Food and Drug Administration for vaccination against pneumococcal infections in children and works by activating helper T cells to interact with B cells for antibody production. [77] Pneumococcal vaccination has been shown to be safe and recommended for cancer patients that will or have undergone chemotherapy. Thus, the use of pneumococcal vaccines presents little if any additional risk to the patient. [78] The use of these vaccines does allow determination of the state of the immune system and its response to the DC based vaccines. Prevnar has been used in two clinical trials for multiple myeloma patients in this manner. These trials included one with adoptive T cell transfer after stem cell transplant[79] and another with lenalidomide. [80] Both trials reported minimal toxicity. In addition there may be a bystander effect on immunity with enhanced lymphocyte trafficking and the response to one antigen leading to enhanced response to anti-tumor antigens. [81]

Preliminary studies performed by Yin and colleagues showed that Prevnar13 enhanced mature DC stimulated CD4 T cell proliferation by approximately 1.75 fold compared to mature DC stimulation alone (Figure 1). Importantly, a review of the results of DC therapy in melanoma suggests that addition of adjuvant capable of T cell help significantly improved overall clinical response. [82] [81] This vaccine will allow us to monitor the immune response prior to and after our intervention. The vaccine will also allow us to quantify any potential in vivo bystander effect due to the combination of DC and pneumococcal vaccine. In the LS1081 study (Vaccine Therapy With or Without Cryosurgery in Treating Patients With Residual, Relapsed, or Refractory B-Cell Non-Hodgkin Lymphoma) no adverse events were seen with Prevnar administrations.

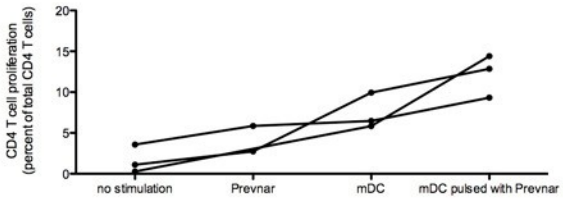


Figure 1. CD4+ T cell proliferation is stimulated with mature dendritic cells pulsed with Prevnar. Monocyte-depleted PBMNC from healthy donors were labeled with CFSE and cultured for 7 days without stimulation or with Prevnar, autologous mature dendritic cells (mDC), or autologous mDC pulsed with Prevnar. At the end of culture, cells were stained with 7-AAD, anti-CD3 and anti-CD4 and analyzed by flow cytometry. Percent of live, CD4 T cells that proliferated are shown here for 3 donors. CD4 T cell proliferation was the greatest in co-culture with Prevnar-pulsed mDC.

1.41 Safety considerations for the use of 13-valent pneumococcal conjugate vaccine (PCV) therapy

PCV (Prevnar®) is approved by the US Food and Drug Administration for vaccination against pneumococcal infections in children and works by activating helper T cells to interact with B cells for antibody production.[77] Pneumococcal vaccination is considered safe for cancer patients undergoing chemotherapy with mild side effects including soreness, low-grade fever and induration at the site of vaccination lasting 1-3 days.[78] It has been used in two clinical trials for multiple myeloma patients, one with adoptive T cell transfer after stem cell transplant [79] and another with lenalidomide.[80] Both trials reported minimal toxicity and some evidence of

improved immune response. As in Rapoport et al, we will use this pneumococcal vaccine to evaluate and monitor the developing immune response. The proposed Prevnar13 (Prevnar) dosing schedule in this study is similar to the Prevnar schedule in LS1081 study. No significant toxicities were observed in 15 patients who received Prevnar along with their DC vaccines. We will use the dose administration and timing as approved for use (Prevnar product insert: http://www.pfizerpro.com/content/showlabeling.asp?id=501).

1.5 Cryoablation therapy

1.51 Cryoablation background

Percutaneous cryoablation systems are currently available from two manufacturers including HealthTronics, Incorporated (Austin, TX) and Galil Medical (St. Paul, MN); each are FDA 510K cleared for treatment of soft-tissue tumors. Since 2003, investigators in this study have used cryoablation for the treatment of renal, lung, soft tissue, and bone tumors, including treatment of patients enrolled in the LS1081 study. Probes may be placed percutaneously using US or CT image guidance.

The mechanism of cryoablation is based on delivery of argon gas through a segmentally insulated probe with expansion of the gas within the probe lumen, resulting in rapid cooling (via Joule Thompson effect). With expansion of the argon gas within the probe, rapid and extreme temperature drops are produced along the distal, uninsulated probe shaft reaching –100 ºC within a few seconds. Adjacent tissues are quickly frozen, resulting in the formation of an iceball. Thawing of the iceball is achieved with either active instillation of helium gas into the cryoprobe instead of argon gas or activation of a small heating element in the distal probe shaft.

Cell death is due to two primary causes. First, rapid freezing immediately adjacent to the probe results in intracellular ice formation, organelle disruption, and subsequent cell destruction. At a further distance from the probe, relative gradual cooling causes osmotic differences across the cell membrane with secondary cellular dehydration and death. Subsequently, further cell death occurs due to thrombosis of affected blood vessels and resultant ischemic necrosis.

The size of the iceball varies depending on the length of uninsulated tip, with commonly used cryoprobes generating an iceball of about 3 cm in diameter and 5 cm in longitudinal length along the probe shaft. The growth and size of the iceball can be controlled by the delivery system through the relative amount of argon passed through the cryoprobe. Importantly, the margins of the iceball are visible with CT, MRI and ultrasound, allowing monitoring of treatment. Given relative cellular tolerance to freezing temperatures, cell death occurs within about 3-5 mm internal to the iceball margin [83, 84]. Following planned cryoprobe placement(s), a target lesion is typically treated with curative intent using two freezing cycles of approximately 10 minutes each, separated by a 5-minute thaw period.

1.52 Safety considerations for cryoablation

Outside of the liver, complications due to cryoablation are uncommon. A large experience exists regarding percutaneous cryoablation of renal tumors, showing a major complication rate of 5-8%. [85,86]. Similar to radiofrequency ablation (RFA), the majority of complications are related to local tissue trauma, including hemorrhage in about 3% of patients. Allowing for a more limited published experience, complications following bone and soft tissue ablation are even more infrequent, ranging from 0- 2% [87 88 89]. Unique to cryoablation, compared to RFA, is the ability to visualize the cytotoxic ice develop using CT imaging, allow the user to precisely control the volume of tissue treated and minimize adjacent tissue injury.

Of particular note, a systemic phenomenon called “cryoshock” has been observed primarily in the cryoablation of liver tumors. Cryoshock is clinically manifested by severe coagulopathy, disseminated intravascular coagulation, and multiorgan failure, possibly related to a systemic inflammatory response syndrome. Historically, this syndrome has been seen in about 1% of cases of hepatic cryotherapy and only 0.04% of prostate cryoablations.[90] More recent experience describing percutaneous cryoablation of hepatic tumors in selected patients showed grade 3 or greater complications in 6% of patients, including cryoshock in 3 of 342 procedures (0.9%) (Littrup et al. Abd Rad 2016; 41:767-780). In these 3 patients, tumors measuring larger than 3cm in size were ablated; cryoshock did not occur in the treatment of smaller hepatic lesions.

1.6 Rationale for the proposed study and research hypothesis

Among patients with advanced melanoma, pembrolizumab administration is associated with an overall objective response rate of 33%, 12-month progression-free survival rate of 35%, and median overall survival of 23 months; [91] approximately 25 % of melanoma patients who demonstrated an objective response to pembrolizumab therapy developed disease progression; results are similar to those reported for nivolumab. Moreover, many patients’ tumors primarily fail to respond to pembrolizumab.

Agents capable of stimulating infiltration and functional activation of both T cells and APCs in such tumors could synergize with PD-1 blockade to increase the frequency of responding patients and expand the range of tumors treatable with PD-1 inhibitors.[92] Such stimulatory agents have the potential to enhance the extent or durability of responses even in patients who have failed to respond to pembrolizumab monotherapy.

Pembrolizumab (MK-3475) is a highly selective, humanized monoclonal IgG4-κ isotype antibody against PD-1 that is approved around the world for the treatment of patients with unresectable or metastatic melanoma with disease progression following ipilimumab and, if BRAFV600 (NCBI accession number NM_004324.2) mutated, a BRAF inhibitor. Pembrolizumab is well-tolerated at 200mg/3 weeks with an acceptable adverse effects profile. This therapy in combination with an anti-melanoma vaccine capable of expanding melanoma-specific T cells might dramatically improve response rated to Anti-PD-1 therapy.

We will conduct a Phase II clinical trial with a safety run-in phase for patients with unresectable or metastatic melanoma to test the hypothesis that PD-1 blockade combined with cryotherapy and a DC vaccine will have an acceptable adverse event profile and will enhance mDC priming of T cells with anti-tumor antigen and activate T cell functions. Combined immunotherapy with pembrolizumab and cryoablation followed by intra-tumoral injection of mDCs will generate anti-tumor immunity and clinical regression of melanoma in patients who have not responded optimally to PD-1 axis-targeting therapy.

2.0 Goals

2.1 Primary

To determine the objective response rate (ORR) of pembrolizumab combined with cryoablation and intratumoral mDCs in patients with metastatic melanoma that has failed to respond or has stopped responding to initial therapy with a PD-1 axis-blocking monoclonal antibody.

2.2 Secondary

2.21 To assess the safety profile of pembrolizumab combined with cryoablation and intratumoral mDCs in patients with metastatic melanoma that have failed to respond or have stopped responding to initial therapy with a PD-1 axis-blocking monoclonal antibody.

2.22 To determine median progression-free survival (PFS) obtained with this approach in this patient population.

2.23 To determine median overall survival (OS) obtained with this approach in this patient population.

2.3 Translational

2.31 To quantitate tumor infiltrating lymphocytes (TILs) in tumor biopsies prior to and following cryoablation and intratumoral mDCs.

2.32 To measure PD-L1 levels in tumor biopsies and blood biopsies prior to and following cryoablation and to assess whether a change in PD-L1 levels differ among those patients who met the criteria for clinical benefit (progression-free and on study for at least 6 months) and those who do not.

2.33 To measure peripheral blood mononuclear cells (PBMC) proliferation and function after coculture with frozen tumor before and after intratumoral mDC injection.

3.0 Patient Eligibility

3.1 Inclusion Criteria

3.11 Age ≥18 years of age on the day of registration.

3.12 Histological or cytologically confirmed diagnosis of unresectable stage III or metastatic melanoma (stage IV) not amenable to curative local therapy.

3.13 Lack of response to therapy with a PD-1- or PD-L1-targeting monoclonal antibody (pembrolizumab, nivolumab, etc.) after at least 18 weeks of therapy OR documented progression of disease at any time after initiation of therapy with a PD-1- or PD-L1-targeting monoclonal antibody.
NOTE: This treatment could have been at any time prior to registration. If given in the adjuvant setting, progression must have been ≤26 weeks after the last dose of therapy.

3.14 ECOG Performance Status (PS) 0 or 1 (Appendix I)

3.15 Minimum of 3 radiographically apparent lesions such that there is:

(1) Minimum of one lesion in areas that have not been previously irradiated that is considered measurable by RECIST 1.1 criteria

AND

(2) Minimum of two lesions in areas that have not been previously irradiated that are determined by Interventional Radiology to be of a size and in a location that a single probe could ablate at least 75% of the lesion.

Note: Hepatic lesions measuring ≤3cm may be treated, as determined by Interventional Radiology.

Note: Brain metastases are not acceptable as lesions defining measurable disease, nor are they candidate lesions for cryoablation.

3.16 Adequate venous access for apheresis as assessed by apheresis team.
NOTE: If a central venous catheter is required for apheresis, the patient is not eligible.

3.17 The following laboratory values obtained ≤14 days prior to registration.

- Absolute neutrophil count (ANC) ≥1000/mm^3
- Absolute lymphocyte count ≥500/mm^3
- Platelet count ≥100,000/mm^3
- Hemoglobin ≥10 g/dL
- Total bilirubin ≤1.5 x upper limit of normal (ULN), unless due to Gilbert's disease
- Aspartate transaminase (AST/SGOT) and alanine transaminase (ALT/SGPT) ≤2.5 x ULN
- Creatinine ≤1.5 x ULN or calculated creatinine clearance ≥60 mL/min for subject with creatinine>1.5 x institutional ULN

3.18 Negative serum pregnancy test for persons of childbearing potential ≤7 days prior to registration.

3.19a Provide written informed consent.

3.19b Willing to return to the enrolling institution for follow-up (during active

treatment and active monitoring phase of the study).

3.19c Willing to provide tissue and blood samples for research purposes (see Sections 6.0, 14.0 and 17.0).

3.19d Willing to use adequate contraception while on the study and until 120 days after the last dose of study drug.

3.2 Exclusion Criteria

3.21 Choroidal melanoma.

3.22 Any of the following because this study involves an investigational agent whose genotoxic, mutagenic and teratogenic effects on the developing fetus and newborn are unknown:
- Pregnant persons
- Nursing persons

3.23 History of HIV, hepatitis B, or hepatitis C,

3.24 Active tuberculosis or active, non-infectious pneumonitis

3.25 Evidence of interstitial lung disease

3.26 Active infection requiring the use of systemic antibiotics

3.27 Symptomatic congestive heart failure (New York Heart Association Classification III or IV cardiovascular disease (Appendix II)),
myocardial infarction ≤6 months prior to registration ,
unstable angina pectoris or cardiac arrhythmia ≤3 months prior to registration, or cardiac arrhythmia.

3.28 Currently receiving or have received any other investigational agent considered as a treatment for the primary neoplasm ≤21 days prior to registration.

3.29a History of other primary malignancy requiring systemic treatment ≤3 years prior to registration. Patients must not be receiving chemotherapy or immunotherapy for another cancer. Patients must not have another active malignancy requiring active treatment.
EXCEPTIONS: Non-melanotic skin cancer or carcinoma-in-situ of the cervix.

3.29b Failure to recover from prior side effects of immune checkpoint inhibitor therapy to ≤Grade 1.
NOTE: Patients will not be excluded for adrenal insufficiency or hypothyroidism secondary to immunotherapy provided they are receiving hormonal replacement

3.29c Major surgery ≤4 weeks prior to registration.

3.29d Prior chemotherapy, targeted therapy, or radiation therapy ≤2 weeks prior to registration or who has not recovered (i.e. to ≤Grade 1 or baseline) from an adverse event due to the previously administered therapy.

3.29e History of hypersensitivity and anaphylactoid reactions to pneumococcal vaccine or any component of the formulation, including diphtheria toxoid.

3.29f Active autoimmune disease such as Crohn's disease, rheumatoid arthritis, Sjögren's disease, systemic lupus erythematosus, or similar conditions requiring systemic treatment within the past 3 months or a documented history of clinically severe autoimmune disease/syndrome difficult to control in the past.

EXCEPTIONS (the following are allowed):

- Vitiligo or resolved childhood asthma/atopy
- Intermittent use of bronchodilators or local steroid injections
- Non-immunosuppressive maintenance treatments in the setting of clinically asymptomatic disease (e.g., sulfasalazine for ulcerative colitis)
- Hypothyroidism stable on hormone replacement,
- Diabetes stable with current management
- History of positive Coombs test but no evidence of hemolysis
- Psoriasis not requiring systemic treatment
- Conditions not expected to recur in the absence of an external trigger
- Secondary adrenal insufficiency from previous hypophysitis, currently on physiologic replacement steroid dosing only.

3.29g Coagulopathy, including the use of therapeutic anticoagulants that cannot be discontinued for the cryoablation procedure.
NOTE: Heparin for line patency without detectable lab abnormalities for coagulation will be allowed.

3.29h Corticosteroid use ≤14 days prior to registration.
NOTE: Patients must be off systemic corticosteroids for at least 2 weeks prior to registration. This includes oral or IV route of administration. Patients on chronic corticosteroids for adrenal insufficiency or other reasons may enroll if they receive less than 10 mg/day of prednisone (or equivalent). Patients receiving inhaled or intranasal or intra-articular steroids are not excluded.

3.29i Active CNS metastasis.
NOTE: Patients with prior brain metastases that are asymptomatic without corticosteroid use and stable or improved ≥90 days after treatment with surgery or radiation are not excluded.

3.29j Receipt of a live vaccine ≤30 days prior to registration.

4.0 Study Schedules

4.1 Test schedule for Melanoma

Tests and Procedures[1]	≤14 days prior to Registration	After Reg	Cycle 1 Day 1	Cycles 2-3	At completion of each cycle (Cycle 3+)	End of Tx
window			±1 day	±3 days	±3 days[2]	±7 days
Physical exam, weight, ECOG performance status	X		X	X	X	X
Height	X					
Serum pregnancy test	X[3]					
Hematology (WBC with differential, Hgb, PLT)	X		X[4]		X	X
Chemistry profile (creatinine, AST, ALT, alkaline phosphatase, T bili, direct bilirubin, sodium, potassium, random glucose)	X				X	X
Thyroid function cascade (TSH, reflex per Mayo standard if abnormal)	X			X	X	X
LDH	X				X	X
Coagulation (INR)	X			X[5]	X[6]	
Adverse event assessment	X				X	X
Radiologic evaluations (CT scan, PET/CT, or MRI)	X[7]				X[8]	X
Venous Access Assessment	X[9]					
Apheresis			X			
Research blood for immune monitoring[R] (See Section 14.0) [10]		X		X[11]	X	X
Research specimen submission: Tumor biopsies[R] (See Section 17.0)				X[12]	X[13]	

[1] **Cycle = 21 days**; All tests and procedures are as clinically indicated, unless noted with an R to indicate funding by research. Hematology, chemistry profile, thyroid function cascade, coagulation tests, and imaging may be performed more frequently at physician discretion.
[2] Window of 3 days applies to Cycles 2-5. After Cycle 5, window is ±7 days.
[3] For persons of childbearing potential only. Must be done ≤7 days prior to registration.
[4] Repeat hematology tests on Cycle 1, Day 1 only if most recent tests were performed >7 days prior to Cycle 1, Day 1.
[5] Coagulation tests should be repeated at the completion of Cycles 1, 2, and 4. If treatment is delayed, coagulation tests should be repeated ≤48 hours prior to tumor biopsy.
[6] Coagulation tests should be repeated at the completion of Cycles 1, 2, and 4. If treatment is delayed, coagulation tests should be repeated ≤48 hours prior to tumor biopsy.
[7] Baseline radiology ≤28 days prior to registration
[8] Imaging is performed at the end of Cycle 4 and then at the end of every fourth cycle of treatment (end of Cycle 8, 12, 16, etc.). Exception: if a patient has PD yet continues protocol treatment, the next imaging should take place two cycles after the imaging showing PD. If that patient continues on protocol treatment, imaging should then take place every 4 cycles.
[9] Assessment of venous access must be done ≥3 days prior to apheresis.
[10] Blood draw for immune monitoring will be collected prior to the start of Cycles 1-5 and prior to the start of each post-imaging cycle thereafter (prior to Cycle 9, 13, 17, etc.)
[11] In addition to the research blood collection on Day 1, research blood will be collected as per Section 14 on Cycle 2, Days 2-5. The collection on Cycle 2, Day 2 should take place 2-4 hours after completion of cryoablation and mDC therapy.
[12] Tumor biopsies will be performed immediately prior to cryoablation on Cycle 2 Day 1 or 2 and Cycle 3 Day 1 or 2
[13] Tumor biopsies will be performed at the end of Cycle 4 after radiologic evaluation

4.2 Event Monitoring/Survival Follow-up

	Event Monitoring Phase[1]				
	q. 3 months until PD	At PD	After PD q. 6 months	Death	New Primary
Event Monitoring	X	X	X	X	At each occurrence

1. If a patient is still alive 5 years after registration, no further follow-up is required.

5.0 Grouping Factor

Cohort: Run-in Phase Ib Dose level 1 vs. Run-in Phase Ib Dose level -1 vs. Continuation Phase (Phase II)

6.0 Registration Procedures

Prior to discussing protocol entry with the patient, call the Mayo Clinic Cancer Center Registration Office (507-284-2753) to confirm study status and insure that a place on the protocol is currently available to the patient

6.1 Registering a patient

To register a patient, access the Mayo Clinic Cancer Center (MCCC) web page and enter the remote registration/randomization application. The registration/randomization application is available 24 hours a day, 7 days a week. Back up and/or system support contact information is available on the Web site. If unable to access the Web site, call the MCCC Registration Office at (507) 284-2753 between the hours of 8 a.m. and 4:30 p.m. Central Time (Monday through Friday).

The instructions for the registration/randomization application are available on the MCCC web page (http://hsrwww.mayo.edu/ccs/training) and detail the process for completing and confirming patient registration. Prior to initiation of protocol treatment, this process must be completed in its entirety and a MCCC subject ID number must be available as noted in the instructions. It is the responsibility of the individual and institution registering the patient to confirm the process has been successfully completed prior to release of the study agent. Patient registration via the registration/randomization application can be confirmed in any of the following ways:

- Contact the MCCC Registration Office (507) 284-2753. If the patient was fully registered, the MCCC Registration Office staff can access the information from the centralized database and confirm the registration.
- Refer to "Instructions for Remote Registration" in section "Finding/Displaying Information about A Registered Subject."

6.2 Verification

Prior to accepting the registration, registration/randomization application will verify the following:

- IRB approval at the registering institution
- Patient eligibility
- Existence of a signed consent form
- Existence of a signed authorization for use and disclosure of protected health information

6.3 Documentation of IRB approval

Documentation of IRB approval must be on file in the Registration Office before an investigator may register any patients.

In addition to submitting initial IRB approval documents, ongoing IRB approval documentation must be on file (no less than annually) at the Registration Office (fax: 507-284-0885). If the necessary documentation is not submitted in advance of attempting patient registration, the registration will not be accepted and the patient may not be enrolled in the protocol until the situation is resolved.

When the study has been permanently closed to patient enrollment, submission of annual IRB approvals to the Registration Office is no longer necessary.

6.4 Correlative studies

A mandatory translational research component is part of this study; the patient will be automatically registered onto this component (Sections 3.19b, 14.0, 17.0).

At the time of registration, the following will be recorded:

- Patient has/has not given permission to store and use his/her sample(s) for future research of cancer at Mayo.
- Patient has/has not given permission to store and use his/her sample(s) for future research to learn, prevent, or treat other health problems.
- Patient has/has not given permission for MCCC to give his/her sample(s) to researchers at other institutions.

6.5 Treatment on protocol

Treatment on this protocol must commence at Mayo Clinic in Rochester, Minnesota under the supervision of a medical oncologist.

6.6 Treatment start

Treatment cannot begin prior to registration and must begin ≤7 days after registration.

6.7 Pretreatment

Pretreatment tests/procedures (see Section 4.0) must be completed within the guidelines specified on the test schedule.

6.8 Baseline symptoms

All required baseline symptoms (see Section 10.6) must be documented and graded.

6.9a Study Conduct

The clinical trial will be conducted in compliance with regulations (21 CFR 312, 50, and 56), guidelines for Good Clinical Practice (ICH Guidance E6), and in accordance with general ethical principles outlined in the Declaration of Helsinki; informed consent will be obtained from all participating patients; the protocol and any amendments will be subject to approval by the designated IRB prior to implementation, in accordance with 21 CFR 56.103(a); and subject records will be stored in a secure location and subject confidentiality will be maintained. The investigator will be thoroughly familiar with the appropriate use of the study drug as described in the protocol and Investigator's Brochure. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

7.0 Protocol Treatment

Use actual weight or estimated dry weight if fluid retention.

Cycle length is 21 days with the exception of Cycle 1 which may be extended an additional 7 days to allow for dendritic cell production.

Dose-limiting toxicity (DLT) is defined as one of the following events occurring during the first two cycles of protocol therapy and attributed as possibly, probably, or definitely related to protocol therapy.

- Any Grade 3+ toxicity
- Grade 2 infusion reaction, acute or chronic kidney disease, or pneumonitis that does not resolve to Grade 0-1 within 3 weeks.

7.1 Drugs used in this study

Pembrolizumab at FDA approved dose for metastatic melanoma of 200mg IV every 21 days

Autologous mature dendritic cells (mDCs) injected into the tumor site

Prevnar13® injected into the tumor site with the DCs

7.2 Run-in Phase Ib

7.21 Schedule 1 (6 patients):

Cycle 1, Day 1: Apheresis (see Section 7.3) and pembrolizumab 200mg IV

Cycles 2 and 3, Day 1: Pembrolizumab 200mg IV

Cycles 2 and 3, either Day 1 or Day 2: cryoablation, injection of 30-60 $x10^6$ mDCs, and injection of 0.5 ml Prevnar13, per Section 7.4

Cycle 4 Day 1 and all subsequent cycles: pembrolizumab 200mg IV

If at most one of the 6 patients enrolled onto Schedule 1 develops a DLT during the first 3 cycles of treatment then Schedule 1 will be carried forward to the continuation phase.

7.22 Schedule -1:

If two or more patients enrolled onto Schedule 1 develop a DLT during the first 3 cycles of treatment, then an additional 6 patients will be treated as follows:

Cycle 1, Day 1: Apheresis and pembrolizumab 200mg IV

Cycles 2 and 3, either Day 1 or Day 2: Cryoablation, injection of 30-60 $x10^6$ mDCs, and injection of 0.5 ml Prevnar13, per Section 7.5.
Note: No pembrolizumab will be given in Cycles 2 and 3 on this schedule.

Cycle 4 Day 1 and all subsequent cycles: pembrolizumab 200mg IV

- If at most one of the 6 patients enrolled onto Schedule -1 develops a DLT during the first 3 cycles of treatment then Schedule -1 will be carried forward to the continuation phase.
- If two or more of the patients enrolled onto Schedule -1 develop a DLT during the first 3 cycles of treatment, enrollment will be discontinued for the study team to review the safety data and consider amending the protocol.

7.3 Phase II

Schedule of pembrolizumab, cryoablation, mDCs, and Prevnar13 will be based on the tolerability findings of the run-in phase

7.4 Apheresis

Patients will undergo apheresis on Cycle 1, Day 1, from which monocytes will be collected by immunomagnetic isolation and cultured *in vitro* under GMP conditions to generate mature DCs (mDCs). The generated mDCs will be frozen and stored until ready for use. Autologous mature dendritic cells (mDCs) will be prepared for administration into the cryoablated tumor. At a minimum, mDCs necessary for 2 injections (30-60 $x10^6$ per injection) will be manufactured. Additional mDCs and lysate will be stored for potential retreatment and immune monitoring assays. If insufficient mDCs are available for treatment, the total mDCs should be split into two doses, and the treating physician may determine whether to continue with protocol treatment or take the patient off study.

NOTE: If first mDC production attempt does not yield any releasable mDCs, then one additional apheresis may be attempted.

7.5 Cryoablation, mDC administration, and Prevnar13 administration

Each patient will undergo percutaneous cryoablation within 36 hours of pembrolizumab treatment on Cycles 2 and 3. (Generally, this will take place on Cycle 2, Day 2, and Cycle 3, Day 2. However, it is acceptable that cryoablation could be performed on Cycle 2, Day 1, and Cycle 3, Day 1.) Two distinct metastatic lesions will be treated with cryoablation, one during Cycle 2, and the other during Cycle 3. Our general cryoablation technique has been previously described (McMenomy et al. JVIR 2013; 24:207-213). Due to the extended length of the procedure, requiring the patient to lie still (expected duration 45 minutes), sedation will be managed by the Department of Anesthesia. Using ultrasound and/or CT guidance, a cryoprobe will be placed in the index melanoma lesion. Research biopsies (6 18-gauge cores) will be obtained immediately prior to freezing the tumor. The lesion will be treated using a conventional freeze-thaw- freeze cycle with the endpoint being the generation of an iceball that measures at least 75% of the diameter of the index tumor but may encompass the entire tumor with a margin of normal tissue.

Following the freezing procedure, the cryoprobe will be actively warmed and then withdrawn. After the lesion has thawed, a 22 G needle will be placed under ultrasound or CT-guidance into the ablated portion of the lesion. The mDC will be administered through this needle over different areas of the cryoablated region and then flushed with 1ml of sterile saline. Prevnar13® will be injected into the ablated portion of the lesion after administration of mDCs. The patient will be transferred to the recovery area and monitored for 2 hours following cryoablation and DC injections for acute toxicity. Given the elevated risk of solid organ cryoablation, patients undergoing ablation of such tumors will be admitted to the hospital for overnight observation, including hematologic assessment prior to discharge.

7.6 Duration of therapy

Patients may remain on pembrolizumab treatment for up to two years. After two years, patients go to event monitoring and may receive pembrolizumab off protocol at the discretion of the treating provider.

8.0 Dosage Modification Based on Adverse Events

8.1 Treatment schedule modifications in patients based on adverse events

Patients will be evaluated by the study team prior to each dose of pembrolizumab for an adverse event check. Determination will be made if the adverse event is treatment-related (possible, probable or definite), i.e. a toxicity (see Section 10; Adverse event monitoring and reporting). If any adverse event has occurred, then the treatment schedule will be modified as in Section 8.2. If an adverse event occurs during Cycle 2 that requires treatment to be held, then omit pembrolizumab for Cycle 3 and resume pembrolizumab with Cycle 4.

8.2 Treatment schedule modifications for pembrolizumab.

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs per Table 8.21 below. See Section 9.0 for supportive care guidelines, including use of corticosteroids.

Table 8.21 Dose Modification Guidelines for Drug-Related Adverse Events

CTCAE System/Organ/Class (SOC)	Adverse Event	Grade	Action
General disorders and administration site conditions	Infusion related reaction	2	Hold all protocol treatment until Grade 0-1 If AE does not resolve to Grade 0-1 within 3 weeks, then permanently discontinue all protocol treatment
		3-4	Permanently discontinue all protocol treatment
Respiratory, thoracic and mediastinal disorders	Pneumonitis	2	Hold all protocol treatment until Grade 0-1 If AE does not resolve to Grade 0-1 within 3 weeks, then permanently discontinue all protocol treatment
		3-4	Permanently discontinue all protocol treatment
Renal and urinary disorders	Acute kidney injury or Chronic kidney disease	2	Hold all protocol treatment until Grade 0-1 If AE does not resolve to Grade 0-1 within 3 weeks, then permanently discontinue all protocol treatment
		3-4	Permanently discontinue all protocol treatment
	All Other Nonhematologic Events	3	Hold all protocol treatment until Grade 0-1 If AE does not resolve to Grade 0-1 within 3 weeks, then permanently discontinue all protocol treatment
		4	Permanently discontinue all protocol treatment

9.0 Ancillary Treatment/Supportive Care

9.1 Full Supportive Care

Patients should receive full supportive care while on this study. This includes blood product support, antibiotic treatment, and treatment of other newly diagnosed or concurrent medical conditions. All blood products and concomitant medications such as antidiarrheals, analgesics, and/or antiemetics received from within 28 days before the study treatment administration until 30 days after the final dose will be recorded in the medical records.

9.11 Antiemetics

Antiemetics may be used at the discretion of the treating physician.

9.12 Non-Steroidal Anti-Inflammatory Drugs (NSAIDS)

NSAIDs use limited to standard non-prescription doses.

9.13 Blood Products and Growth Factors

Blood products and growth factors should be utilized as clinically warranted and following institutional policies and recommendations. The use of growth factors should follow published guidelines of the American Society of Clinical Oncology: Recommendations for the Use of WBC Growth Factors: American Society of Clinical Oncology Practice Guidelines Update. Journal of Clinical Oncology 2015;33:3199-3212..

9.2 Suggested supportive care measures for the management of adverse events with potential immunologic etiology

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. 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 does not need to follow the treatment guidance (as outlined below). Refer to Section 8.0 for dose modification.

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

- **Pneumonitis**:
  - For Grade 2 events, treat with systemic corticosteroids at 0.5 -1 mg/kg of prednisone or equivalent. Monitor O2 saturations. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
  - For Grade 3 events. Rule out infectious events, but do not hold steroids while doing so. Consider hospitalization, start steroids at 2 mg/kg of prednisone or equivalent. Monitor O2 and administer oxygen as needed. Once symptoms

improve to grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Consider bronchoscopy, to evaluate for infection.

- For Grade 4 events, patients should be hospitalized in an intensive care unit, and started on high dose IV steroids at 1 gram of methylprednisolone. Rule out infectious etiology, but do not hold steroids while doing so. Consult Pulmonology and patients should undergo bronchoscopy. 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). Any patients with these symptoms should immediately undergo CT of the abdomen to rule out bowel perforation.

  - 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, lasting >one week patients should be started on budesonide 12 mg QD. Additionally infectious etiologies should be ruled out. Do not hold steroids while ruling out infection. For patients who do not respond to budesonide or have progressive diarrhea, these patients should be started on systemic steroids at 0.5-1 mg/kg of prednisone or equivalent.
  - For Grade 3 diarrhea/colitis, patients should be started on budesonide at 12 mg daily (if not already started) and 2 mg/kg of prednisone or equivalent. IF no response within 3 days, patient should be hospitalized and started on IV steroids (methylprednisolone 500 mg-1000mg/daily). Beware of rebound diarrhea.
  - For Grade 4 diarrhea/colitis patients should be hospitalized and started on 1 gram of methylprednisolone daily, and 12 mg budesonide QD, for up to 3 days (then reduce to 2 mg/kg of prednisone or equivalent) and consult with GI immediately. Patients who do not respond to steroids, may consider infliximab (under direction of GI). Patients who respond initially to high dose IV steroids can be transitioned to high dose oral steroids (2mg/kg). Beware of rebound diarrhea.
  - For all patients, 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.**
  - For T1DM or Grade 3-4 Hyperglycemia
    - Insulin replacement therapy is recommended for Type I diabetes mellitus, treat based on grade of hyperglycemia as below.
    - For Grade 3 Hyperglycemia- Consider hospitalization, insulin therapy is required. Monitor glucose closely, and refer to Endocrinology for further management.
    - For Grade 4 hyperglycemia associated with metabolic acidosis or ketonuria. Hospitalize patient (consider admission to an ICU), immediate insulin therapy is necessary, and refer to endocrinology for further management.

- Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

- **Hypophysitis:**
  - For Grade 2 events, treat with corticosteroids 0.5mg/kg. When symptoms improve to Grade 1 or less, steroid taper should be started and be rapidly tapered to physiologic steroid dosing provided patient remains asymptomatic (some patients may require a slower taper). Replacement of appropriate hormones may be required as the steroid dose is tapered. Refer to Endocrinology for further management.
  - For Grade 3-4 events, treat with high dose (1-2 mg/kg) of prednisone or equivalent). When symptoms improve to Grade 1 or less, rapidly taper to physiologic steroid dosing provided patients remains asymptomatic (some patients may require a slower taper). Replacement of appropriate hormones may be required as the steroid dose is tapered. Refer to endocrinology for further management.

- **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:
    - Non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
  - Grade 3-4 hyperthyroidism
    - As above- Additionally patients should be hospitalized and may need possible thyroid suppression therapy. Patients should be immediately referred to an endocrinologist for treatment.
  - Hypothyroidism (TSH <10)
    - Asymptomatic- no intervention needed, continue to monitor TFTs
    - Symptomatic- thyroid replacement indicated.
  - Hypothyroidism (TSH >10)
    - Thyroid replacement indicated.

- **Hepatic:**
  - For Grade 2 events, monitor liver function tests twice weekly until returned to Grade 1 and/or baseline values.
    - Treat with oral steroids at 1 mg/kg of prednisone or equivalent. Once levels return to grade 1 or less, begin steroid taper and taper over at least 4 weeks.
  - For Grade 3:
    - Rule out other causes (i.e., progressive disease)
    - Asymptomatic treat with 1-2 mg/kg of prednisone or equivalent
    - Symptomatic- treat with IV steroids, 500 mg of methylprednisolone or equivalent, Consider hospitalization. Once resolved to grade 1 taper steroids over at least 4 weeks.
  - For Grade 4:
    - Rule out other causes (i.e., progressive disease)

        - Hospitalize, and treat with intravenous corticosteroids (1 gram methylprednisolone) for 24 to 48 hours. Once stable, may switch to oral high dose steroids.
    - When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.
    - For patients who are refractory to steroids, consider hepatology consult and starting mycophenolate.
- **Renal Failure or Nephritis:**
    - For Grade 2 events, treat with steroids at 1 mg/kg prednisone or equivalent. Once resolved to Grade 1, may start taper over one month.
    - For Grade 3 events, consider hospitalization, consult nephrology, renal biopsy may be indicated. Administer steroids at 2 mg/kg prednisone or equivalent.
    - For Grade 4 - hospitalization, consult nephrology, renal biopsy as indicated, treat with IV systemic corticosteroids 1 gram of methylprednisolone. Dialysis may be indicated.
    - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
- **Rash**
    - Grade 2 - Treat symptomatically with topical steroid cream, as well as antihistamines (i.e., loratidine, diphenhydramine, etc.). For patients that do not respond, consider starting steroids at 0.5 mg/kg prednisone or equivalent.
    - Grade 3 - As above for grade 2 and Start steroids at 1 mg/kg prednisone or equivalent.
    - If any signs of Steven Johnson Syndrome (SJS) or Toxic Epidermal Necrolysis (TENS), immediately hospitalize patient and treat with high dose IV steroids (1 gram methylprednisolone) and consult dermatology.
- **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 9.21 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab.

Table 9.21 Infusion reaction treatment guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for ≤24 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 adverse events despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be pre-medicated 1.5h (±30 minutes) prior to infusion of pembrolizumab with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine) Acetaminophen 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.		

10.0 Adverse Event (AE) Reporting and Monitoring

The site principal investigator is responsible for reporting any/all serious adverse events to the sponsor as described within the protocol, regardless of attribution to study agent or treatment procedure.

The sponsor/sponsor-investigator is responsible for notifying FDA and all participating investigators in a written safety report of any of the following:

- Any suspected adverse reaction that is both serious and unexpected.
- Any findings from laboratory animal or *in vitro* testing that suggest a significant risk for human subjects, including reports of mutagenicity, teratogenicity, or carcinogenicity.
- Any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies, whether or not conducted under an IND and whether or not conducted by the sponsor, that suggest a significant risk in humans exposed to the drug
- Any clinically important increase in the rate of a serious suspected adverse reaction over the rate stated in the protocol or Investigator's Brochure (IB).

Summary of SAE Reporting for this study
(please read entire section for specific instructions):

WHO:	WHAT form:	WHERE to send:
All sites	Pregnancy Reporting http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/PregnancyReportFormUpdated.pdf	Mayo Sites – attach to MCCC Electronic SAE Reporting Form
Mayo Clinic Sites	Mayo Clinic Cancer Center SAE Reporting Form: http://livecycle2.mayo.edu/workspace/?startEndpoint=MC4158-56/Processes/MC4158-56-Process.MC4158-56 AND attach MedWatch 3500A: http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM048334.pdf	Will automatically be sent to CANCERCROSAFETYIN@mayo.edu

Definitions

Adverse Event
Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Suspected Adverse Reaction
Any adverse event for which there is a reasonable possibility that the drug caused the adverse event.

Expedited Reporting
Events reported to sponsor within 24 hours, 5 days or 10 days of study team becoming aware of the event.

Routine Reporting
Events reported to sponsor via case report forms

Events of Interest
Events that would not typically be considered to meet the criteria for expedited reporting,

but that for a specific protocol are being reported via expedited means in order to facilitate the review of safety data (may be requested by the FDA or the sponsor).

Unanticipated Adverse Device Event (UADE)
Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects

10.1 Adverse event characteristics

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site: (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm)

a. Identify the grade and severity of the event using the CTCAE version 4.0.
b. Determine whether the event is expected or unexpected (see Section 10.2).
c. Determine if the adverse event is related to the study intervention (agent, treatment or procedure) (see Section 10.3).
d. Determine whether the event must be reported as an expedited report. If yes, determine the timeframe/mechanism (see Section 10.4).
e. Determine if other reporting is required (see Section 10.5).
f. Note: All AEs reported via expedited mechanisms must also be reported via the routine data reporting mechanisms defined by the protocol (see Sections 10.6 and 18.0).

NOTE: A severe AE is NOT the same as a serious AE, which is defined in Section 10.4.

10.2 Expected vs. unexpected events

Expected events - are those described within the Section 15.0 of the protocol, the study specific consent form, package insert (if applicable), and/or the investigator brochure, (if an investigator brochure is not required, otherwise described in the general investigational plan).

Unexpected adverse events or suspected adverse reactions are those not listed in Section 15.0 of the protocol, the study specific consent form, package insert (if applicable), or in the investigator brochure (or are not listed at the specificity or severity that has been observed); if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan.

Unexpected also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs but have not been observed with the drug under investigation.

An investigational agent/intervention might exacerbate the expected AEs associated with a commercial agent. Therefore, if an expected AE (for the commercial agent) occurs with a higher degree of severity or specificity, expedited reporting is required.

NOTE: *The consent form may contain study specific information at the discretion of the Principal Investigator; it is possible that this information may NOT be included in the protocol or the investigator brochure. Refer to protocol or IB for reporting needs.

10.3 Attribution to agent(s) or procedure

When assessing whether an adverse event (AE) is related to a medical agent(s) medical or procedure, the following attribution categories are utilized:

Definite - The AE *is clearly related* to the agent(s)/procedure.
Probable - The AE *is likely related* to the agent(s)/procedure.
Possible - The AE *may be related* to the agent(s)/procedure.
Unlikely - The AE *is doubtfully related* to the agent(s)/procedure.
Unrelated - The AE *is clearly NOT related* to the agent(s)/procedure.

10.31 EXPECTED Serious Adverse Events: Protocol Specific Exceptions to Expedited Reporting

For this protocol only, the following Adverse Events/Grades are expected to occur within this population and do not require Expedited Reporting. These events must still be reported via Routine Reporting (see Section 10.6).*

*Report any clinically important increase in the rate of a serious suspected adverse reaction (at your study site) over that which is listed in the protocol or investigator brochure as an expedited event.

*Report an expected event that is greater in severity or specificity than expected as an expedited event.

CTCAE System/Organ/Class	Adverse Event	CTCAE Grade at which the event will not be reported in an expedited manner[1]
Blood and lymphatic system disorders	Anemia	≤Grade 4
Investigations	Lymphocyte count decreased	≤Grade 4
	Neutrophil count decreased	≤Grade 4
	Platelet count decreased	≤Grade 4
	White blood cell decreased	≤Grade 4

1 These exceptions only apply if the adverse event does not result in hospitalization. If the adverse event results in hospitalization, then the standard expedited adverse events reporting requirements must be followed.
Specific protocol exceptions to expedited reporting should be reported expeditiously by investigators ONLY if they exceed the expected grade of the event.

The following hospitalizations are not considered to be SAEs because there is no "adverse event" (*i.e.*, there is no untoward medical occurrence) associated with the hospitalization:

- Hospitalizations for respite care
- Planned hospitalizations required by the protocol
- Hospitalization planned before informed consent (where the condition requiring the hospitalization has not changed post study drug administration)
- Hospitalization for elective procedures unrelated to the current disease and/or treatment on this trial
- Hospitalization for administration of study drug or insertion of access for

administration of study drug
- Hospitalization for routine maintenance of a device (*e.g.*, battery replacement) that was in place before study entry
- Hospitalization, or other serious outcomes for signs and symptoms of progression of the cancer.]

10.4 Expedited Reporting Requirements for IND/IDE Agents

10.41 **Late Phase 2 and Phase 3 Studies**: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention[1,2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria **MUST** be immediately reported to the sponsor within the timeframes detailed in the table below.

<table>
<tr><th>Hospitalization</th><th>Grade 1 Timeframes</th><th>Grade 2 Timeframes</th><th>Grade 3 Timeframes</th><th>Grade 4 & 5 Timeframes</th></tr>
<tr><td>Resulting in Hospitalization ≥24 hrs</td><td colspan="3">7 Calendar Days</td><td rowspan="2">24-Hour 3 Calendar Days</td></tr>
<tr><td>Not resulting in Hospitalization ≥24 hrs</td><td colspan="2">Not required</td><td>7 Calendar Days</td></tr>
</table>

Expedited AE reporting timelines are defined as:
- "24-Hour; 3 Calendar Days" - The AE must initially be reported within 24 hours of learning of the AE, followed by a complete expedited report within 3 calendar days of the initial 24-hour report.
- "7 Calendar Days" - A complete expedited report on the AE must be submitted within 7 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-hour notification followed by complete report within 3 calendar days for:
- All Grade 4, and Grade 5 AEs

Expedited 7 calendar day reports for:
- Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization
- Grade 3 adverse events

[2] For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

NOTE: Refer to Section 10.31 for exceptions to Expedited Reporting

10.42 General reporting instructions

The Mayo IND Coordinator will assist the sponsor-investigator in the processing of expedited adverse events and forwarding of suspected unexpected serious adverse reactions (SUSARs) to the FDA and IRB.

Use Mayo Expedited Event Report form http://livecycle2.mayo.edu/workspace/?startEndpoint=MC4158-56/Processes/MC4158-56-Process.MC4158-56 for investigational agents or commercial/investigational agents on the same arm.

10.43 Reporting of re-occurring SAEs

ALL SERIOUS adverse events that meet the criteria outlined in table10.41 MUST be immediately reported to the sponsor within the timeframes detailed in the corresponding table. This reporting includes, but is not limited to SAEs that re-occur again after resolution.

10.5 Other Required Reporting

10.51 Unanticipated Problems Involving Risks to Subjects or Others (UPIRTSOS)

Unanticipated Problems Involving Risks to Subjects or Others (UPIRTSOS) in general, include any incident, experience, or outcome that meets **all** of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
2. Related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
3. Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Some unanticipated problems involve social or economic harm instead of the physical or psychological harm associated with adverse events. In other cases, unanticipated problems place subjects or others at increased *risk* of harm, but no harm occurs.

Note: If there is no language in the protocol indicating that pregnancy is not considered an adverse experience for this trial, and if the consent form does not indicate that subjects should not get pregnant/impregnate others, then any pregnancy in a subject/patient or a male patient's partner (spontaneously reported) which occurs during the study or within 120 days of completing the study should be reported as a UPIRTSO.

Mayo Clinic Cancer Center (MCCC) Institutions:

If the event meets the criteria for IRB submission as a Reportable Event/UPIRTSO, provide the Reportable Event coversheet and appropriate

documentation to CANCERCROSAFETYIN@mayo.edu. The Mayo Regulatory Affairs Office will review and process the submission to the Mayo Clinic IRB.

10.52 Death

Note: A death on study requires both routine and expedited reporting regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.

Any death occurring within 30 days of the last dose, regardless of attribution to an agent/intervention under an IND/IDE requires expedited reporting within 24-hours.

Any death occurring greater than 30 days with an attribution of possible, probable, or definite to an agent/intervention under an IND/IDE requires expedited reporting within 24-hours.

Reportable categories of Death

- Death attributable to a CTCAE term.
- Death Neonatal: A disorder characterized by cessation of life during the first 28 days of life.
- Death NOS: A cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Sudden death NOS: A sudden (defined as instant or within one hour of the onset of symptoms) or an unobserved cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Death due to progressive disease should be reported as **Grade 5 "Neoplasms benign, malignant and unspecified (including cysts and polyps) – Other (Progressive Disease)"** under the system organ class (SOC) of the same name. Evidence that the death was a manifestation of underlying disease (e.g., radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Per NIH OBA Appendix M, should a patient die during the study or study follow-up, no matter what the cause, the study doctor will ask the patient's family for permission to perform an autopsy. If permission is granted, a copy of the autopsy report will be sent to the sponsor after all identifying information has been removed. An autopsy will help the researchers learn more about the safety and efficacy of the treatment. Patients should advise their families about their wishes regarding autopsy.

10.53 Secondary Malignancy

- A ***secondary malignancy*** is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.
- All secondary malignancies that occur following treatment with an agent under an IND/IDE will be reported. Three options are available to describe the event:
    - Leukemia secondary to oncology chemotherapy (e.g., Acute Myeloctyic Leukemia [AML])

- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

- Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

10.54 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is NOT a metastasis from the initial malignancy). Second malignancies require ONLY routine reporting unless otherwise specified.

10.55 Pregnancy, Fetal Death, and Death Neonatal

If a female subject (or female partner of a male subject) taking investigational product becomes pregnant, the subject taking should notify the Investigator, and the pregnant female should be advised to call her healthcare provider immediately. The patient should have appropriate follow-up as deemed necessary by her physician. If the baby is born with a birth defect or anomaly, a second expedited report is required.

Prior to obtaining private information about a pregnant woman and her infant, the investigator must obtain consent from the pregnant woman and the newborn infant's parent or legal guardian before any data collection can occur. A consent form will need to be submitted to the IRB for these subjects if a pregnancy occurs. If informed consent is not obtained, no information may be collected.

In cases of fetal death, miscarriage or abortion, the mother is the patient. In cases where the child/fetus experiences a serious adverse event other than fetal death, the child/fetus is the patient.

NOTE: When submitting Mayo Expedited Adverse Event Report reports for "Pregnancy", "Pregnancy loss", or "Neonatal loss", the potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agent(s) should be documented in the "Description of Event" section. Include any available medical documentation. Include this form: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/PregnancyReportFormUpdated.pdf

10.551 Pregnancy

Pregnancy should be reported in an expedited manner as **Grade 3 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy)"** under the Pregnancy, puerperium and perinatal conditions SOC. Pregnancy should be followed until the outcome is known.

10.552 Fetal Death

Fetal death is defined in CTCAE as "A disorder characterized by death in utero; failure of the product of conception to show evidence of respiration, heartbeat, or definite movement of a voluntary muscle after expulsion from the uterus, without possibility of resuscitation."

Any fetal death should be reported expeditiously, as **Grade 4 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy loss)"** under the Pregnancy, puerperium and perinatal conditions SOC.

10.553 Death Neonatal

Neonatal death, defined in CTCAE as "A disorder characterized by cessation of life occurring during the first 28 days of life" that is felt by the investigator to be at least possibly due to the investigational agent/intervention, should be reported expeditiously.

A neonatal death should be reported expeditiously as **Grade 4 "General disorders and administration - Other (neonatal loss)"** under the General disorders and administration SOC.

10.6 Required Routine Reporting

10.61 Baseline and Adverse Events Evaluations

Pretreatment symptoms/conditions to be graded at baseline and adverse events to be graded at each evaluation.
Grading is per CTCAE v4.0 **unless** alternate grading is indicated in the table below:

CTCAE System Organ Class (SOC)	Adverse event/Symptoms	Baseline	Each evaluation
Blood and lymphatic system disorders	Anemia	X	X
Endocrine disorders	Hypothyroidism	X	X
Gastrointestinal disorders	Baseline # stools	X	
	Diarrhea		X
General disorders	Fatigue	X	X
	Fever		X
	Pain	X	X
Immune system disorders	Allergic reaction		X
	Anaphylaxis		X
Investigations	Alanine aminotransferase increased	X	X
	Aspartate aminotransferase increased	X	X
	Blood bilirubin increased	X	X
	Creatinine increased	X	X
Metabolism and nutrition disorders	Hyperglycemia	X	X
Respiratory, thoracic and mediastinal disorders	Dyspnea	X	X
Skin and subcutaneous tissue disorders	Rash, maculo-papular	X	X

10.62 All other AEs

Submit via appropriate MCCC Case Report Forms (i.e., paper or electronic, as applicable) the following AEs experienced by a patient and not specified in Section 10.6:

10.621 Grade 2 AEs deemed *possibly, probably, or definitely* related to the study treatment or procedure.

10.622 Grade 3 and 4 AEs regardless of attribution to the study treatment or procedure.

10.623 Grade 5 AEs (Deaths)

10.6231 Any death within 30 days of the patient's last study treatment or procedure regardless of attribution to the study treatment or procedure.

10.6232 Any death more than 30 days after the patient's last study treatment or procedure that is felt to be at least possibly treatment related must also be submitted as a Grade 5 AE, with a CTCAE type and attribution assigned.

10.7 Late Occurring Adverse Events

Refer to the instructions in the Forms Packet (or electronic data entry screens, as applicable) regarding the submission of late occurring AEs following completion of the Active Monitoring Phase (i.e., compliance with Test Schedule in Section 4.0).

11.0 Treatment Evaluation

11.1 Treatment evaluation using RECIST 1.1 guidelines

The first scan after treatment initiation will be performed at the completion of Cycle 4; scans will be then performed at the completion of every fourth cycle of treatment until disease progression is confirmed or withdrawn of consent. If a patient experiences PD yet continues protocol treatment, the next imaging should take place 2 cycles after the imaging showing PD. If that patient continues on protocol treatment, imaging should then take place every 4 cycles (See Section 4.0).

Disease progression for this protocol is defined as meeting the RECIST criteria for disease progression on two consecutive evaluations at least 6 weeks apart.

11.2 Definitions of measurable and non-measurable disease

11.21 Measurable disease

11.211 A non-nodal lesion is considered measurable if its longest diameter can be accurately measured as ≥1.0 cm with CT scan, CT component of a PET/CT, or MRI.

11.212 A malignant lymph node is considered measurable if its short axis is ≥1.5 cm when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm).

NOTE: Tumor lesions in a previously irradiated area are not considered measurable disease.

11.22 Non-measurable disease

All other lesions (or sites of disease) are considered non-measurable disease, including pathological nodes (those with a short axis ≥1.0 to <1.5 cm). Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable as well.

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

Lymph nodes that have a short axis <1.0 cm are considered non- pathological (i.e., normal) and should not be recorded or followed.

11.3 Guidelines for evaluation of measurable disease

11.31 Measurement methods:

- All measurements should be recorded in metric notation (i.e., decimal fractions of centimeters) using a ruler or calipers.
- The same method of assessment and the same technique must be used to characterize each identified and reported lesion at baseline and during follow-up. For patients having only lesions measuring at least 1 cm to less than 2 cm must use CT imaging for both pre- and post-treatment tumor assessments.

11.32 Acceptable modalities for measurable disease:

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness.

- As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. The lesions should be measured on the same pulse sequence. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.
- PET-CT: If the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time.
- FDG-PET: FDG-PET scanning is allowed to complement CT scanning in assessment of progressive disease [PD] and particularly possible 'new' disease. A ‘positive’ FDG-PET scanned lesion is defined as one which is FDG avid with an update greater than twice that of the surrounding tissue on the attenuation corrected image; otherwise, an FDG-PET scanned lesion is considered ‘negative.’ New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:
    a. Negative FDG-PET at baseline with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
    b. No FDG-PET at baseline and a positive FDG-PET at follow-up:
        i. If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD.
        ii. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT at the same evaluation, additional follow-up CT scans (i.e., additional follow-up scans at least 4 weeks later) are needed to determine if there is truly progression occurring at that site. In this situation, the date of PD will be the date of the initial abnormal PDG-PET scan.
        iii If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, it is not classified as PD.
- The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

- Cytologic and histologic techniques can be used to differentiate between PR and CR in rare cases (e.g., residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain.)

11.4 Measurement of effect

11.41 Target lesions & target lymph nodes

- Measurable lesions (as defined in Section 11.21) up to a maximum of 5 lesions, representative of all involved organs, should be identified as "Target Lesions" and recorded and measured at baseline. These lesions can be non-nodal or nodal (as defined in 11.21), where no more than 2 lesions are from the same organ and no more than 2 malignant nodal lesions are selected.

  Note: If fewer than 5 target lesions and target lymph nodes are identified (as there often will be), there is no reason to perform additional studies beyond those specified in the protocol to discover new lesions.

- Target lesions and target lymph nodes should be selected on the basis of their size, be representative of all involved sites of disease, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion (or malignant lymph node) does not lend itself to reproducible measurements in which circumstance the next largest lesion (or malignant lymph node) which can be measured reproducibly should be selected.

- Baseline Sum of Dimensions (BSD): A sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes will be calculated and reported as the baseline sum of dimensions (BSD). The BSD will be used as reference to further characterize any objective tumor response in the measurable dimension of the disease.

- Post-Baseline Sum of the Dimensions (PBSD): A sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes will be calculated and reported as the post-baseline sum of dimensions (PBSD). If the radiologist is able to provide an actual measure for the target lesion (or target lymph node), that should be recorded, even if it is below 0.5 cm. If the target lesion (or target lymph node) is believed to be present and is faintly seen but too small to measure, a default value of 0.5 cm should be assigned. If it is the opinion of the radiologist that the target lesion or target lymph node has likely disappeared, the measurement should be recorded as 0 cm.

- The minimum sum of the dimensions (MSD) is the minimum of the BSD and the PBSD.

11.42 Non-target lesions & non-target lymph nodes

Non-measurable sites of disease (Section 11.22) are classified as non- target lesions or non-target lymph nodes and should also be recorded at base line. These lesions and lymph nodes should be followed in accord with 11.433.

11.43 Response criteria

11.431 All target lesions and target lymph nodes followed by CT/MRI/PET-CT must be measured on re-evaluation at evaluation times specified in Section 11.1. Specifically, a change in objective status to either a PR or

CR cannot be done without re-measuring target lesions and target lymph nodes.

Note: Non-target lesions and non-target lymph nodes should be evaluated at each assessment, especially in the case of first response or confirmation of response. In selected circumstances, certain non-target organs may be evaluated less frequently. For example, bone scans may need to be repeated only when complete response is identified in target disease or when progression in bone is suspected.

11.432 Evaluation of target lesions

Complete Response (CR):	All of the following must be true: a. Disappearance of all target lesions. b. Each target lymph node must have reduction in short axis to <1.0 cm.
Partial Response (PR):	At least a 30% decrease in PBSD (sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes at current evaluation) taking as reference the BSD (*see* Section 11.41).
Progression (PD):	At least one of the following must be true: a. At least one new malignant lesion, which also includes any lymph node that was normal at baseline (<1.0 cm short axis) and increased to ≥1.0 cm short axis during follow-up. b. At least a 20% increase in PBSD (sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes at current evaluation) taking as reference the MSD (Section 11.41). In addition, the PBSD must also demonstrate an absolute increase of at least 0.5 cm from the MSD. c. See Section 11.32 for details in regards to the requirements for PD via FDG-PET imaging.
Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR, nor sufficient increase to qualify for PD taking as reference the MSD.

11.433 Evaluation of Non-Target Lesions & Non-target Lymph Nodes

Complete Response (CR):	All of the following must be true: a. Disappearance of all non-target lesions.

b. Each non-target lymph node must have a reduction in short axis to <1.0 cm.

Non-CR/Non-PD: Persistence of one or more non-target lesions or non-target lymph nodes.

Progression (PD): At least one of the following must be true:

a. At least one new malignant lesion, which also includes any lymph node that was normal at baseline (<1.0 cm short axis) and increased to ≥1.0 cm short axis during follow-up.

b. Unequivocal progression of existing non-target lesions and non-target lymph nodes. (NOTE: Unequivocal progression should not normally trump target lesion and target lymph node status. It must be representative of overall disease status change.)

c. See Section 11.32 for details in regards to the requirements for PD via FDG-PET imaging.

11.44 Overall objective status

The overall objective status for an evaluation is determined by combining the patient's status on target lesions, target lymph nodes, non-target lesions, non-target lymph nodes, and new disease as defined in the following tables:

Target Lesions & Target Lymph Nodes	**Non-Target Lesions & Non-Target Lymph Nodes**	**New Sites of Disease**	**Overall Objective Status**
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	CR Non-CR/Non-PD	No	PR
CR/PR	Not All Evaluated*	No	PR
SD	CR Non-CR/Non-PD Not All Evaluated*	No	SD
Not all Evaluated	CR Non-CR/Non-PD Not All Evaluated*	No	Not Evaluated (NE)
PD	Unequivocal PD CR Non-CR/Non-PD Not All Evaluated*	Yes or No	PD

Target Lesions & Target Lymph Nodes	Non-Target Lesions & Non-Target Lymph Nodes	New Sites of Disease	Overall Objective Status
CR/PR/SD/PD/Not all Evaluated	Unequivocal PD	Yes or No	PD
CR/PR/SD/PD/Not all Evaluated	CR Non-CR/Non-PD Not All Evaluated*	Yes	PD

*See Section 11.431

11.45 Symptomatic Deterioration

Patients with global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time, and not either related to study treatment or other medical conditions, should be reported as PD due to "symptomatic deterioration." Every effort should be made to document the objective progression even after discontinuation of treatment due to symptomatic deterioration. A patient is classified as having PD due to "symptomatic deterioration" if any of the following occur that are not either related to study treatment or other medical conditions:

- Weight loss >10% of body weight.
- Worsening of tumor-related symptoms.
- Decline in performance status of >1 level on ECOG scale.

12.0 Descriptive Factors: None

13.0 Treatment/Follow–up Decision at Evaluation of Patient

13.1 Continuation of treatment

Patients who have not had disease progression and have experienced acceptable toxicity are to continue treatment per protocol until confirmed PD, unacceptable toxicity or refusal. And then they will go to event monitoring until death or a maximum of 5 years post-registration.

13.2 Progressive Disease (PD)

The first instance in which a patient's disease status meets the RECIST criteria for PD will continue two additional cycles of treatment per protocol and then undergo disease revaluation. If the initial documented progression is not confirmed by this scan, patients will continue treatment per protocol. If the initial documented progression is confirmed by this scan, patients will go to the event-monitoring phase until death or a maximum of 5 years post-registration.

Exception: Patients who develop PD in the CNS should discontinue study treatment and go to Event Monitoring.

13.3 Criteria for discontinuation of protocol therapy include:

- Disease progression: Where disease progression is: the development of a new metastatic lesion or an objective status of disease progression (as defined by RECIST criteria) on two consecutive evaluations at least 6 weeks apart (Section 13.2).

Exception: Patients who develop PD in the CNS should discontinue study treatment.

- Request by patient to discontinue study treatment
- Unacceptable toxicity
- Intercurrent illness that would, in the judgment of the investigator, affect assessments of clinical status to a significant degree or require discontinuation of drug
- Administration of radiotherapy, non-protocol chemotherapy, immunotherapy, biological agents, or an experimental drug during the trial
- Development of new primary cancer
- Ineligibility

Patients who discontinue treatment due to progression or development of a second primary, desire for non-protocol treatment, intolerability, patient request, physician decision, or inter-current illness preventing further administration of protocol treatment will proceed to event monitoring phase of the trial where patient and disease status until death or a maximum of 5 years post-registration

13.4 Ineligible

A patient is deemed *ineligible* if after registration, it is determined that at the time of registration, the patient did not satisfy each and every eligibility criteria for study entry.

- If the patient received any protocol treatment, all data (except biospecimens) up until the point of confirmation of ineligibility must be submitted. Event monitoring will be required per Section 18.0 of the protocol.
- If the patient never received any protocol treatment, on-study material (except biospecimens) and the End of Active Treatment/Cancel Notification Form must be submitted. No further No further data submission is necessary.

13.5 Withdrawn

A patient who withdraws consent before any study treatment is given. On-study material (except biospecimens) and the End of Active Treatment/Cancel Notification Form must be submitted. No further data submission is necessary.

13.6 Subsequent Treatment

Subsequent treatment is at the discretion of the attending physician.

13.7 Major violation

A patient is deemed a *major violation*, if protocol requirements regarding treatment in cycle 1 of the initial therapy are severely violated that evaluability for primary end point is questionable. All data up until the point of confirmation of a major violation must be submitted. The patient will go directly to the event-monitoring phase of the study. The patient may continue treatment off-protocol at the discretion of the physician as long as there are no safety concerns, and the patient was properly registered. Event monitoring will be required per Section 4.0 of the protocol.

13.8 Cancel

A patient is deemed a *cancel* if he/she is removed from the study for any reason before any study treatment is given. On-study material and the End of Active Treatment/Cancel Notification Form must be submitted. No further data submission is necessary.

14.0 Body Fluid Biospecimens

14.1 Summary Table of Research Blood/Blood Products to Be Collected for This Protocol

Collect and process all blood/blood products according to instructions and table below. For immunological monitoring both Panel 1 and Panel 2 will be collected.

14.11 Collection During Treatment

Correlative Study (Section for more information)	Mandatory or Optional	Blood or Body Fluid being Collected	Component being harvested	Type of Collection Tube (color of tube top)	Volume to collect per tube (# of tubes to be collected)	Day 1 of designated cycles[1]	Days 2-5 of Cycle 2	Process at collecting site? (Yes or No)	Temperature Conditions for Storage/ Shipping[2]
Immune Monitoring	Mandatory	Whole Blood	Plasma, leukocytes	Heparin (green)	10 mL (7)	X		No	Room temperature
				EDTA (purple)	6 mL (1) 4 mL (1)				
Immune Monitoring	Mandatory	Whole Blood	Plasma, leukocytes	EDTA (purple)	6 mL (1)		X[3]	No	Room temperature

1. Research blood should be collected per Section 4.0.
2. All specimens should be sent to Dr. Lin's laboratory c/o Anping Chen(pager 4-8864), Stabile 3-25
3. Specimens may be collected at any laboratory. If not collected at Mayo Clinic, specimens should be shipped overnight at room temperature to Dr. Lin's laboratory c/o Anping Chen(pager 4-8864), Stabile 3-25, Mayo Clinic, 200 First St SW, Rochester MN 55905

14.2 Background/Methodology

14.21 T cell functional assays

If available, we will keep samples of mDC from each patient and use these cells to stimulate peripheral blood collected as per the test schedule and assay for anti-tumor response via proliferation, ELIspot, or intracellular cytokine expression. We will also stimulate T cells with a cocktail of multiple peptides from common melanoma antigens.

14.22 Immune phenotyping

We will immune phenotype blood and tumor for immune cells including but not limited to Tregs, central memory and effector memory T cells, quantitative T, B, and NK cell panel, dendritic cells and immune suppressor cells (CD14+DRneg) and other immune modulating cells per our prior experience. PD-1, PD-L1 and PD- L2 expression on leukocyte subset will be analyzed.

14.23 Tetramer assays

We will compare pre- and post-treatment frequencies of melanoma-specific CD8 T cells via tetramer staining in HLA-A2-positive patients. PBMCs will be labeled with HLA-A2 tetramers complexed with melanoma-associated antigen peptides (MART-1_{27-35}, $GP100_{209-217}$, survivin-- ELTLGEFLKL, and $tyrosinase_{368-376}$), and control peptides. PBMCs will be counterstained with anti-CD8 (BD Biosciences, San Jose, CA). The stained samples will be analyzed by flow cytometry (FACScan and CellQuest software, Becton-Dickinson).

14.24 Plasma cytokine assay

We will profile the plasma cytokine changes as a result of vaccine administration using commercially available cytokine multiplex assays. We will also assess plasma levels of soluble PD-L1 via ELISA.

15.0 Drug Information

15.1 Pembrolizumab (MK-3475, Keytruda®) (commercial supply)

15.11 Background

Highly selective anti-PD-1 humanized monoclonal antibody which inhibits programmed cell death-1 (PD-1) activity by binding to the PD-1 receptor on T-cells to block PD-1 ligands (PD-L1 and PD-L2) from binding. Blocking the PD-1 pathway inhibits the negative immune regulation caused by PD-1 receptor signaling. Anti-PD-1 antibodies reverse T-cell suppression and induce antitumor responses.

15.12 Formulation

Commercially available for injection 25mg/ml (4ml) [contains polysorbate 80] in a one-vial formulation

15.13 Preparation and storage

Appropriate dose required volume should be added to an IV bag of either 0.9% sodium chloride or dextrose 5% in water, and the final concentration should be between 1 mg/ml to 10 mg/ml. Mix diluted solution by gentle inversion. Discard any unused portion left in the vial. Store bag at room temperature for no more than 6 hours (includes duration of infusion) and refrigerate [2°C to 8°C or 36°F to 46°F] for no more than 24 hours. Allow solution to come to room temperature prior to administration.

15.14 Administration:

Infuse over 30 minutes through a 0.2 to 5 micron sterile, nonpyrogenic, low-protein binding inline or add-on filter. Do not infuse other medications through the same infusion line. Compatible in either 0.9% sodium chloride or dextrose 5% in water.

15.15 Pharmacokinetic information:

Note: Clearance is ~20% lower at steady state than with the first dose. With weight-based dosing (2 mg/kg), pembrolizumab concentrations in pediatric patients are comparable to those of adults (at the same dose).
Distribution: V_{dss}: 6.1 L
Excretion – mean estimated $t_{1/2}$ values from the 28-day single dose profiles ranged from 14.1 to 21.6 days. Clearance is nonlinear and saturable.

15.16 Potential Drug Interactions

There are no known significant drug interactions.

15.17 Known potential toxicities:

Consult the package inset for the most current and complete information.

Common known potential toxicities, >10%:
Cardiovascular: Facial edema
Central nervous system: Fatigue
Dermatologic: Pruritus, skin rash
Endocrine & metabolic: Hyperglycemia, hyponatremia, hypoalbuminemia, hypertriglyceridemia, hypocalcemia, decreased sodium bicarbonate
Gastrointestinal: Nausea, decreased appetite, constipation, diarrhea, vomiting,

abdominal pain
Hematologic & oncologic: Anemia, lymphocytopenia
Hepatic: Increased serum AST, increased serum ALT, increased serum alkaline phosphatase
Neuromuscular & skeletal: Arthralgia
Respiratory: Cough, dyspnea
Miscellaneous: Fever

Less common known potential toxicities, 1% - 10%:
Central nervous system: Confusion, peripheral neuropathy
Endocrine & metabolic: Hypothyroidism, hyperthyroidism
Gastrointestinal: Colitis
Immunologic: Antibody development
Neuromuscular and skeletal: Weakness, arthritis
Respiratory: Pneumonitis, pleural effusion, pneumonia, respiratory failure

Rare known potential toxicities, <1% (Limited to important or life-threatening):
Adrenocortical insufficiency (immune-mediated), Bullous pemphigoid (immune-mediated), chronic inflammatory demyelinating polyradiculoneuropathy, diabetic ketoacidosis, exfoliative dermatitis (immune-mediated), Guillain-Barre syndrome (immune-mediated), hemolytic anemia (immune-mediated), hepatitis (including autoimmune hepatitis), hypophysitis, infusion-related reaction, interstitial nephritis (with renal failure), myasthenia gravis (immune-mediated), myositis (immune-mediated), nephritis (autoimmune), pancreatitis (immune-mediated), partial epilepsy (immune-mediated; in a patient with inflammatory foci in brain parenchyma), thyroiditis, type I diabetes mellitus, uveitis (immune-mediated), vasculitis (immune-mediated)

15.18 Drug procurement:

Commercial supplies. Pharmacist or clinics shall obtain supplies from normal commercial supply or wholesaler.

15.19 Nursing guidelines

15.191 Pembrolizumab side effects vary greatly from those of traditional chemotherapy and can vary in severity from mild to life threatening. Instruct patients to report any side effects to the study team immediately. Side effects may be immediate or delayed up to months after discontinuation of therapy. Most side effects are reversible with prompt intervention of corticosteroids.

15.192 Diarrhea can be seen however is less common than that seen with anti-CTLA-4 agents. However it can be severe, leading to colonic perforation. Instruct patients to report ANY increase in the number of stools and/or change in baseline, blood in the stool, abdominal pain to the study team immediately.

15.193 Rash/pruirits/dermatitis is seen. Patients should report any rash to the study team. Treat per section 9.0 and monitor for effectiveness.

15.194 Monitor LFTs closely as elevations in these levels could indicate early onset autoimmune hepatitis. Patients should also be instructed to report any jaundice, or right upper quadrant pain to the study team immediately.

15.195 Pneumonitis can be seen and may be mild (only seen on imaging) to severe. Patients should be instructed to report any SOB, dyspnea, cough, chest pain, etc. to the study team immediately. Patients reporting these symptoms should have a pulse ox checked and consider immediate imaging per the treating MD.

15.196 Endocrinopathies (including hypopituitarism, hypothyroidism, hypophysitis, and adrenal insufficiency) are seen with this agent. Patients may present only with the vague sense of fatigue and "not feeling well." Additional symptoms may be that of nausea, sweating and decreased activity tolerance. Instruct patients to report these signs or symptoms immediately and obtain appropriate labs as ordered by MD.

15.197 Patients who are started on steroid therapy for any side effects of pembrolizumab toxicity should be instructed to take the steroids as ordered, and not to discontinue abruptly as symptoms may return and be severe. Patients may be on steroid therapy for weeks. Instruct patients to report any increase or change in side effects with any dosage decrease as patients may need a slower taper.

15.198 Fatigue is common and may or may not be associated with immune related side effects. Assess patient's fatigue level prior to each cycle of therapy and report any changes to the study team.

15.199a Patients should avoid receiving live vaccines within 30 days of study drug administration or per other study guidelines.

15.199b Patients who have undergone an allogeneic bone marrow transplant have an increased risk of severe complications including early GVHD and venoocclusive disease, if they have previously been treated with pembrolizumab.

15.199c Myocarditis has been reported and associated with pembrolizumab. Instruct patients to report chest pain, SOB, or dyspnea to study team immediately and/or seek emergency medical attention.

15.199d Autoimmune hematologic disorders including ITP and hemolytic anemia have been reported. Monitor blood counts closely and report any abnormalities to the study team.

15.199e Rare neurologic disorders including Guillain-Barre syndrome and myasthenia gravis have been reported. Instruct patients to report any neurologic symptoms including weakness, parasthesias or numbness, tingling to the study team immedicately.

15.2 Mature Dendritic Cells (IND#17522)

15.21 Background

The DC used in this study is patient's autologous dendritic cells (mDC). Dendritic cells are cells manufactured in lab from monocytes removed from patients' blood by leukapheresis. The dendritic cells need to acquire tumor antigens in order to stimulate anti-tumor immunity. Patients will be treated with DC placed into a tumor killed using cryoablation where tumor antigen exposure will occur *in vivo*.

15.22 Formulation

DCs are supplied as recently thawed cells prepared from patient material. Cells are manufactured and released by the Human Cellular Therapy Lab, Mayo Clinic Rochester. Before released for use, cells will undergo testing for sterility and potency.

15.23 Preparation and storage

mDCs will be prepared and stored at the Human Cellular Therapy Lab, Mayo Clinic Rochester according to approved SOPs included in the IND. The drug will be supplied to the appropriate administration area by personnel from the Human Cellular Therapy Lab/Immune, Progenitor, and Cellular Therapy (IMPACT) Unit.

15.24 Administration:

See Section 7.5.

15.25 Pharmacokinetic information

NOTE: this drug is a biologic made up of cells from the patient. Classical drug pharmacokinetics do not apply.

15.26 Potential Drug Interactions: Unknown.

15.27 Known potential toxicities: Unknown.

15.28 Drug procurement:

Drug is manufactured on site in the Immune, Progenitor, and Cell Therapeutics (IMPACT) Laboratory (507) 284-4010

15.3 Pneumococcal 13-valent Conjugate Vaccine (Prevnar13®)

15.31 Background:

Pneumococcal 13-valent Conjugate Vaccine (Diphtheria CRM_{197} Protein), is a sterile solution of saccharides of the capsular antigens of *Streptococcus pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F individually linked to non-toxic diphtheria CRM_{197} protein. Each serotype is grown in soy peptone broth.

Potency of the formulated vaccine is determined by quantification of each of the saccharide antigens, and by the saccharide to protein ratios in the individual glycoconjugates.

15.32 Formulation

Supplied as ready-to-use prefilled syringes (10 x 0.5-mL prefilled syringes per package). Pneumococcal 13-valent Conjugate Vaccine is manufactured as a liquid preparation for intramuscular injection. Each 0.5 mL dose of the vaccine is formulated to contain approximately 2.2 µg of each of *Streptococcus pneumoniae* serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F, 23F saccharides, 4.4 µg of 6B saccharides, 34 µg CRM_{197} carrier protein, 100 µg polysorbate 80, 295 µg succinate buffer and 125 µg aluminum as aluminum phosphate adjuvant.

15.33 Preparation and storage:

Pneumococcal 13-valent Conjugate Vaccine is stored at refrigerated temperatures of 2ºC to 8ºC (36ºF to 46ºF) away from freezer compartment. DO NOT FREEZE. Discard if frozen.

15.34 Administration

Shake vigorously immediately prior to use to obtain a uniform suspension in the vaccine container. The vaccine should not be used if it cannot be resuspended. After shaking, the vaccine is a homogeneous, white suspension. Do not mix the vaccine with other products in the same syringe.

15.35 Potential Drug Interactions

Patients receiving therapy with immunosuppressive agents (large amounts of corticosteroids, antimetabolites, alkylating agents, cytotoxic agents) may not respond optimally to active immunization.

As with other intramuscular injections, Pneumococcal 13-valent Conjugate Vaccine should be given with caution to patients on anticoagulant therapy.

See complete prescribing information for information regarding co-administration of Pneumococcal 13-valent Conjugate Vaccine with other vaccines.

15.36 Known potential toxicities

There is no safety data available in the adult patient population. Please see Prevnar13® prescribing information for comprehensive toxicity data.

The following systemic events were noted within 2-3 days of the Pneumococcal 13-valent Conjugate Vaccine injection in pediatric patients: fever, irritability, drowsiness, restless sleep, decreased appetite, vomiting, diarrhea, and urticaria-like rash. The following local reactions occurred within 3 days of immunization pediatric patients: erythema, induration, tenderness and interference with limb movement.

15.37 Drug procurement

Prevnar13 will be purchased with study funds and provided free of charge to patients.

16.0 Statistical Considerations and Methodology

16.1 Study Design

Dose-limiting toxicity (DLT) is defined as one of the following events occurring during the first two cycles of protocol therapy and attributed as possibly, probably, or definitely related to protocol therapy.

- Grade 3+ infusion reactions, acute kidney injury, chronic kidney disease, pneumonitis; or Grade 2 infusion reactions, acute kidney injury, chronic kidney disease or pneumonitis that does not resolve to Grade 0-1 within 21 days.

Primary Endpoint: The primary endpoint is tumor response rate (RR) defined as the number of patients whose disease meets the RECIST criteria for a partial (PR) or complete (CR) on two consecutive evaluations at least 4 cycles (approximately 84 days) apart divided by the total number of patients who started protocol treatment at the continuation dose level. (That is, patients in the run-in phase assigned the dose level chosen enrolled onto the schedule carried forward into the continuation phase will be for the continuation phase will be included in the analysis of this endpoint). A 90% exact binomial confidence interval for the tumor response rate will be constructed.

16.11 Run-in phase (Phase Ib)

Six patients will be enrolled onto Schedule 1. If at most one of these 6 patients develops a DLT during the first 3 cycles of treatment then Schedule 1 will be carried forward to the continuation phase (Phase II).

If two or more patients enrolled onto Schedule 1 develop a DLT during the first 3 cycles of treatment, then an additional 6 patients will be treated on Schedule -1 (See Section 7.2).

16.12 Continuation phase (Phase II)

Schedule of pembrolizumab, cryoablation, mDCs, and Prevnar13 will be based on the tolerability findings of the run-in phase

16.13 Adverse Event Stopping Rules

Enrollment to the trial will temporarily suspended for close examination of treatment safety and tolerability:

- at each instance of a treatment related death;
- if 2 or more of the first 6 patients develops one of the following toxicities that is considered to be possibly, probably, or definitely related to protocol treatment: a Grade 4 hematologic toxicity or Grade 3 or worse non-hematologic toxicity;
- or at any time after the first 6 patients have been enrolled, if 25% or more patients develops one of the following toxicities that is considered to be possibly, probably or definitely related to protocol treatment: a Grade 4 hematologic toxicity or Grade 3 or worse non- hematologic toxicity.

All toxicity data will be examined to assess whether changes should be made in the treatment or eligibility criteria to increase the tolerability of the treatment. All modifications to the protocol will be forwarded to the IRB for review.

16.2 Sample Size and accrual rate

If a cohort of 33 patients is enrolled onto the schedule carried forward into the continuation phase, a one-sided exact binomial test of proportions with significance level of 0.10 will have a 90% chance of rejecting the null hypothesis that the tumor response rate is at most 12% when the alternative hypothesis that the tumor response rate is at least 30% is true.

Thus, the minimum sample size for this trial will be 33 patients (if at most 1 DLT is seen on Schedule 1in the run-in phase) or a maximum of 39 patients (if 2 or more DLTs are seen on Schedule 1 and at most 1 DLT is seen on Schedule -1)

It is anticipated that 2 patients per month will be enrolled onto this study with a temporarily break in enrollment after the first 6 patients are enrolled onto Schedule 1 (and if necessary after the first 6 patients are enrolled onto Schedule -1) to establish a schedule to be carried forward into the continuation phase.

16.3 Secondary endpoints

16.31 Safety profile:

All eligible patients that have initiated treatment will be considered evaluable for assessing the safety profile. Toxicities will be graded in terms of severity and relationship to study treatment using CTC-CAE criteria. For each patient who initiated treatment, the maximum grade of each toxicity noted during treatment will be recorded. Frequency tables will be constructed by treatment schedule.

16.32 Feasibility

A point estimate of the proportion of patients who received both intra-tumoral DC injections among those who initiated treatment will be determined. A 95% exact binomial confidence interval for this proportion will be constructed.

16.33 Clinical Benefit Rate (CBR)

Clinical Benefit Rate (CBR) is defined as the proportion of patients who have completed 6 cycles of treatment without disease progression (that is, their objective disease status is a CR, PR, or stable for 6 cycles or more) A 90% exact binomial confidence interval for this proportion will be constructed.

16.34 Progression-free survival (PFS)

Time from randomization to the first 2 consecutive evaluations approximately 6 weeks apart documenting disease progression. The distribution of progression-free survival times will be estimated using the Kaplan-Meier approach.

16.35 Overall survival (OS)

Time from registration to death due to any cause. The distribution of overall survival times will be estimated using the Kaplan-Meier approach

16.4 Correlative endpoints

A time series plot of individual patient TILs will be constructed. Also, the change in the number of tumor infiltrating lymphocytes (TILs) in tumor biopsies taken prior to and following cryoablation and intra-tumoral mDCs on Cycle 2 and on Cycle 3 will be determined. For a given cycle, the percent change in the number of TILs following cryoablation and intra-tumoral mDCs from pre- cryoablation and intra-tumoral mDCs levels will be determined.

A times series plot of individual patient PD-L1 levels found in tumor biopsies as well as blood biopsies will be constructed and visually assess for trends within and between biopsy types. Wilcoxon rank sum tests will be used to assess whether the amount of the change in PD-L1 levels after first cryoablation and intra-tumor or the PD-L1 levels at completion of second cryoablation and intra-tumor differ among those patients who met the criteria for clinical benefit (progression-free and on study for at least 6 months) and those who do not.

16.5 Data & Safety Monitoring:

The study statistician will provide the Mayo Clinic Cancer Center (MCCC) Data Safety Monitoring Board (DSMB) a report encompassing accrual, adverse events, and feasibility

issues that might be developing at least twice a year. After every cohort of 10 patients is treated at the continuation phase dose level, a formal safety check will be carried out. If 3 or more patients in a given cohort of 10 patients develop a DLT, enrollment will be temporarily suspended to assess whether changes should be made in the treatment or eligibility criteria to increase the tolerability of the treatment. All modifications to the protocol will be forwarded to the IRB for approval.

16.6 Subset Analyses for Minorities

16.61 Study availability

This study will be available to all eligible patients, regardless of gender, race or ethnic origin.

16.62 Statistical analysis by subset

There is no information currently available regarding differential effects of this regimen in subsets defined by race, gender, or ethnicity, and there is no reason to expect such differences to exist. Therefore, although the planned analyses will look for differences in treatment effect based on racial groupings, the sample size is not increased in order to provide additional power for subset analyses.

16.63 Regional population

The geographical region served by MCCC has a population which includes approximately 3% minorities. Based on prior MCCC studies involving similar disease sites, we expect about 3-5% of patients will be classified as minorities by race and about 33% of patients will be women. Expected sizes of racial by gender subsets are shown in the following table:

Accrual Targets			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	0	1	1
Not Hispanic or Latino	15	23	38
Ethnic Category: Total of all subjects*	**15**	**24**	**39**
Racial Category			
American Indian or Alaskan Native			
Asian			
Black or African American			
Native Hawaiian or other Pacific Islander			
White	15	24	39
Racial Category: Total of all subjects*	**15**	**24**	**39**

Ethnic Categories:

Hispanic or Latino – a person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. The term “Spanish origin” can also be used in addition to “Hispanic or Latino.”

Not Hispanic or Latino

Racial Categories:

American Indian or Alaskan Native – a person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliations or community attachment.

Asian – a person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Philippine Islands have been recorded as Pacific Islanders in previous data collection strategies.)

Black or African American – a person having origins in any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black or African American."

Native Hawaiian or other Pacific Islander – a person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.

White – a person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

17.0 Pathology Considerations/Tissue Biospecimens:

17.1 Summary Table of Research Tissue Specimens to be collected for this Protocol

Correlative Study (Section for more information)	Mandatory or Optional	Type of Tissue to Collect	Block, Slides, Core, etc. (# of each to submit)	Cycles 2 and 3	Prior to Cycle 5	Process at site? (Yes or No)	Temperature Conditions for Storage/ Shipping[1]
Immunohistochemistry, immunofluorescence, gene sequencing for tumor antigen and T cell receptors (17.2)	Mandatory	tumor in normal saline	18 gauge core needle biopsy (6 cores)	X	X	No	2 tissue samples for Cryostore. 4 tissue samples for formalin-fixed paraffin-embedded (FFPE)

1. All specimens should be sent to PRC for processing (into FFPE) and then to Dr. Block's laboratory c/o Courtney Erskine (pager 127-14057, Guggenheim 3-23

17.2 Background and Methodology

17.21 Analysis of tumor biopsies

We will examine tumors for tumor-infiltrating lymphocyte and dendritic cell phenotype and compare changes prospectively over time. Immuno-histochemistry, flow, and gene sequencing analysis will be performed. Exploratory biomarkers to predict treatment response include but are not limited to tumor-infiltrating CD4 and CD8 T cells, NK cells, and PD-1/PDL1/PDL2 expression on melanoma and stromal cells. Leftover samples may be used for genomic or proteomic analysis.

18.0 Records and Data Collection Procedures

18.1 Submission Timetable

Data submission instructions for this study can be found in the Case Report Form packet.

18.2 Event monitoring

See Section 4.0 and Data Submission Table in the Case Report Form packet for the event monitoring schedule.

18.3 CRF completion

This study will use Medidata Rave® for remote data capture (rdc) of all study data.

18.4 Site responsibilities

Each site will be responsible for insuring that all materials contain the patient's initials, MCCC registration number, and MCCC protocol number. Patient's name must be removed.

18.5 Supporting documentation

This study requires supporting documentation of histologic diagnosis and staging which includes the most recent tumor tissue biopsy pathology report, bone marrow biopsy report, and imaging reports. To submit these materials, they can be uploaded into the Supporting Documentation form in Medidata Rave®. These reports should be submitted within 14 days of registration.

For response to treatment, supporting documentation includes imaging reports and, if applicable, a bone marrow biopsy report.

For patients who progress after study therapy, supporting documentation including imaging reports is required.

18.6 Labelling of materials

Each site will be responsible for insuring that all materials contain the patient's initials, MCCC registration number, and MCCC protocol number. Patient's name must be removed.

18.7 Incomplete materials

Any data entered into a form will result in that form being marked as "received." However, missing data will be flagged by edit checks in the database.

18.8 Overdue lists

A list of overdue materials is automatically available to each site at any time. A list of overdue materials and forms for study patients will be generated monthly. The listings will be sorted by location and will include the patient study registration number. The appropriate co-sponsor/participant will be responsible to obtain the overdue material.

18.9 Corrections forms

If a correction is necessary the QAS will query the site. The query will be sent to the appropriate site to make the correction in the database and respond back to the QAS.

19.0 Budget

19.1 Costs charged to patient: Routine clinical care, pembrolizumab (including administration)

19.2 Tests to be research funded:

- Leukapheresis
- Cryoablation/Anesthesia
- DC and Prevnar13 injections
- Correlative studies outlined in Section 14.0 and 17.0

19.3 Other budget concerns: None

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Appendix I ECOG Performance Status

ECOG PERFORMANCE STATUS*

Grade	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any selfcare. 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 ECOG Performance Status is in the public domain therefore available for public use. To duplicate the scale, please cite the reference above and credit the Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

From http://www.ecog.org/general/perf_stat.html

Appendix II New York Heart Association Classification of Congestive Heart Failure

NYHA Class	Symptoms
I	Cardiac disease, but no symptoms and no limitation in ordinary physical activity, e.g. no shortness of breath when walking, climbing stairs etc.
II	Mild symptoms (mild shortness of breath and/or angina) and slight limitation during ordinary activity.
III	Marked limitation in activity due to symptoms, even during less-than-ordinary activity, e.g. walking short distances (20–100 m). Comfortable only at rest.
IV	Severe limitations. Experiences symptoms even while *at rest*. Mostly bedbound patients.

Adapted from Dolgin M, Association NYH, Fox AC, Gorlin R, Levin RI, New York Heart Association. Criteria Committee. Nomenclature and criteria for diagnosis of diseases of the heart and great vessels. 9th ed. Boston, MA: Lippincott Williams and Wilkins; March 1, 1994.
Original source: Criteria Committee, New York Heart Association , Inc. Diseases of the Heart and Blood Vessels. Nomenclature and Criteria for diagnosis, 6th edition Boston, Little, Brown and Co. 1964, p 114.