Masonic Cancer Center
University of Minnesota

A Phase 2 Study of Palliative Radiation and Combination Sequential Immunotherapy for Metastatic Cutaneous Melanoma and Ocular Melanoma
CPRC # 2018LS110

Principal Investigator:
Evidio Domingo-Musibay, MD
Department of Medicine
Division of Hematology, Oncology and Transplantation

Co-Investigators:
Division of Hematology, Oncology and Transplantation
Naomi Fujioka, MD*
Shernan Holtan, MD
Martin Felices, PhD*

Department of Surgery
Todd Tuttle, MD*
Jane Hui, MD, MS*

Department of Interventional Radiology
Shamar Young, MD*

Department of Radiation Oncology
Jianling Yuan, MD PhD*
Chris Wilke, MD, PhD*

Department of Radiology
Daniel J Steinberger, MD*

Laboratory Medicine and Pathology
Alessio Giubellino, MD, PhD*
Bartosz Grzywacz, MD*

Division of Epidemiology and Community Health
Heather H Nelson MPH, PhD*

*will not consent patients

Biostatistician:
Saonli Basu, PhD

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Revision History

Revision #	Version Date	Summary Of Changes	Consent Changes
	09/12/2018	Original to CPRC	n/a
	11/12/2018	Response to CPRC stips including updating the title and adding background information on ocular melanoma Other minor edits and clarfications including: • Synospsis – correct IL-2 dose • Schema – clarify treatment days for each cohort, change the ocular melanoma cohort treatment Day 7 to treatment Day 8 (changed throughout the protocol) • Section 9 – Schedule of patient activities – clarify that the schedule of visits will be identical for both cohorts (although treatment will occur at different visits based on cutaneous vs ocular melanoma); in Section 9.2, add a Day 8 time point for Cycles 2 and 3 to match clinical calendar	n/a
	03/05/2019	In response to IRB's initial review: • Section 9.1 - Clarify that for study eligibility a patient must have either archived tumor tissue or a fresh tumor biopsy must be done – SOC – charge to insurance • Section 9.2 – Clarify that a patient must have a fresh tumor biopsy prior to treatment start – if a fresh tumor biopsy is done for eligibility per Section 9.1, the baseline biopsy in Section 9.2 is not required; however if a patient has archived tissue, a fresh tumor biopsy is required per Section 9.2 and charged to research. • Synopsis and Section 1.3 - Add a correlative objective for the stool samples and add a new section (Section 9.2.3) regarding sample collection Edits and clarifications from the Clinical Care Planning Meeting: • Minor clarifications to the inclusion and exclusion criteria • Section 9.2 greatly reduce the number of times the FACT QOL survey is completed and stool samples collected • Other minor edits through-out	yes
1	07/02/2019	• Synopsis, Schema, Sections 4 and 7.1: For patients receiving palliative RT outside of the study shorten the window to within 30 days (previously 60) and clarify 30 days is based on the time between the last dose of RT and 1st dose of aldesleukin. • Synopsis, Schema, Section 7.4: Update the nivolumab/ impilimab dosing for ocular cohort based on new information – rationale added to Section 2.4 • Section 9.2: Remove stool sample collection from x chart, delete Section 9.2.3 and correlative objective related to stool collection • Sections 9.1 and 9.2 – clarify a fresh tumor biopsy is required at baseline on all patients, but if patient has	yes

Revision #	Version Date	Summary Of Changes	Consent Changes
		archived tissue, biopsy will be charged for research Standardize the maintenance therapy language that it is independent of the study and at the discretion of the treating phyisician • Section 9.2: reduce the frequency of research related blood samples Other edits and minor clarifications: • Section 5.1.10 and Appendix I: Inclusion criteria add "if given" for time requirement since previous anti-tumor therapy and RT start • Section 5.1.11: Inclusion criteria add "if applicable" regarding recovery from previous cancer treatment side effects • Synopsis: Correct wording in the inclusion criteria in regarding prior treatment to match Section 5.1.10 and appendix I • Section 4: delete treatment details are repeat of information in schema, synopsis and Section 7 • Section 9.1: added pregnancy testing to the x-chart to match exclusion criteria Section 5.1.2. • Replace IL-2 with aldesleukin at several places in the protocol for drug name consistency (although also retain reference to IL-2 when using as a general term and in the consent form) • Other minor edits and formatting changes as tracked	
2	02/18/2020	• Increase the allowable time between last dose of radiation therapy to 1st dose of IL-2 from 30 days to 90 days for patients enrolling on the study after palliative therapy. • Remove all tests and evaluations under the radiation column • Add windows around individual treatments without affecting future treatment schedule and allow longer delays with adjustment to future dosing as appropriate. • Delete Eligibility Checklist as Appendix I and renumbering remaining appendices (and update numbering in the protocol text). • Other minor edits including adding > 80 years of age must be approved by PI to synopsis to match other protocol sections, adding to missing x's bloodwork • Add Chris Wilke, MD, PhD as co-I	Yes – Timing of previous RT, delete blood work during RT

Principal Investigator Contact Information:

Evidio Domingo-Musibay, MD
University of Minnesota Department of Medicine
Division of Hematology, Oncology, and Transplantation
420 Delaware Street SE MMC 480
Minneapolis, MN 55455
Phone: 612-624-5373 Email: musib024@umn.edu

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KEY ABBREVIATIONS

AE	Adverse event
CRF	Case report form
DLCO	diffusing capacity of lung
eGFR	estimated glomerular filtration rate
EOT	End of Treatment
FACT-M	Functional Assessment of Cancer Therapy – Melanoma
GKR	Gamma Knife Radiosurgery
iDOR	duration of response based on iRECIST
IL-2	Interleukin-2 (Aldesleukin)
I-O	immuno-oncology
irAE	Immune related adverse event
iRECIST	immune response evaluation criteria in solid tumours
IV	intravenous
LVEF	Left Ventricular Ejection Fraction
MTD	maximum tolerated dose
ORR	objective response rate
OS	overall survival
PBMC	Peripheral blood mononuclear cells
PD	progressive disease
PFS	progression free survival
QOL	Quality of life
RECIST	response evaluation criteria in solid tumours
SBRT	Stereotactic Body Radiation Therapy
ULN	Upper limit of normal
WBRT	whole brain radiation therapy

PROTOCOL SYNOPSIS

A Phase 2 Study of Palliative Radiation and Combination Sequential Immunotherapy for Metastatic Cutaneous Melanoma and Ocular Melanoma

Study Design:

This is a Phase 2 study designed to evaluate the combination of checkpoint blockade and aldesleukin (IL-2) therapy after a course of standard of care palliative radiation in the management of unresectable metastatic melanoma. To be eligible, a patient must have a minimum of 3 (preferably >5) radiographically distinct, measurable (>1.5 cm) lesions based on RECIST 1.1. Metastatic cutaneous melanoma must be refractory to standard immunotherapy drugs, molecular targeted agents and/or chemotherapy. Patients with ocular melanoma subtypes may enroll in this study without prior therapy, as there is no standard front-line therapy for this subset of patients.

Two treatment cohorts will be used based on disease origin:
Cohort 1: Cutaneous melanoma
Cohort 2: Ocular melanoma

Each cohort enrolls 22 patients employing a separate Simon's two stage design and separate monitoring for excessive toxicity.

Standard of care palliative radiation therapy is delivered to a maximum of 3 metastatic tumors over no more than 3 weeks. A minimum of 2 lesions must remain non-irradiated for disease response assessments. Patients who have received palliative radiation therapy prior to study enrollment also may be considered for the study provided the 1st dose of aldesleukin is given within 90 days after the last dose of RT.

Direct participation consists of 3 cycles of study treatment over 16 weeks and an End of Trial (EOT) visit.

Disease re-assessment includes physical examination and radiographic response assessment by irRECIST and RECIST v1.1 of non-irradiated target lesions using CT (or PET/CT) and/or at MRI during Week 6 of Cycle 1 and again after Cycle 3 as part of the End of Trial (EOT) visit.

Direct study participation ends with the EOT; however, patients may continue receiving nivolumab every 2 weeks at 240 mg IV as maintenance therapy independent of this study at the discretion of the treating physician OR nivolumab 480 mg IV every 4 weeks per 2018 FDA approval.

Determinaton of disease progression for the study requires confirmation of progression with scans obtained at least 4-6 weeks later that qualify as progressive disease (PD) by irRECIST due to pseudoprogression and late responses seen with immunotherapy. Therapy after progression remain at the discretion of the treating physician and study participant.

All patients are followed for progression-free and overall survival endpoints for two years from the start of aldesleukin.

Primary Objective:

Determine the objective response rate (ORR) and confirm safety and tolerability of sequential combination immunotherapy

Secondary Objectives:

- Estimate 6-month Progression-Free Survival (PFS)
- Estimate 12-month Overall Survival (OS)

Correlative Objectives:

- Assessment of tumor microenvironment changes and systemic immune parameters, identification of biomarkers of response, identification of autoimmune toxicities associated with therapy, and characterization of resistance and response mechanisms.
- Assess the effect of natural genetic variation (SNPs) in selected genes including PD-1, PD-L1/L2, CTLA-4, TIM-3, LAG-3, OX40/OX40L as well as HLA, NKG2D and NKG2D Ligand genes.
- Evaluate Health Related Quality of Life as assessed by the FACT-M

Disease Status

Biopsy-proven unresectable, metastatic melanoma refractory to standard front-line therapy with a **minimum of 3 radiographically distinct metastases (>1.5 cm)** measurable by RECIST 1.1.

- o At least 2 lesions must remain non-irradiated for systemic response assessments
- o A maximum of 3 metastases will be treated by palliative radiation (max 2 metastases per treated organ may be targeted, but must be separated by more than 5 cm of normal tissue)

  Note: Appropriate candidates with lung metastases may be considered for ablative hypofractionation. Appropriate liver metastases may be considered for ablative hypofractionation or Y-90 radioembolization. Suitable brain metastases will be treated using Gamma Knife Radiosurgery (GKR) or whole brain radiation therapy (WBRT) per the treating radiation oncologist. Total radiation dose and number of fractions will be determined by the treating radiation oncologist based on anatomic and dosing constraints.

Archival tissue block and/or newly obtained core, punch, or excisional biopsy of tumor tissue.

Prior recent palliative radiation: enrollment is permissible if the last dose of palliative radiation therapy was given within 90 days of the 1st dose of aldesleukin and at least 2 radiographically distinct non-irradiated metastases (>1.5 cm) remain for systemic response assessment

Ocular melanoma: patients with metastatic ocular melanoma may enroll in this study without prior therapy, given no standard front-line therapy available for this subset of patients.

Key Inclusion:

- Age 18 through 80 years, >80 years of age must be approved by the PI
- ECOG performance status 0 or 1
- Willingness to undergo two (2) separate biopsies (core and/or excisional) during the study period; an accessible site is always preferred.
- Adequate organ function as evidenced by testing within 14 days of study enrollment defined as:
  - o Hematologic: leukocytes ≥ 2,000/mcL, ANC ≥ 1,000/mcL, hemoglobin ≥ 9.0 g/dL, platelets ≥ 100,000/mcL
  - o Renal: creatinine ≤ 1.8 mg/dL, if > 1.5 mg/dL must have eGFR of ≥ 35 mL/min
  - o Hepatic: AST, ALT, ALP ≤3 x upper limit of normal (≤5 x UNL if know or suspected liver mets), total bilirubin ≤ 2.0 mg/dL
  - o Pulmonary: oxygen saturation ≥90% on room air; corrected DLCO and FEV1, ≥60% predicted
  - o Cardiac: absence of clinical decompensated congestive heart failure or uncontrolled arrhythmia; LVEF ≥ 40%. QTc < 450 ms in males and < 470 ms in females.
- A minimum of 1 week between last anti-tumor treatment if given, and 1st dose of radiation therapy (not applicable for patients enrolling after palliative radiation therapy)
- Recovery from previous cancer treatment if applicable (acute toxicity ≤ Grade 1 by CTCAE 5.0 criteria) prior to first radiation treatment
- Women of childbearing potential and males with partners of childbearing potential must agree to the use of barrier methods of contraception, hormonal contraceptives, or to abstain from heterosexual activity for the duration of study participation. Women of childbearing potential must have a negative pregnancy test within 14 days of study enrollment.
- Ability to understand and provide voluntary written consent

Key Exclusion Criteria:

- Concurrent use of high-dose steroids **Note**: chronic or maintenance steroids (< 2 mg dexamethasone equivalent) permissible
- Any concurrent malignancy requiring active treatment.
- Severe and active autoimmune diseases requiring systemic immunosuppression.
- Prior organ allograft or allogeneic transplantation.

Treatment Plan

After completion of palliative radiation therapy:

Cycle 1 (6 week duration):
Days 1-5 and Days 15-19 (all patients):
Aldesleukin (IL-2) (600,000 U/kg/dose) given as a bolus infusion once every 8 to 12 hours over 5 days or until no longer tolerated (to a maximum of 10 doses). Administered as an inpatient.

The reminder of treatment is given as an outpatient:
On Day 29 Cohort 1 (cutaneous): Nivolumab 240 mg IV

On Day 29 Cohort 2 (ocular):

- Nivolumab 3 mg/kg IV and Ipilimumab 1 mg/kg IV
  OR for a high risk patient with an excellent performance status, the following regimen may be given at the discretion of the treating investigator:
- Nivolumab 1 mg/kg IV and Ipilimumab 3 mg/kg IV

Cycle 2 (6 week duration):
Cohort 1: Nivolumab 240 mg IV on Day 1, 15 and 29.

Cohort 2: Nivolumab followed by Ipilimumab on Day 8 and on Day 29 using the same dose regimen as used Cycle 1 Day 29.

Cycle 3 (4 week duration):
Cohort 1: Nivolumab 240 mg IV on Day 1 and 15.

Cohort 2: Nivolumab followed by Ipilimumab on Day 8 using the same dose regimen as used Cycle 1 Day 29.

After Cycle 3 and the End of Trial (EOT) assessment is completed, direct study participation ends. Patients may continue maintenance nivolumab 240 mg IV every 2 weeks OR nivolumab 480 mg IV every 4 weeks per 2018 FDA approval at discretion of the treating physician and independent of this study.

All patients are followed for progression-free and overall survival endpoints for two years from the start of aldesleukin by phone/electronic communications.

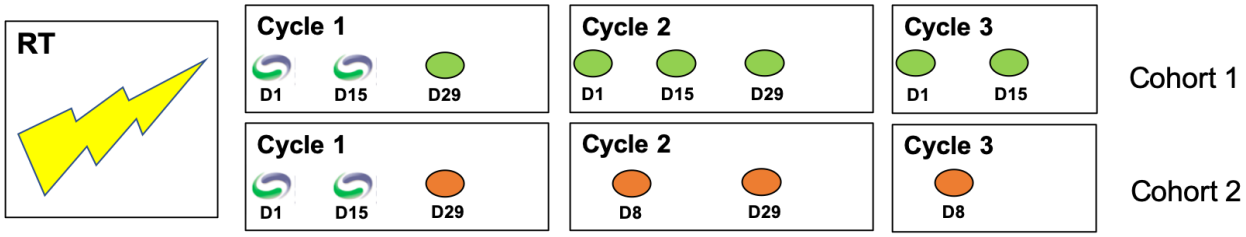
Enrollment: Enrollment for each melanoma cohort will be independent of the other:

Stage 1: enroll 16 patients – if 4 or more patients have a disease response, activate stage 2. If 3 or fewer responses in these 16 patients, the cohort will be stopped for futility.

Stage 2: enroll 6 additional patients for a total accrual of 22

Approximately 44 patients will be enrolled over a period of 24 months; based on an average of 1-2 patients per month.

STUDY SCHEMA



Aldesleukin (IL-2)

Nivolumab 240 mg

Nivolumab
Ipilimumab

Cohort		CPI
1	Doublet	Nivolumab (240 mg)
2	Triplet	Nivolumab and Ipilimumab

Palliative Radiation administered per standard of care. Patients who have received recent palliative radiation may be considered for the study provided the 1st dose of aldeskeukin can be given within 90 days of the last dose of palliative radiation therapy.

Aldesleukin (IL-2) 600,000 U/kg is administered as an intravenous infusion via a central catheter (either PICC line or port) over 15 minutes approximately once every 8 hours per institutional guidelines and standard clinical practice. Patients may receive a maximum of 10 doses, no longer than 24-hours apart, on Days 1 through 5 and Days 15 through 19 of Cycle 1.

Cohort 1 only (Cutaneous Melanoma):
Nivolumab 240 mg IV is administered every 2 weeks after IL-2 treatments for six doses (Cycle 1 Day 29 and Cycle 2 Day 1, 15, and 29, and Cycle 3 Day 1 and 15).

Cohort 2 only (Ocular Melanoma):

- **Nivolumab** 3 mg/kg IV and **Ipilimumab** 1 mg/kg IV

OR for a high risk patient with an excellent performance status, the following regimen may be given at the discretion of the treating investigator:

- **Nivolumab** 1 mg/kg IV and **Ipilimumab** 3 mg/kg IV

Dosing occurs every 3 weeks after IL-2 treatments for four doses (Cycle 1 Day 29 and Cycle 2 Day 8 and 29, and Cycle 3 Day 8).

After Cycle 3 and an End of Trial (EOT) assessment 2-4 weeks after last dose of study drug is completed, direct study participation ends.

Patients may continue maintenance nivolumab 240 mg IV every 2 weeks OR nivolumab 480 mg IV every 4 weeks per 2018 FDA approval at discretion of the treating physician and independent of this study.

1. OBJECTIVES

1.1. Primary Objective

The primary objective of this study is to determine the objective response rate (ORR) and confirm safety and tolerability of sequential combination immunotherapy in patients with metastatic melanoma after palliative radiation therapy.

1.2. Secondary Objectives

- Estimate 6-month Progression-Free Survival (PFS)
- Estimate 12-month Overall Survival (OS)

1.3. Correlative Objectives

- Assessment of tumor microenvironment changes and systemic immune parameters, identification of biomarkers of response, identification of autoimmune toxicities associated with therapy, and characterization of resistance and response mechanisms.
- Assess the effect of natural genetic variation (SNPs) in selected genes including PD-1, PD-L1/L2, CTLA-4, TIM-3, LAG-3, OX40/OX40L as well as HLA, NKG2D and NKG2D Ligand genes.
- Evaluation of Health Related Quality of Life as assessed by the FACT-M

2. BACKGROUND

2.1. Metastatic Melanoma and Standard Treatments

Melanoma is the deadliest form of skin cancer affecting people of all ages. In the United States, melanoma incidence rates have been increasing for the last 40 years and it is estimated in 2017 approximately 87,110 patients will be diagnosed with malignant melanoma, and 9,730 deaths are expected from the disease(1).

Melanoma metastasis is associated with a poor prognosis, with historical 5-year survival rates of less than 10%(2). While the use molecularly targeted agents and routine use of checkpoint inhibition has improved the outlook for many patients, still 6 in 10 patient with metastatic cutaneous melanoma fail to respond to front-line immunotherapy with anti-PD1 antibodies(3).

Fortunately, for approximately 50% of cutaneous melanoma cases harboring a BRAF mutation, targeted BRAF/MEK inhibition demonstrates high initial response rates. However, responses are short-lived due to rapidly acquired resistance. Median duration of response to dabrafenib and trametinib is 12 months(4), and nearly 25% of patients recur with a metastasis to brain(5).

Brain metastasis is a common finding in cutaneous melanoma patients, with nearly 37% of patients eventually developing brain metastasis and autopsies showing 75% of melanoma deaths associated with brain metastasis(6). Currently, asymptomatic patients may be initially managed with systemic therapy, but symptomatic brain metastasis is primarily treated with surgery and radiation as standard brain directed

therapy(7). Particularly for patients with heavily pre-treated CNS metastatic disease, there is no standard immunotherapy approach for subsequent management.

The standard of care for refractory metastatic melanoma is clinical trial participation. The two most widely used historical chemotherapy agents, temozolomide and dacarbazine, are associated with response rates of 13% after 2 cycles of therapy, and median overall survival times of 7.7 and 6.4 months, for each respectively(8). The primary toxicities associated with dacarbazine and temozolomide are hematologic including Grade 3 to 4 neutropenia (16%), lymphopenia (9%), leukopenia (8%), and thrombocytopenia (6%). Common non-hematologic toxicity includes Grade 3 to 4 fatigue (5%), nausea (3%), and vomiting (2%)(9).

For ocular melanoma subtypes, the malignant cells initiate within the uveal tract of the eye. Patients with localized uveal melanoma are typically managed with local therapy first, using radiation, laser therapy, or surgery (enucleation), followed by observation or clinical trial participation. There are no approved adjuvant therapies, and following local therapy greater than 50% of patients develop metastatic disease. Tumor cells spread hematogenously to liver and patients typically die from hepatic infiltration and liver failure.

Despite the significant success of immune checkpoint blockade in the management of metastatic cutaneous melanoma, similar success has not been seen in treatment of ocular melanoma. Ocular melanoma is a unique subset with distinct biology and natural history compared with cutaneous melanoma. While characteristic BRAF mutation in cutaneous melanoma is targetable, mutations in the G-coupled receptor subunits GNA11 and GNAQ seen in >50% of uveal melanoma tumors, are not effective drug targets. A randomized phase II study of the MEK inhibitor, selumetinib, versus chemotherapy demonstrated no significant improvement in overall survival(10).

Similarly, less than 10% of uveal melanoma tumors express the biomarker PD-L1(11). Ocular melanoma treatment outcome with standard anti-PD1 monotherapy is poor, with ORR of 3.6%, median PFS of 2.6 months, and OS of 7.6 months(12). Standard practice is to treat metastatic ocular melanoma patients with approved immunotherapy agents for cutaneous melanoma, with ipilimumab and nivolumab being the most common front-line regimen for fit patients with this disease. Efficacy is limited in this disease, though, and this is a population of patients with high unmet need. New immunotherapy approaches are needed and novel immunologic therapeutic strategies should be further investigated in this population.

The sequential administration of immunostimulatory Interleukin-2 (IL-2) following radiation therapy for palliation, brain directed therapy, or liver directed therapy (ocular melanoma) offers a rational immunologic priming strategy to sustain and expand antigen priming of T cells under growth promoting effects. The sequential addition of checkpoint inhibition strategies after IL-2 also offers rational timing of anti-PD1 and anti-CTLA-4 blockade to inhibit activity of regulatory and exhausted T cells following IL-2 based immunotherapy.

2.2. Pre-clinical Results of Radiation and Immunotherapy Combinations

Radiation therapy is known to be an effective palliative treatment for melanoma(13), and it is used routinely and safely in the multidisciplinary management of advanced, metastatic disease(14, 15). It has long been observed that radiation can also cause tumor regressions outside of the field of radiation; a phenomenon termed the abscopal effect(16). Studies have shown that ionizing radiation enhances immunogenicity of tumor associated antigens(17) by inducing immunogenic cell death (ICD) and activating host innate immune signaling and APC processing and antigen presentation to stimulate adaptive T-cell mediated immunity(18, 19) creating strong rationale for combination radiation and immunotherapy.

Pre-clinical models have also demonstrated abscopal effects require an intact immune system, and ionizing radiation to melanoma tumors generates large quantities of antigen that induce APC maturation and migration into draining lymph nodes where they can activate naïve T cells that subsequently traffic to distant sites as TILs(20). Further studies have also shown that T-cell priming in draining lymph nodes leads to regression of distant non-irradiated metastases in a cytotoxic CD8+ T cell dependent fashion(21).

Advances in the planning and delivery of radiation therapy now allow for highly conformal treatment that spares normal adjacent tissues and structures. Technological improvements have also made it possible to deliver higher doses of radiation per fraction using image-guided radiation therapy (IGRT) and stereotactic body radiation therapy (SBRT) techniques. For patients with melanoma brain metastasis, stereotactic techniques like Gamma Knife can spare patients the late effects of whole brain radiation therapy.

2.3. Clinical Experience with Radiation and Combination Immunotherapy

Strategies aimed at potentiating immunologic effects of local therapy in cancer management offers patients the potential to maintain quality of life and improve immunotherapy outcomes, even in refractory metastatic disease.

Metastatic melanoma to brain affects 30-50% of metastatic melanoma patients, and combinations of radiotherapy and IL-2 in that setting have demonstrated clinical efficacy and long-term disease control using both WBRT and stereotactic radiosurgical techniques(22). A single-institution retrospective study from the University of Minnesota identified 15 patients receiving high-dose IL-2 therapy, two of which had brain metastases treated with WBRT followed by IL-2, in both cases resulting in durable complete responses(23). Gamma Knife radiosurgery combined with Ipilimumab has also been associated with improvement in outcomes and survival rates equivalent to melanoma patients without brain metastases(24).

Most radiotherapy combinations reported in the literature have used Ipilimumab, the first checkpoint inhibitor approved for treatment of metastatic melanoma based on improvement in overall survival(25, 26). Early evidence for immune enhancement came in case report format describing abscopal effects in a patient receiving

Ipilimumab during radiotherapy for melanoma(27). The addition of radiation to Ipilimumab was followed by distant tumor regressions and changes in peripheral blood immune cell subsets and led to antibody production against cancer-testis antigen NY-ESO-1(27).

However, newer evidence has shown that anti-PD1 therapy has synergy with radiation. In a recent retrospective review of patients treated with concurrent immune checkpoint inhibitor therapy and radiotherapy after initial progression on nivolumab, further nivolumab treatment was associated with an overall response rate of 30%(28). Given the potential for synergy between radiation and immunotherapy there is a need for additional prospective clinical trials to optimize *in situ* vaccination effects, improve responses, and reveal mechanistic details concerning the underlying immunobiology of radiation induced *in situ* vaccination.

Optimal radiation dose and fractionation schemes have not yet been fully defined, but fractionated radiation as opposed to single dose radiotherapy appears best able to induce abscopal responses(29). A recent retrospective systematic review of metastatic melanoma patients treated with Ipilimumab and fractionated palliative radiation suggests a threshold effect may be seen at around 2 - 3 Gy per fraction. In that study median radiotherapy dose was 26 Gy (range: 8-68 Gy) and median fraction size was 4 Gy (range 1.8 – 25 Gy), and though the benefit of combined radiation and immunotherapy was highest for fractions < 3 Gy benefits were seen regardless of the radiation dose used(30).

2.4. Clinical Experience with Aldesleukin and Checkpoint Inhibition

Interleukin-2 (IL-2) is a key human cytokine involved in the activation and proliferation of T cells(31). Aldesleukin is a recombinant version of IL-2 approved by the Food and Drug Administration (FDA) for treatment of metastatic melanoma. In the first-line setting, high-dose Aldesleukin results in durable complete response (CR) in 6% and partial response (PR) in 10% of patients, and durable responses seen out past 40 months(32).

Importantly, radiation induced abscopal effects appear critically dependent on cytotoxic T lymphocytes(21), and IL-2 is known to potently stimulate the expansion and activation of CD8+ cytotoxic T cells as well as NK cells. Exogenous IL-2 reduces minimal TCR signaling requirements on CD8+ T cells thereby allowing for proliferation under conditions of suboptimal TCR stimulation(33) and increase bystander killing activity via the release of granzyme-B and perforins. Therapeutic approaches using autologous tumor infiltrating lymphocytes (TILs) and high-dose intravenous interleukin-2 (IL-2) are also associated with response rates in excess of 50% and durable complete responses in 20% of patients(34).

Current strategies using checkpoint inhibition depend on a pre-existing anti-tumor immune response that subsequently becomes tolerogenic. Chronic antigenic exposure generates antigen-specific signal 1 and 2 required for activation of T cells, and the eventual loss of co-stimulation and induction of CTLA-4, PD-1 and other

checkpoints lead to the state of immune suppression that characterizes the immunologic state of exhaustion. Use of checkpoint inhibitors reverses T cell exhaustion in patients responding to these approaches.

In MDX010-20, the phase 3 study of Ipilimumab with or without glycoprotein 100 (gp100) peptide vaccine, the Ipilimumab monotherapy arm received 3 mg/kg Ipilimumab every 3 weeks for four doses(35). In that trial median overall survival was 10.0 months among patients receiving Ipilimumab plus the gp100 vaccine. Similarly, in a randomized phase 2 clinical trial of Ipilimumab (10 mg/kg) with or without Sargramostim (GM-CSF) improvements in overall survival were seen, with 1year OS of 68.9% (95% CI, 60.6-85.5%) vs. 52.9% (95% CI, 43.6-62.2%), respectively(36).

In CheckMate-069, a randomized phase 2 study with n=142 patients with advanced melanoma and previously untreated BRAF-WT tumors, ORR was 61% Nivolumab (1 mg/kg) and Ipilimumab (3 mg/kg) versus 11% for Ipilimumab (3 mg/kg) and placebo(37). A recent phase IIIb/IV study evaluating nivolumab 3 mg/kg plus ipilimumab 1 mg/kg in previously untreated stage III or IV melanoma (CheckMate- 511) demonstrated significantly lower incidence of treatment-related Grade 3-5 adverse events with no differences in efficacy(38). For this reason, either dosing scheme may be used in this study based on treating physician judgement. Similarly, safety of Ipilimumab and high-dose IL-2 has also been demonstrated in a phase I/II trial conducted at the NIH Surgery Branch, National Cancer Institute(39). In that study, Ipilimumab was given every 3 weeks for 3 doses, with the 2nd and 3rd doses followed within 24 hours by high-dose IL-2 administration(39). No radiation therapy was used in the trial and expected toxicity events were encountered with the combination.

2.5. Summary of Safety and Pharmacologic Considerations

The sequencing strategy employed in this trial using high dose IL-2 and anti-PD1 and anti-CTLA-4 checkpoint blockade aims to promote activation of anti-melanoma T cells after radiation therapy induced tumor antigen release. Inhibition of CTLA-4 mediated signaling can reduce regulatory T cell function, which may contribute to increased potency of IL-2 mediated T cell dependent anti-tumor immune responses. Anti-PD1 checkpoint inhibition similarly works on effector T cells to decrease physiologic inhibition of the exhausted T cell.

In the phase I/II trial conducted at the NIH Surgery Branch, in which Ipilimumab every 3 weeks was followed by high-dose IL-2, 14% of patients developed grade III/IV toxicities including 4 cases of enterocolitis and 1 case or arthritis and of uveitis. In the 3 mg/kg dose group, one of five (20%) responders experienced grade III/IV autoimmunity attributable to anti-CTLA-4 therapy(39). Anti-CTLA-4 checkpoint blockade and its effects on regulatory T cell activation suggests toxicity was most likely due to the de-inhibition of T reg activity by repetitive anti-CTLA-4 blockade and breaking of peripheral tolerance mechanisms.

In the larger phase 3 study of Ipilimumab with or without gp100 vaccine (MDX010-20) drug related adverse events were seen in 79% of patients receiving Ipilimumab. In the

monotherapy arm there were Grade 3/4 events in 21% and Grade 5 events in 3/131 (2%) of patients(35). The most frequent AEs for all arms of the trial were rash (30%), pruritis (33%), diarrhea (33%), colitis (8%), endocrine disorders (9%), AST/ALT elevation (2%), and hepatitis (1%). Any grade immune related adverse event rate was 60%, and the Grade 3/4 immune related adverse event rate was 13% with the most frequent adverse events being diarrhea (5%), colitis (5%), rash (2%), and endocrine disorders (3%)(35).

The pharmacokinetics of Ipilimumab was studied in 785 patients with unresectable or metastatic melanoma who received doses of 0.3, 3, or 10 mg/kg once every 3 weeks for 4 doses(40). Peak concentration (Cmax), trough concentration (Cmin), and area under the plasma concentration versus time curve (AUC) of Ipilimumab increased dose proportionally within the dose range examined. Upon repeated dosing every 3 weeks, the clearance (CL) of Ipilimumab was found to be time-invariant, and systemic accumulation was 1.5-fold or less. Steady-state concentrations of Ipilimumab were reached by the third dose; the mean Cmin at steady-state was 19.4 mcg/mL following repeated doses of 3 mg/kg. The mean value (% coefficient of variation) generated through population PK analysis for the terminal half-life (t1/2) was 15.4 days (34%) and for CL was 16.8 mL/h (38%). Notably, no clinically important effect on the CL of Ipilimumab was seen based on: age (range: 23–88 years), gender, performance status, renal impairment, mild hepatic impairment, previous cancer therapy, and baseline lactate dehydrogenase (LDH) levels(40).

In this study, steady-state concentrations of Ipilimumab will be reached in cohort 2, and the terminal half-life (t1/2) of the drug is anticipated to be approximately 15 days. The expected in vivo degradation pattern of the anti-CTLA-4 and anti-PD1 monoclonal antibodies is to small peptides and amino acids via biochemical pathways independent of cytochrome P450 enzymes.

Due to the potential for clinically meaningful AEs requiring early recognition and prompt intervention, management algorithms have been developed for suspected pulmonary toxicity, GI and hepatotoxicity, endocrinopathy, skin toxicity, neurological toxicity and nephrotoxicity related to the incorporation of Ipilimumab (see Appendix 3). Additional guidelines are available and published separately by the National Comprehensive Cancer Network (NCCN) and Society for Immunotherapy of Cancer (SITC).

2.6. Correlative Studies

The sequence of IL-2 administration and anti-PD1 and anti-CTLA-4 checkpoint blockade aims to promote sequential activation of anti-melanoma T cells after radiation therapy induced antigenic stimulation. Correlative studies associated with this trial aim to characterize the cooperative interactions between radiation therapy and combination immunotherapy.

Central to the goal of the study is assessment of the temporal sequence of immunologic activation and the counter-regulatory mechanisms elicited by this

approach. Correlative laboratory studies have been designed to help elucidate mechanisms of resistance to checkpoint inhibitor strategies. We hypothesize that hypofractionated radiation induces immunogenic cell death and that sequential Interleukin-2 allows for priming of anti-melanoma tumor immunity in the refractory disease setting. We also hypothesize that checkpoint inhibition in sequence with IL-2 will decrease suppressor T cell activity and exhaustion and increase T effector cell subsets. We will also assess the degree to which Nivolumab monotherapy and dual checkpoint inhibition with Nivolumab and Ipilimumab affects clinical outcome and therapy associated toxicities.

Several planned studies will also attempt to answer the following questions:

1. What immunologic checkpoints (i.e. PD1/Tim-3/Lag-3) are induced in the primary T cell response to radiation and IL-2 based therapy? Can the approach sensitize patients to anti-PD1 blockade, or other agents directed against T and NK cell targets?
2. Is baseline or early intratumoral T-cell infiltrate a marker of response to therapy? Can radiation therapy and IL-2 induce TIL mediated anti-tumor rejection? What is clonality of TILs in responder patients versus nonresponders?
3. Does anti-PD1 and anti-CTLA4 checkpoint blockade impact generation and functional activity of regulatory T cells? What immune evasion mechanisms are active in tumors failing to respond combination therapy approaches? Can gene expression profiling of tumors differentiate immunogenic versus non-immunogenic tumors?
4. Does radiation therapy lead to formation of neoantigens, and can whole-exome/transcriptome sequencing analysis and MHC binding prediction techniques predict tumor regression or subsequent response to checkpoint inhibition?
5. Do cytokine profiles and/or plasma soluble analytes (ctDNA, miRNA) predict primary or secondary responses to combination sequential radioimmunotherapy? Can ctDNA profiling identify tumor neoantigens recognized by T cells?
6. Does radiation and IL-2 therapy induce a meaningful anti-melanoma CTL response in peripheral blood and/or in TIL subsets? Can anti-tumor humoral immunity be induced against tumor-associated antigens?
7. Does combination sequential radioimmunotherapy lead to CMV reactivation and generation of adaptive NK cells (CD57+NKG2C+)? Can anti-CMV T and NK cells or other anti-viral T cells in peripheral blood and the tumor microenviroment predict treatment response or identify biomarkers of response to T and NK cell therapeutics in refractory melanoma patients?

2.6.1. Evaluation of Tumor Biopsies

Tumor infiltrating lymphocytes (TILs) and other immune cells are associated with clinical outcome, and this study will collect tumor biopsy specimens to quantify TILs in all formalin-fixed paraffin-embedded tumor specimens.

We will use immunohistochemistry (IHC) and proteomics techniques to determine the extent and nature of immune infiltrates at baseline and after treatment. Only non-irradiated lesions will be biopsied to allow for characterization of therapy-induced systemic immunologic responses. We are particularly interested in the relationship of treatment with Nivolumab monotherapy versus dual checkpoint inhibition and function of T regs, and other T cell subsets.

Additional immune cells of interest include natural killer (NK) cells, monocytes, dendritic cells, myeloid derived suppressor cells (MDSCs), vascular and lymphovascular endothelial cells, and stromal cells. The following cellular markers will be analyzed using IHC and/or similar technique: CD3, CD8, PD-1, CTLA-4, MHC-I, CD4 (T cells), CD56 and CD16 (NK and NK T cells) CD45 (leukocytes), CD19 (B cells), CD68, glucocorticoid-induced tumor necrosis factor receptor family related gene (GITR), TIM-3, forkhead box P3 (FOXP3), and lymphocyte-activation gene 3 (LAG3).

We hypothesize that combination sequential radioimmunotherapy increases the pool of effector and memory T cells against melanoma tumor-antigens and induces molecules such as CTLA-4, PD-1, LAG-3, and TIM-3 on the surface of tumor infiltrating lymphocytes.

Tumor tissue will be processed for DNA/RNA analysis and RNA-seq will be used for deep characterization of treatment related responses and identification of signatures predicting primary and secondary immunologic responses (UMN Genomics Center).

2.6.2. Melanoma Specific Cellular Immune Responses

Correlative studies will help answer several immunologic questions regarding the ability of radiation and IL-2 based therapy to prime anti-tumor immunity in refractory populations. Studies will assess induction of regulatory T cells and other suppressor cells, and the ability of checkpoint inhibition to inhibit these processes.

Peripheral blood mononuclear cells (PBMC) will be collected and processed for flow cytometric analyses and cryostorage for later batch studies. The effect of radiation therapy and IL-2 on lymphocyte repertoire, numbers and phenotype will be assessed at baseline and at defined time points during the trial. The frequency and percentage of PBMC and T-cell subsets (CD4 and CD8 effector memory, central memory and Treg subsets), NK cells and myeloid-derived suppressor cells will be determined.

We hypothesize that T cells primed by the combination sequential radioimmunotherapy approach undergo induction of normal homeostatic regulatory checkpoints that sensitize a subset of patients to later checkpoint inhibitor based therapy with currently approved standard agents.

Peripheral blood tumor-specific T cell subsets will be evaluated by flow cytometry in cooperation with the Translational Therapy Laboratory, Masonic Cancer Center. Cytotoxic T lymphocytes (CD8+ T cells) and CD4+ helper T cells will be isolated using fluorescence-activated cell sorting (FACS) from cryopreserved cells followed by mRNA expression analysis to assess gene expression changes resulting from therapy. Testing will be performed in collaboration with the University of Minnesota Flow Cytometry and Genomics Core Laboratories.

Peripheral blood mononuclear cells (PBMC) may also be tested in functional assays (i.e. IFN-γ ELISpot) for responses to known melanoma antigens, viral antigens, and predicted neoantigens from whole-exome/transcriptome sequencing of melanoma tumors. All patients will also undergo HLA testing to enable epitope discovery using available MHC binding prediction algorithms.

3. STUDY RATIONALE

There is a growing body of pre-clinical and clinical evidence that points to a synergistic relationship between radiation therapy and immunotherapy. Preclinical studies have shown that irradiation of tumor cells leads to immunogenic cell death via upregulation of major histocompatibility (MHC) molecules, DC cross-presentation of tumor antigens, and T-cell priming in draining lymph nodes(20, 21).

Preclinical studies combining Ipilimumab with radiation demonstrate enhanced abscopal responses in several cancer types(29). Similar abscopal effects have been reported in multiple instances of patients receiving radiation during concurrent checkpoint inhibition with anti-PD1 and anti-CTLA4 antibodies for metastatic disease(17, 30, 41, 42).

It is known that T cells require distinct sets of signals to proliferate and differentiate into effector T cells capable of mounting effective adaptive immune responses(43). The classical two-signal model of T cell activation first involves stimulation of a rearranged T cell receptor (TCR) with specificity for antigenic peptide within the groove of the MHC molecule. This is followed by signal 2, the co-stimulatory interactions of CD28 on the surface of naïve T cells with CD80 and CD86 ligands on the surface of antigen presenting cells.

Following the activation of T cells, expression of cytotoxic T-lymphocyte associated protein 4 (CTLA-4) is induced on the surface of activated T cells to restrain the CD28 activation pathway. The CTLA-4 checkpoint molecule is largely restricted to CD4 and CD8 T cells(44). However, CTLA-4 is also constitutively expressed in Foxp3+ regulatory (Treg) subsets(45), where CTLA-4 competes for CD80 and CD86 ligand interactions to blunt T-cell activation. It follows that administration of anti-CTLA-4 therapy leads to an increased risk of immune related adverse events and autoimmune toxicity. To minimize toxicity from anti-CTLA-4 therapy there is interest in reducing Ipilimumab dosing and dosage of Ipilimumab. The overarching goal of these strategies is to maintain clinical activity while reducing immune related adverse events.

This clinical study aims to sequence immunotherapy treatments after radiation therapy to coincide with antigen release and uptake and processing by APCs. After completion of radiation therapy patients receive high dose Aldesleukin (IL-2) to support *in vivo* priming and expansion of endogenous T cell populations against melanoma antigens. Importantly, primary T cell activation is tightly regulated and the sequential signals required to direct and amplify T cells must be delivered in-sequence to avoid paralyzing effects on T cell dependent immunity seen with out-of sequence delivery(46).

This combination sequential radioimmunotherapy approach builds on phase 1 study data demonstrating safety and preliminary efficacy signals of combination stereotactic ablative radiotherapy (SABR) and IL-2 as first-line treatment for metastatic melanoma and renal cell carcinoma(47). The current trial adds checkpoint inhibition following aldesleukin to maximize induced anti-tumor responses in an immunotherapy refractory patient population.

4. STUDY DESIGN

This is a Phase 2 study designed to evaluate the combination of checkpoint blockade and aldesleukin (IL-2) therapy after a course of standard of care palliative radiation in the management of unresectable metastatic melanoma refractory to standard immunotherapy drugs, molecular targeted agents and/or chemotherapy. To be eligible, a patient must have a minimum of 3 (preferably >5) radiographically distinct, measurable (>1.5 cm) lesions based on RECIST 1.1. Ocular melanomas may enroll in this study without prior therapy as there is no standard front-line therapy for this subset of patients.

Standard of care palliative radiation therapy is delivered to a maximum of 3 metastatic tumors over no more than 3 weeks. A minimum of 2 lesions must remain non-irradiated for disease response assessments. Patients who have completed palliative radiation therapy within 90 days prior to the 1st dose of aldesleukin also may be considered for the study.

Two treatment cohorts (treatment arms in OnCore) will be used based on disease origin:
Cohort 1: cutaneous melanoma – aldesleukin followed by nivolumab every 2 weeks for 6 doses.
Cohort 2: ocular melanoma – aldesleukin followed by nivolumab/ipilimumab given 3 weeks for 4 doses.

Each cohort enrolls 22 patients employing a separate Simon's two stage design and separate monitoring for excessive toxicity.

Disease re-assessment includes physical examination, as well as radiographic response assessment (RECIST v1.1) of non-irradiated target lesions using CT (or PET/CT) and/or MRI at the 6 week (end of Cycle 1) and the end of trial (EOT) visit.

A final determination of disease progression for the study requires confirmation of progression with scans obtained at least 4-6 weeks after the EOT visit, in order to qualify

as progressive disease (PD) by RECIST 1.1 due to observed pseudoprogression and late responses seen with immunotherapy treatments. Therapy after progression is at the discretion of the patient and primary physician.

Direct study participation ends after the End of Trial (EOT) visit approximately 4 weeks after the last dose of study drug; however, nivolumab maintenance therapy may continue at the treating physician's discretion independent of this study.

After the EOT visit, patients are monitored for progression and survival endpoints via medical record review or phone/electronic communications every 3 months for two years from the start of aldesleukin.

5. PATIENT SELECTION

The study is open to persons 18 years through and including 80 years of age regardless of gender, race or ethnic background. While there will be every effort to seek out and include women and minority patients, the patient population is expected to be no different than that of other advanced melanoma studies at the University of Minnesota.

5.1. Inclusion Criteria

5.1.1 Biopsy-proven unresectable, metastatic melanoma refractory to standard immunotherapy drugs or regimens, including prior treatment with Aldesleukin (IL-2), GM-CSF, Ipilimumab, Nivolumab, Pembrolizumab, and/or Imlygic (T-VEC).

Prior clinical trial participation or treatment with molecularly targeted agents (i.e. Vemurafenib/Cobimetinib, Dabrafenib/Trametinib) or chemotherapy (i.e. Temozolomide, Dacarbazine, Platinum, or Taxanes) is permitted.

Patients with ocular melanoma may enroll (Cohort 2) without prior therapy as there is no standard 1st line therapy for this subset of melanoma.

5.1.2 Must have a minimum of 3 radiographically distinct (>1.5 cm) lesions measurable by RECIST 1.1 at time of study enrollment (>5 preferred).

- A maximum of 2 metastases per treated organ may be targeted for palliative radiation, but must be separated by more than 5 cm of normal tissue
- At least 2 non-irradiated lesions are required for systemic response assessments

5.1.3 Pulmonary metastases: Pulmonary metastasis permissible. Appropriate candidates with lung lesions may be considered for ablative hypofractionation using stereotactic body radiation therapy (SBRT).

5.1.4 Hepatic metastases: Hepatic metastasis permissible. Appropriate candidates with metastasis to liver may be considered for ablative hypofractionation using stereotactic body radiation therapy (SBRT).

5.1.5 Brain metastases: Brain metastases may be treated using Gamma Knife Radiosurgery (GKR) or whole brain radiation therapy (WBRT) per the treating radiation oncologist. Total radiation dose and number of fractions will be determined by the treating radiation oncologist based on anatomic and dosing constraints. MRI of the vertebral column is required for all patients with suspected epidural tumor extension.

5.1.6 Must have sufficient archival tissue block material (1.5 x 1.5 x 1.5 cm) and/or newly obtained core or excisional biopsy of tumor tissue; minimum of 2 cores.

5.1.7 ECOG performance status 0 or 1 (Appendix 1)

5.1.8 Age 18 through 80 years of age; > 80 years of age must be approved by the Principal Investigator.

5.1.9 Adequate organ function within 14 days of enrollment (30 days for pulmonary and cardiac assessments) defined as:

Hematologic: leukocytes ≥ 2,000/mcL, ANC ≥ 1,000/mcL, hemoglobin ≥ 9.0 g/dL, platelets ≥ 100,000/mcL unsupported by transfusions

Renal: Serum creatinine ≤ 1.8 mg/dL; for patients with a creatinine > 1.5 mg/dL or a history of renal dysfunction, an estimated glomerular filtration rate ≥ 35 mL/min/1.73 m^2 is required

Hepatic: AST, ALT, and alkaline phosphatase ≤ 3 x upper limit of normal (≤ 5 x ULN if known or suspected liver mets) and total bilirubin ≤ 2.0 mg/dL

Pulmonary: oxygen saturation ≥90% on room air; corrected DLCO and FEV1, ≥ 60% predicted

Cardiac: Absence of clinical decompensated congestive heart failure or uncontrolled arrhythmia; left ventricular ejection fraction (echocardiogram within 6 months permitted) ≥ 40%. QTc must be < 450 ms in males and < 470 ms in females.

5.1.10 A minimum of 1 week between last anti-tumor treatment if given, and 1st dose of radiation therapy (not applicable for patients enrolling after palliative radiation therapy).

5.1.11 Recovery from previous cancer treatment if applicable defined as ≤ Grade 1 (by CTCAE 5.0 criteria) at enrollment

5.1.12 Women of childbearing potential and males with partners of childbearing potential must agree to the use of barrier methods of contraception, hormonal contraceptives, or abstain from heterosexual activity for the duration of study treatment and for 3 months after the last dose of study drug.

5.1.13 Ability to understand and provide voluntary written consent

5.2. Exclusion Criteria

5.2.1 Pregnant or breast feeding. The agents used in this study have the potential to harm a fetus. Radiation is a known teratogen. There is insufficient information regarding potential for fetal harm during immunotherapy at this time. Biological females of childbearing potential must have a negative pregnancy test within 14 days of enrollment.

5.2.2 Concurrent use of high dose steroids; chronic steroid use of < 2 mg dexamethasone or equivalent per day is permissible

5.2.3 Concurrent malignancy requiring active treatment, except basal cell carcinoma of the skin, squamous cell carcinoma of the skin or carcinoma in situ

5.2.4 Severe and active autoimmune diseases requiring systemic immunosuppression

5.2.5 Prior organ allograft or allogeneic transplantation

5.2.6 Other contraindication to IL-2, nivolumab, ipilimumab, or combination immunotherapy per treating medical oncologist

5.2.7 Live vaccines within 30 days prior to the first dose of IL-2 and while participating in the trial. Examples of live vaccines include, but are not limited to, measles, mumps, rubella, chicken pox, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally inactivated virus vaccines and are allowed. Intranasal influenza vaccine (eg, Flu - Mist®) is a live attenuated vaccine, and is not allowed.

6. PATIENT SCREENING AND STUDY ENROLLMENT

Written consent must be obtained prior to the performance of any research related tests or procedures. Consent is obtained before eligibility is confirmed.

6.1. Enrollment with the University of Minnesota Clinical Trials Office

Any patient who has been consented is to be entered in OnCore by the site Study Coordinator or designee. If a patient is consented but is not enrolled, the patient's record is updated in OnCore as a screen failure and reason for exclusion recorded.

6.2. Patient Enrollment in OnCore

To be eligible for study enrollment, the patient must sign the treatment consent and meet each inclusion criteria and none of the exclusion criteria on the eligibility checklist based on an eligibility assessment documented in the patient's medical record.

The Study Coordinator or designee assigns the study treatment arm (Cohort 1 – cutaneous melanoma or Cohort 2 – ocular melanoma).

6.3. Patients Who Do Not Begin Study Treatment

If a patient is registered to the study and is later found unable to begin study treatment (1st dose of IL-2) the patient will be removed from study and treated at the physician's discretion. The study staff will update OnCore of the patient's non-treatment status (off study). The reason for removal from study prior to starting study treatment will be clearly indicated in OnCore. The patient will be replaced to complete enrollment.

7. TREATMENT PLAN

7.1. Palliative Radiation Therapy

When palliative radiation has been received off-study or delivered outside of the University of Minnesota, patients may go on to receive IL-2 and checkpoint inhibitor on study as described in Section 7.2 and Section 7.3 provided the 1st dose of aldesleukin is given within 90 days of the last dose of RT. If additional palliative radiation is clinically warranted, the patient would be treated as described below.

A maximum of 3 metastatic tumors will be targeted for palliative radiation; up to 2 metastases per treated organ may be targeted for hypofractionated palliative radiation but must be separated by more than 5 cm of normal tissue. Appropriate candidates with lung and liver metastases may be considered for ablative hypofractionation. For liver directed therapy, patients will have an MRI of the liver for radiation treatment planning. Patient must have at least one non-irradiated hepatic metastasis amenable to percutaneous biopsy. Treatment for hepatic metastasis must also spare a critical hepatic volume (volume receiving <21 Gy) of 700 ml. Appropriate candidates with liver metastases may be considered for Y-90 radioembolization.

Brain metastases may be treated with surgery followed by Gamma Knife (GK) to the resection cavity or alternatively as part of definitive brain directed therapy. Whole brain radiation therapy (WBRT) may also be used at the discretion of the treating radiation-oncologist.

For sites not amenable to ablative radiotherapy, fractions will be delivered at a minimum of 3 Gy per fraction.

At least 2 non-irradiated lesions >1.5 cm are required for systemic response assessments. The total radiation dose and number of fractions will be determined by the treating radiation oncologist based on anatomic and dosing constraints; however when palliative radiation therapy is received on study it must not exceed 3 weeks and treatment must be administered in such a way that that patient can be admitted to the hospital within 5 days of the last radiation fraction to start high-dose IL-2.

7.2. Aldesleukin (IL-2) – All Participants

After palliative radiation is complete, patients are admitted to the hospital to begin Aldesleukin (IL-2) therapy. Treatment is administered as an inpatient according to the University of Minnesota guidelines for Aldesleukin administration.

Aldesleukin (IL-2) at 600,000 U/kg will be administered as an intravenous infusion via a central catheter (either PICC line or port) over 15 minutes approximately once every 8 to 12 hours, per institutional guidelines and standard clinical practice. Patients may receive a maximum of 10 doses, no longer than 24-hours apart, on Days 1 through 5 and Days 15 through 19.

The dosing calculations should be based on the body weight. If the subject's weight on the day of dosing differs by > 10% from the weight used to calculate the dose, the dose must be recalculated. All doses should be rounded to the nearest milligram.

As it recognized that most patients experience severe toxicity with IL-2 and often are not able to complete all planned doses, the number of doses an individual patient receives will be based on personal experience and the treating physician's clinical judgement. Even if a patient is unable to receive IL-2 as planned, the study calendar for disease reassessment and research sample collections should be followed.

Patients will have routine vital signs and laboratory tests measured daily during aldesleukin therapy. IL-2 is associated with a decrease in systemic vascular resistance and low blood pressure from development of capillary leak syndrome (CLS). Increased permeability of blood vessels results in extravasation of plasma proteins and fluid into extravascular compartments and spaces, necessitating maintenance IV fluid hydration and aggressive fluid boluses to support blood pressure and maintain normal organ perfusion.

Standard institutional practices govern IL-2 administration. Briefly, bolus infusions continue until the development of CTCAE v5 Grade 3 or 4 expected toxicities based on patient tolerance. The most common Grade 4 events are pulmonary and renal impairment, and mental status changes. The anticipated toxicities require supportive measures during treatment, but toxicities are generally acute and reversible and the majority of patients have no long-term sequelae from intensive IL-2 treatment. Development of side effects from IL-2 is anticipated and therefore will not be considered serious adverse event for safety analyses. However, abnormal lab values must plateau and begin trending toward baseline prior to hospital discharge following IL-2 treatments.

Since IL-2 treatment can cause a transient decrease in neutrophil chemotaxis antibiotic prophylaxis is given (i.e. penicillin or cephalosporin) to minimize the incidence and risk of serious bacterial infections.

7.3. Nivolumab Monotherapy (Cohort 1 - Cutaneous)

Nivolumab 240 mg IV is administered over 30 minutes every 2 weeks as maintenance therapy.

For any individual dose a ±3 day window is permitted without affecting (no adjustment to) future doses. This window is primarily for scheduling issues (i.e. holidays, inclement weather, etc).

Longer delays are permitted, if in the opinion of the treating investigator, a delay in therapy is in the best interest for the patient and their ability to complete the planned treatment. In the instance of longer delays (i.e. 1 week or longer), the reminder of the treatment schedule will be shifted to keep proper spacing between the remaining doses. Section 8.3 provides guidance on delays for significant immune related toxicity; however, delays may be for other reasons.

The 1st dose is given Day 29 of Cycle 1 and continues in Cycle 2 on Days 1, 15, and 29 of a 6 week cycle.

Cycle 3 nivolumab is given on Day 1 and Day 15 of a standard 4 week cycle.

Cycle 3 is the end of the planned study treatment; however patients may continue maintenance nivolumab 240 mg IV every 2 weeks OR nivolumab 480 mg IV every 4 weeks per 2018 FDA approval at discretion of the treating physician and independent of this study.

Nivolumab is administered in the outpatient setting; however, inpatient administration is permitted as medically appropriate if a patient is hospitalized. No premedication is required. Monitoring will be according to institutional guidelines with interruption or slowing of rate of infusion with Grade 1 to 2 infusional toxicities and discontinuation of nivolumab with Grade 3 to 4 infusional toxicities.

The dose is calculated on a pre-treatment (baseline) weight within 7 days of treatment start. The dose must be re-calculated in a new treatment cycle if a greater than 10% weight change occurs from baseline.

No dose modifications are permitted for nivolumab but dose may be withheld based on occurrence of significant immune-related AE Grade 2 or higher based on NCI Common Terminology Criteria for Adverse Events (CTCAE) v5.0, as described in Section 8.3.

7.4. Nivolumab and Ipilimumab (Cohort 2- Ocular)

Nivolumab and Ipilimumab is administered sequentially as an intravenous infusion of nivolumab (3 mg/kg) over 30 minutes followed by an intravenous infusion of ipilimumab (1 mg/kg) over 90 minutes.

Alternatively, at the discretion of the treating investigator, a high risk patient with an excellent performance status may receive nivolumab (1 mg/kg) over 30 minutes followed by an intravenous infusion of ipilimumab (3 mg/kg) over 90 minutes.

Nivolumab and ipilimumab is given for 4 doses every 3 weeks (on Day 29 of Cycle 1, Day 8 and 29 of Cycle 2, and Day 8 of Cycle 3).

For any individual dose a ±3 day window is permitted without affecting (no adjustment to) future doses. This window is primarily for scheduling issues (i.e. holidays, inclement weather, etc).

Longer delays are permitted, if in the opinion of the treating investigator, a delay in therapy is in the best interest for the patient and their ability to complete the planned treatment. In the instance of longer delays (i.e. 1 week or longer), the reminder of the treatment schedule will be shifted to keep proper spacing between the remaining doses. Section 8.3 provides guidance on delays for significant immune related toxicity; however, delays may be for other reasons.

On Day 1 of Cycle 4 the patient may begin nivolumab maintenance as a single agent per Section 7.3.

Diphenhydramine and/or acetaminophen may be administered as prophylactic pre-medications.

Nivolumab and Ipilimumab is administered in the outpatient setting; however, inpatient administration is permitted as medically appropriate if a patient is hospitalized. No premedication is required. Monitoring will be according to institutional guidelines with interruption or slowing of rate of infusion with grade 1 to 2 infusional toxicities and discontinuation of nivolumab and ipilimumab with grade 3 to 4 infusional toxicities.

The dose is calculated on a pre-treatment (baseline) weight within 7 days of treatment start. The dose must be re-calculated in a new treatment cycle if a greater than 10% weight change occurs from baseline.

No dose modifications are permitted for nivolumab and ipilimumab but doses may be withheld based on occurrence of significant immune-related AE Grade 2 or higher based on NCI Common Terminology Criteria for Adverse Events (CTCAE) v5.0, as described in Section 8.3.

7.5. Supportive Care

In order to provide optimal patient care and to account for individual medical conditions, investigator discretion may be used in the prescribing of all supportive care drug therapy (i.e. acetaminophen, diphenhydramine, etc.), and will not be considered a protocol deviation.

7.6. Duration of Study Treatment

Treatment is given over approximately 16 weeks. Treatment continues in the absence of documented progression, unacceptable side effects or patient refusal/noncompliance. A final determination of disease progression for the study requires confirmation of progression with radiographic scans obtained at least 4-6 weeks after the EOT visit, in order to qualify as progressive disease (PD) by RECIST 1.1 due to observed pseudoprogression and late responses seen with immunotherapy treatments.

Palliative radiation is administered to most patients over a 3 week period just prior to starting aldesleukin infusions, although patients may be considered for the study if they have completed palliative radiation within 90 days of the 1st dose of aldesleukin.

Patients may continue maintenance nivolumab 240 mg IV every 2 weeks OR nivolumab 480 mg IV every 4 weeks per 2018 FDA approval at discretion of the treating physician and independent of this study.

7.7. Duration of Study Participation

Direct study participation ends after the End of Trial (EOT) visit approximately 4 weeks after last dose of study directed treatment. If in the opinion of the investigator there is ongoing toxicity at least possibly related to the study treatment, the patient will be followed as medically appropriate until resolution or stabilization of the toxicity.

Follow-up for disease status and survival will continue for up to 2 years from study enrollment by electronic/phone contact every 3 months (± 2 weeks) unless the patient withdraws consent.

8. EXPECTED SIDE EFFECTS AND MANAGEMENT OF SELECTED TOXICITIES

8.1. Palliative Radiation Therapy

Adverse events related to radiation therapy for the treatment of metastases are dependent on the location of the metastases treated as well as from exposure of surrounding normal tissues. For all treated metastases, fatigue is likely to occur and should be transient lasting < 8 weeks. Other adverse events are likely to be related to the specific metastatic location receiving radiation therapy. All radiation therapy associated adverse events should be graded according to the Common Terminology Criteria for Adverse Events (CTCAE), v. 5.

8.1.1. Lung (Central and Peripheral), Mediastinal/Cervical Lymph Node Metastases

- Cardiac and Pericardial Injury: uncommon in conventionally fractionated course of radiation therapy, but with large doses per fraction of SBRT myocarditis, pericarditis and other side-effects can be seen.
- Gastrointestinal/Esophageal Injury: The radiation effects on the esophagus can be acute: esophagitis (i.e. dysphagia) typically improves soon after RT course is completed, or chronic: esophageal stenosis, ulceration, or perforation (rarely in extreme cases).
- Central Airway/Bronchial Injury: Bronchial injury may cause subsequent focal collapse of lung impairing overall pulmonary status. Collapsed/atelectatic lung may make further assessment of tumor response more difficult. Bronchial toxicity may lead to cough, dyspnea, hypoxia, impairment in pulmonary function test parameters, pleural effusion, or pleuritic pain.
- Lung Injury: Radiation pneumonitis is a subacute (weeks to months from treatment) inflammation of the end bronchioles and alveoli. Radiation fibrosis is a late manifestation of radiation injury to the irradiated lung. Infiltrates on chest x-ray characteristically include the area treated to high

dose, but may extend outside of the region and appear “geometric” in appearance, corresponding to the radiation portal.

- Mild radiation pneumonitis may be treated with nonsteroidal anti-inflammatory agents or steroid inhalers.
- Moderate to severe radiation pneumonitis will be treated with systemic steroids, bronchodilators, and pulmonary hygiene.

8.1.2. Liver and Abdominal-Pelvic Metastases

- Radiation-induced liver disease (RILD): Not likely (<5%), but serious. Classic RILD is a clinical diagnosis of anicteric ascites, hepatomegaly and elevation of alkaline phosphatase relative to other transaminases that may occur 2 weeks to 3 months following radiation to the Liver; non-classic RILD includes elevation of liver enzymes and/or any decline in liver function within 12 weeks from start of therapy. There is increased risk of liver toxicity in patients with large tumors and in patients with pre-existing liver disease.
- Permanent thrombocytopenia (<1%), which may lead to bleeding
- Kidney injury (<1%), which may lead to changes on imaging studies.

8.1.3. Spinal Metastases

- Radiation myelitis: Given proximity and position of spinal cord in relation to the radiosurgery target, every effort should be made to minimize radiation dose to the spinal cord. Radiation myelitis is a subacute or chronic clinical syndrome after radiation. Symptoms may include paresthesia, sensory changes, and motor weakness including paralysis. There is no active treatment for radiation myelitis, therefore it is important to prevent any injury to the spinal cord.
- Radiation esophagitis: Thoracic spine treatment will likely lead to esophageal mucositis within the first 2 weeks. Symptoms generally subside with time. Hydration is important given that dehydration may result due to consequences of esophageal toxicity i.e. dysphagia, cough, swallowing difficulty. Dysphonia and fistula are rare.
- Radiation Laryngitis or pharyngitis: Cervical spine treatment will likely lead to laryngopharyngeal mucositis within the first 2 weeks. Symptoms generally subside with time. Hydration is important given that dehydration may result due to consequences of radiation toxicity i.e. dysphagia, cough, swallowing difficulty. Dysphonia and fistula are rare.
- Compression Fracture of treated Vertebra: Radiation doses in excess of 19 Gy for a single fraction have been associated with higher rates of vertebral body compression (Saghal 2013) and will be avoided.

8.1.4. Osseous metastases

- Erythema, desquamation and alopecia are common side effects from radiation therapy for osseous metastases; other effects based on location of metastasis and may include pain, edema, and neuralgia.

8.2. Aldesleukin (IL-2)

The development of anticipated side effects from IL-2 is physiologic and therefore side effects are not considered serious adverse events for safety analyses. However, abnormal lab values must plateau and begin trending toward baseline prior to Hospital discharge following IL-2 treatments.

Aldesleukin (IL-2) Expected Risks		
Most common	**Common**	**Less Common**
● diarrhea ● fever and chills ● flu-like symptoms ● capillary leak syndrome (hypotension and tachycardia from decreased intravascular volume) ● heartburn ● indigestion ● itching skin ● nausea ● rash ● severe stomach pain, cramping, or burning ● unusual tiredness or weakness ● vomiting ● dizziness ● double vision ● drooping eyelids ● drowsiness ● swelling of the face, feet, or lower leg ● unusual weight gain	● abdominal or stomach tenderness ● anxiety ● blistering, crusting, irritation, itching, or reddening of the skin ● blurred vision ● burning or itching eyes ● burning, tingling, numbness or pain in the hands, arms, feet, or legs ● chest pain ● clay-colored stools ● cough ● cracked or scaly skin ● dark urine ● decreased appetite ● decreased frequency or amount of urine ● eye discharge or excessive tearing ● eye pain or sensitivity to light ● fainting ● headache ● hives or welts ● loss of appetite ● mental depression ● muscle pain or stiffness ● muscle weakness ● painful or difficult urination ● redness, pain, or swelling of the eye, eyelid, or inner lining of the eyelid ● sensation of pins and needles ● severe tiredness ● shortness of breath ● sore throat ● sores, ulcers, or white spots on the lips or in the mouth ● stabbing pain ● swollen glands ● trouble breathing	● bloody or cloudy urine ● bloody, black or tarry stools ● bloating ● blue or pale skin ● chest pain, possibly moving to the left arm, neck, or shoulder ● confusion ● constipation ● difficulty with moving ● fast heartbeat ● general feeling of discomfort or illness ● increased blood pressure ● increased thirst ● lower back or side pain ● pain or burning in the throat ● pain, swelling, or redness in the joints ● pains in the stomach or side, possibly radiating to the back ● sores, welting, or blisters on the skin ● stiff neck or back ● unusual bleeding or bruising ● yellow eyes or skin ● vomiting of material that looks like coffee grounds, severe and continuing ● difficulty breathing, chewing, swallowing, or talking

Treatment Guidelines for High-Dose Aldesleukin (IL-2) Toxicity Management

- Acetaminophen, naproxen (as needed), and H2 blocker therapy will be used to manage anticipated side effects of high-dose IL-2 therapy, and will generally be continued for 8-12 hours after last dose of IL-2 to avoid development of high fever.
- Meperidine (25-50 mg) may be given intravenously if severe chills develop.

- Additional antiemetic therapy will be administered for breakthrough nausea and/or vomiting.
- Supportive care measures are outlined in Appendix 2, as indicated for expected toxicities from high-dose IL-2.

8.3. Ipilimumab and Nivolumab Related Toxicity Management

Immune-related adverse events (irAEs) from ipilimumab and nivolumab will be managed depending on severity (NCI CTCAE V5.0 grading) as outlined below.

Immune-Related (ir) Adverse Events Management – General Guidelines

Grade 1 to 2: Treat symptomatically or hold checkpoint inhibitors. Consideration of moderate dose steroids (0.5 mg/kg), and more frequent monitoring.

Grade 1 to 2 (persistent): Manage similar to Grade 3 to 4 irAE

Grade 3 to 4: Treat with high dose corticosteroids, typically 1-2 mg/kg of prednisone or IV steroid equivalent and

Grade 4 irAEs permanently discontinue treatment with nivolumab or nivolumab and ipilimumab

Grade 3 irAEs requires withholding nivolumab or nivolumab and ipilimumab except for any of the following: Transient (≤ 6 hours) Grade 3 flu-like symptoms or fever, which is controlled with medical management, transient (≤ 24 hours) Grade 3 fatigue, local reactions, headache, nausea, emesis that resolves to Grade ≤ 1 and single laboratory values out of normal range (excluding Grade ≥ 3 liver function test increase) that are unlikely related to study treatment according to the Investigator, do not have any clinical correlate, and resolve to Grade ≤ 1 within 7 days with adequate medical management

Any Grade 2 irAEs should be managed as follows:
If a Grade 2 irAE resolves to Grade ≤ 1 by the next planned cycle, treatment may continue.

If a Grade 2 irAE does not resolve to Grade ≤ 1 by the next planned cycle, treatment should be withheld at next cycle. If at the end of the following cycle the event has not resolved to ≤ Grade 1, the subject should be treated with high-dose steroids or discontinue treatment with nivolumab or nivolumab and ipilimumab (except for hormone insufficiencies, which can be managed by replacement therapy).

Upon the recurrence of the same Grade 2 irAE (except for hormone insufficiencies that can be managed by replacement therapy) in the same subject, treatment with nivolumab or nivolumab and ipilimumab should be permanently discontinued.

Treatment of gastrointestinal, dermatological, pulmonary, hepatic and endocrine irAEs should follow guidelines in the following table.

GI irAEs		
Severity of Diarrhea/Colitis	**Management**	**Follow-up**
Grade 1 Diarrhea: < 4 stools/day over baseline Colitis: asymptomatic	Continue checkpoint inhibitor therapy. Symptomatic treatment (for example, loperamide)	Close monitoring for worsening symptoms Educate subject to report worsening immediately If worsens: Treat as Grade 2 or 3 to 4
Grade 2 Diarrhea: 4 to 6 stools per day over baseline; IV fluids indicated < 24 hours; not interfering with ADL Colitis: abdominal pain; blood in stool	Withhold checkpoint inhibitor therapy for the current cycle Symptomatic treatment	If improves to Grade ≤1: Resume checkpoint inhibitor If worsens: treat as Grade 3 to 4
Grade 3 to 4 Diarrhea (Grade 3): ≥ 7 stools per day over Baseline; incontinence; IV fluids ≥ 24 hrs.; interfering with ADL Colitis (Grade 3): severe abdominal pain, medical intervention indicated, peritoneal signs Grade 4: life-threatening, perforation	Grade 3: Withhold checkpoint inhibitor for the current cycle Grade 4 or recurrent Grade 3: permanently discontinue checkpoint inhibitor Begin methylprednisolone 1.0 to 2.0 mg/kg/day IV or equivalent Add prophylactic antibiotics for opportunistic infections Consider colonoscopy if needed	If improves, continue steroids until Grade < 1, then taper over at least 1 month, Resume checkpoint inhibitor on Day 1 of the next cycle If worsens, persists > 3 to 5 days, or recurs after improvement, add infliximab 5 mg/kg (if no contraindication), Note: Infliximab should not be used in cases of perforation or sepsis
Dermatological irAEs		
Grade of Rash	**Management**	**Follow-up**
Grade 1 to 2 Covering for Grade 3 30% body surface area	Symptomatic therapy (for example, antihistamines, topical steroids) Continue checkpoint inhibitor	If persists > 1 to 2 weeks or recurs: Consider skin biopsy Consider methylprednisolone 0.5 to 1.0 mg/kg/day IV or oral equivalent. Once improving, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections. If worsens. treat as Grade 3 or 4
Grade 3 to 4 Covering > 30% Grade 4: body surface area; life threatening consequences	Grade 3: Skip checkpoint inhibitor for the current cycle Grade 4 or recurrent Grade 3: Permanently discontinue checkpoint inhibitor Consider skin biopsy Dermatology consult methylprednisolone 1.0 to 2.0 mg/kg/day IV or IV equivalent	If improves to ≤ Grade 1, taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections Resume checkpoint inhibitor therapy Day 1 of next cycle for Grade 3

Pulmonary irAEs		
Grade of Pneumonitis	**Management**	**Follow-up**
Grade 1 Radiographic changes only	Permanently discontinue checkpoint inhibitor Monitor for symptoms every 2 to 3 days Consider pulmonary and Infectious disease consults	If worsens, treat as Grade 2 or 3 to 4
Grade 2 Mild to moderate new symptoms	Permanently discontinue checkpoint inhibitor Pulmonary and Infectious Disease consults Monitor symptoms daily, consider hospitalization methyl-prednisolone 1.0 mg/kg/day IV or oral equivalent Consider bronchoscopy, lung biopsy	When symptoms improve to Grade ≤1, taper steroids over at least 1 month If not improving after 2 weeks or worsening, treat as Grade 3 to 4
Grade 3 to 4 Severe new symptoms; New / worsening hypoxia; life-threatening	Permanently discontinue checkpoint inhibitor Hospitalize Pulmonary and Infectious Disease consults methyl-prednisolone 2 to 4 mg/kg/day IV or IV equivalent Add prophylactic antibiotics for opportunistic infections Consider bronchoscopy, lung biopsy	When symptoms improve to Grade ≤ 1, taper steroids over at least 1 month If not improving after 48 hours or worsening:, add additional immunosuppression (for example, infliximab, cyclophosphamide, IV immunoglobulin, or mycophenolate mofetil)
Hepatic irAEs		
Grade of Liver Test Elevation	**Management**	**Follow-up**
Grade 1 Grade 1 AST or ALT > ULN to 3.0 x ULN and/or total bilirubin > ULN to 1.5 x ULN	Continue checkpoint inhibitor	Continue liver function monitoring If worsens, treat as Grade 2 or 3 to 4
Grade 2 AST or ALT > 3.0 to ≤ 5 x ULN and / or total bilirubin > 1.5 to ≤ 3 x ULN	Skip checkpoint inhibitor for the current cycle Increase frequency of monitoring to every 3 days	If returns Grade ≤ 1, resume routine monitoring, resume checkpoint inhibitor on Day 1 of the next cycle If elevations persist > 7 days or worsens, methylprednisolone 0.5 to 1 mg/kg/day or oral equivalent and when LFT returns to Grade ≤ 1 taper steroids over at least 1 month, resume checkpoint inhibitor on Day 1 of next cycle
Grade 3 to 4 AST or ALT > 5 x ULN and / or total bilirubin > 3 x ULN	Permanently discontinue checkpoint inhibitor Increase frequency of monitoring to every 1 to 2 days methylprednisolone 1.0 to 2.0 mg/kg/day IV or IV equivalent Consult gastroenterologist Consider obtaining MRI/CT scan of liver and liver biopsy if clinically warranted	If returns to Grade ≤1, taper steroids over at least 1 month If does not improve in > 5 days, worsens or rebounds, add mycophenolate mofetil 1 gram (g) twice daily If no response within an additional 3 to 5 days, consider other immunosuppressants per local guidelines

Cardiac irAEs		
Myocarditis	**Management**	**Follow-up**
New onset cardiac signs/symptoms and/or new laboratory cardiac biomarker elevations (e.g. troponin, CK-MB, BNP) or cardiac imaging suggestive of myocarditis	Skip checkpoint inhibitor for the current cycle Hospitalize. Cardiology consult to establish etiology and rule-out immune-mediated myocarditis, consider myocardial biopsy	If symptoms improve and immune-mediated etiology is ruled out, resume checkpoint inhibitor Day 1 of next cycle. If symptoms do not improve or worsen, and immune-mediated etiology is suspected or confirmed manage as immune-mediated myocarditis.
Immune-mediated myocarditis	Permanently discontinue checkpoint inhibitor Methylprednisolone 1 to 2 mg/kg/day.	Once improving, taper steroids over at least 1 month If no improvement or worsening, consider additional immunosuppressants (e.g. azathioprine, cyclosporine A)
Endocrine irAEs		
Endocrine Disorder	**Management**	**Follow-up**
Asymptomatic TSH abnormality	Continue checkpoint inhibitor If TSH < 0.5 x LLN, or TSH > 2 x ULN, or consistently out of range in 2 subsequent measurements: include T4 at subsequent cycles as clinically indicated; consider endocrinology consult	
Hypophysitis **Grade 2 or 3**	Evaluate endocrine function Consider pituitary scan Withhold checkpoint inhibitor 1 mg/day prednisone equivalents Initiate appropriate hormone therapy as indicated Consider Endocrinology consult	If improves (with or without hormone replacement): Taper steroids over at least 1 month Resume checkpoint inhibitor on Day 1 of next cycle
Grade 4	Permanently discontinue checkpoint inhibitor	
Adrenal Insufficiency **Grade 2**	Withhold checkpoint inhibitor Evaluate endocrine function Consider Endocrinology consult	If improves to Grade ≤1, Resume checkpoint inhibitor Day 1 of next cycle
Grade 3 or 4	Permanently discontinue checkpoint inhibitor Consider Endocrinology consult prednisone 1 to 2 mg/kg/day or equivalents	

9. SCHEDULE OF PATIENT ACTIVITIES

Scheduled evaluations/drug dosing during treatment may be performed ±3 days from the targeted date. In addition, targeted days may be altered as clinically appropriate. Follow-up is by record review, phone/electronic communication every 3 months (±2 weeks).

For any individual dose a ±3 day window is permitted without affecting (no adjustment to) future doses. This window is primarily for scheduling issues (i.e. holidays, inclement weather, etc).

Longer delays are permitted, if in the opinion of the treating investigator, a delay in therapy is in the best interest for the patient and their ability to complete the planned treatment. In the instance of longer delays (i.e. 1 week or longer), the reminder of the treatment schedule be shifted to keep proper spacing between the remaining doses.

Section 8.3 provides guidance on delays for significant immune related toxicity; however, delays may be for other reasons.

Note: Although the treatment days for Cohort 1 and 2 differ, the clinic visit schedule is the same.

9.1. Required Clinical Care

	Baseline	RT (if given)	Cycle 1 (6 week duration)						Cycle 2 (6 week duration)				Cycle 3 (4 week duration)			End of Trial (EOT) visit[2]	Optional nivolumab maintenance[3]	Every 3 months until 2 years from 1st dose of IL-2
			Day 1	Day 8	Day 15	Day 22	Day 29	Day 36	Day 1	Day 8	Day 15	Day 29	Day 1	Day 8	Day 15			
Consent	X																	
Screening Assessment	X																	
Medical History including other malignancies, concomitant medications	X																	
RAD-ONC/IR Consult	X																	
Physical Exam	X		Daily during IL-2	X	Daily during IL-2	X	X	X	X	X	X	X	X	X	X	X		
Vital Signs, O2 sats	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Fluid I/O (inpt only)			X		X													
Weight	X		X		X			X	X	X	X	X	X	X		X		
Height	X																	
Documentation of lesions and ID of non-irradiated lesions for disease response	X		X					X								X		
Archived Tumor Tissue	X[2]																	
Performance Status	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Toxicity Assessment/Stopping Rule Event	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X		
CBC with Diff, Plt	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Basic metabolic panel (BMP) – unless CMP done			Daily during IL-2		Daily during IL-2													
Comprehensive metabolic panel (CMP)	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X		
ACTH and TSH w/ reflex free T4	X															X		
eGFR	X																	
LDH			X													X		
Pregancy test (serum or urine) per Section 5.2.1	X																	
Disease Staging/Status	X							X								X		X
Tumor Biopsy	X[2]							X								X		
PFTs	X																	
EKG	X																	
Echocardiogram	X																	
Disease and Survival Status																		X
	Study Treatment																	
Aldesleukin (IL-2) - both Cohorts			Day 1-5		Day 15-19													
Nivolumab (Cohort 1)							X		X		X	X	X		X			
Nivolumab/Ipilimumab (Cohort 2 only)							X			X		X		X				
Nivolumab maintenance – both Cohorts																	X	

1. Within 30 days of study enrollment except for labs required for eligibility must be within 14 days of study enrollment
2. As part of this protocol's eligibility, a patient must have sufficient archival tissue block material (1.5 x 1.5 x 1.5 cm) If archived tissue not available, a fresh tumor biopsy is required (core or excisional biopsy of tumor tissue; minimum of 2 cores) for study eligibility; however, if archived tissue is available, the fresh tumor biopsy will be charged to research – refer to Section 9.2
3. End of trial clinical visit and disease reassessment 2-4 weeks after Cycle 3 Day 15 (cohort 1) or 2-4 weeks after Cycle 3 Day 8 (cohort 2)

9.2. Research Related Sample Collection

If a patient discontinues aldesleukin early, the research calendar and disease reassessment should continue to follow the schedule below using the 1st dose of aldesleukin as the calendar anchor.

	Baseline	Day 7 of RT (if given)	Day 1 and 5	Day 15 and 19	Cycle 1 Day 36	Cycle 2 Day 29	End of Trial (EOT) visit	At disease progression if feasible
Toxicity Notation	X	X	X	X	X	X	X	
FACT-M questionnaire	X	X	Day 1	Day 15	X	X	X	
Whole blood for Plasma (CMV DNA and cytokine testing) and PBMC (Immunophenotyping and functional studies) (10 mL x 5, green top, heparinized) Collect prior to Day's dosing (except 8 hour post IL-2 sample)	X	X[1]	X[2]	X[2]	X	X	X	
Tumor biopsy (4 cores); FFPE (x2) and Frozen (x2) -80 C	X[3]				X		X	X

1 – On RT treatment Day 7 (±3 days),

2 – before the 1st dose of aldesleukin and 8 to 12 hours after the last dose of aldesleukin administered

3- a fresh tumor biopsy is requried at baseline; however, it is done only once. If the patient has archived tissue and does not require a biopsy for study eligibility per footnote #2 in Section 9.1, a tumor biopsy will be done and charged to research per Section 9.2.

All research samples go to the Masonic Cancer Center's Translational Therapy Lab (TTL) unless otherwise specified on the lab slip.

Research sample collection and questionnaire completion are tied to the clinical care schedule of events and their associated window for performance. Therefore, if a clinical time point does not occur or is altered, the research related time point will be adjusted (or eliminated) as appropriate.

It is recognized that with novel therapies as used in this study, the timing of protocol directed research samples may miss important patient specific events. For this reason, up to 3 extra samples for a total of 180 ml of blood may be drawn at additional time points that are not specified above.

9.2.1. Collection of Tumor Biospecimens

As part of this protocol's eligibility, a patient must have sufficient archival tissue block material (1.5 x 1.5 x 1.5 cm) and/or newly obtained core or excisional biopsy of tumor tissue; minimum of 2 cores. A biopsy is performed around Day 36 of cycle 1 and again at the EOT visit soley for research purposes. In patients with confirmed radiologic progression, a biopsy sample of a tumor would ideally be obtained near the time of progression if feasible and safe (core, punch or excisional biopsy). Biopsy confirmation at time of progression is a standard of care practice.

Pre- and post-treatment levels of CD8+ T-cell tumor infiltrates will be assessed by IHC, in the Department of Laboratory Medicine and Pathology and with the assistance of BIONET. PD-1 and PD-L1, LAG-3, and TIM-3 expression will be

tested using Immunohistochemical reagents and techniques. RNA sequencing (RNA-Seq) analysis will be performed from unstained tissue slide material. Gene expression analysis will be performed by the University of Minnesota Genomics Core (UMGC).

9.2.2. Collection of Blood Specimens

Research sample processing and specialized testing will be provided by the Translational Therapy Shared Resource, at the Masonic Cancer Center, University of Minnesota. When appropriate, samples from normal donors will be used in comparative analyses.

We will collect whole blood for cytokine studies, DNA/RNA and Immune cell gene expression, and for PBMC isolation at various time points. For immune cell phenotype and functional studies we will collect 5 x 10 mL Green top (Heparinized) tubes for detailed NK cell and T-cell subset analysis and for plasma isolation and PBMC storage at -80°C.

To determine if tetramer reagents would be applicable for a given patient, a low-resolution human leukocyte antigen (HLA) Class I typing assay will also be performed at study enrollment.

For a complete listing of correlative objectives and tissue and assays planned, see Appendix 5.

9.2.3. Quality of Life (QOL) Measurement, FACT-Melanoma

The Functional Assessment of Cancer Therapy (FACT) subscale specific for melanoma, FACT-M, is a melanoma-specific quality of life (QOL) assessment instrument. The FACT–Melanoma subscale was developed to assess and identify the unique concerns of patients with melanoma.

The FACT-Melanoma questionnaire is a reliable and valid instrument for patients with melanoma that can be used for the assessment of QOL in clinical trials(48).

During the course of the study patients will fill out the FACT-Melanoma QOL assessment instrument to monitor patient wellbeing in multiple domains (Appendix 4).

9.3. Imaging Assessments

Tumor response will be defined by the Response Evaluation Criteria in Solid Tumors (RECIST 1.1) according to the schedule outlined in Section 9.1.

Based on immune related RECIST (irRECIST 1.1), a modification of RECIST, the study requires confirmation of progressive disease (PD) with an additional scan 4 to 6 weeks later that also meets criteria for progression per RECIST 1.1. (see Appendix 6)

10. ADVERSE EVENT REPORTING

Toxicity and adverse events will be classified according to NCI's Common Terminology Criteria for Adverse Events V 5.0 (CTCAE). A copy of the CTCAE can be downloaded from the CTEP home page https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

10.1. Definitions

The following definitions are based on the Code of Federal Regulations Title 21 Part 312.32 (21CFR312.32(a)).

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. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Life-Threatening Adverse Event Or Life-Threatening Suspected Adverse Reaction: An adverse event or suspected adverse reaction is considered “life-threatening” if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Serious Adverse Event Or Serious Suspected Adverse Reaction: An adverse event or suspected adverse reaction is considered “serious” if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate 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

Unexpected adverse event or unexpected suspected adverse reaction: An adverse event or suspected adverse reaction is considered “unexpected” if it is not listed in the investigator brochure or is not listed at the specificity or severity that has

been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Attribution of the AE:

- Definite – The AE is clearly related to the study treatment.

- Probable – The AE is likely related to the study treatment.

- Possible – The AE may be related to the study treatment.

- Unlikely – The AE is doubtfully related to the study treatment.
- Unrelated – The AE is clearly NOT related to the study treatment.

The following definitions are from the Masonic Cancer Center's Standard Operating Procedure (SOP) Deviation Reporting:

Major Deviation: A deviation or violation that impacts the risks and benefits of the research; may impact subject safety, affect the integrity of research data and/or affect a subject's willingness to participate in the research. Deviations that place a subject at risk, but do not result in harm are considered to be major deviations.

Minor Deviation: A deviation or violation that does not impact subject safety, compromise the integrity of research data and/or affect a subject's willingness to participate in the research.

10.2. Adverse Event Monitoring and Documentation

The monitoring period for adverse events begins with the administration of the 1st dose of aldesleukin (IL-2) through the End of Trial visit.

Patients who are withdrawn due to an AE should have an AE assessment completed 28 days or more after their last trial treatment. All AEs will be captured on the AE CRF.

For the purposes of this study, adverse event documentation requirements will be determined based on grade, expectedness and relationship to the study treatment:

	Grade 1	Grade 2		Grade 3		Grade 4 and 5
	Expected or Unexpected	**Expected**	**Unexpected**	**Expected**	**Unexpected**	**Expected or Unexpected**
Unrelated Unlikely	Not required	Not required	Not required	Not required	Required	Required
Possible Probable Definite	Not required	Not required	Required	Required	Required	Required

The following events count toward an early stopping rule event per Section 12:

- Any Grade 2 or greater drug-related uveitis, eye pain, or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within 7 days of checkpoint inhibitor OR requires systemic treatment with corticosteroids
- Any Grade 3 or greater non-skin, drug-related adverse event lasting > 7 days, including uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic toxicity, hypersensitivity reactions, and infusion reactions with the following exceptions:
    - Grade 3 or greater drug-related laboratory abnormalities are not considered a stopping rule event if they constitute an expected side effect that is reversible and corrects with usual supportive measures.

In addition, the trial would be stopped and re-evaluated if there is ≥ 1 treatment-related mortality event.

10.3. Required Reporting: IRB and Masonic Cancer Center's SAE Coordinator

Agency	Criteria for reporting	Timeframe	Form to Use	Submission address/ form
U of MN IRB	Unexpected Death, Information that indicates a new or increased risk, or a safety issue.	Within 5 business days of event discovery	RNI	ethos
Masonic Cancer Center SAE Coordinator	Events that count an early study stopping rule.	At time of reporting	Stopping Rule Form	SAE Coordinator mcc-saes@umn.edu

The SAE Coordinator will provide the Masonic Cancer Center's Data and Safety Monitoring Council (DSMC) with the SAE in an appropriate format depending on the individual SAE (as reported or in a summary format).

11. STUDY DATA COLLECTION AND MONITORING

11.1. Data Management

This study will collect regulatory and clinical data using University of Minnesota CTSI's instance of OnCore® (Online Enterprise Research Management Environment). The Oncore database resides on dedicated secure and PHI compliant hardware. All relevant Academic Health Center – Information Systems (AHC-IS) procedures related for PHI compliant servers (as required by the Center of Excellence for HIPAA Data) apply to Oncore databases.

Additional immune monitoring data about correlative laboratory samples generated by the Masonic Cancer Center Translational Therapy Laboratory (TTL) from the protocol-

directed correlative research samples is stored in their Laboratory Information Management System (LIMS). The LIMS database application is also stored on a production server located in the UMN datacenter (WBOB) and is managed by the Academic Health Center.

Key study personnel are trained on the use of OnCore and will comply with protocol specific instructions embedded within the OnCore.

11.2. Case Report Forms

Participant data will be collected using protocol specific electronic case report forms (e-CRF) developed within OnCore based on its library of standardized forms. The e-CRF will be approved by the study's Principal Investigator and the Biostatistician prior to release for use. The Study Coordinator or designee will be responsible for registering the patient into OnCore at time of study entry, completing e-CRF based on the patient specific calendar, and updating the patient record until patient death or end of required study participation.

11.3. Data and Safety Monitoring Plan (DSMP)

The study's Data and Safety Monitoring Plan will be in compliance with the University of Minnesota Masonic Cancer Center's Data & Safety Monitoring Plan (DSMP), which can be accessed at http://z.umn.edu/dmsp.

For the purposes of data and safety monitoring, this study is classified as moderate risk.

Therefore the following requirements will be fulfilled:

- The Masonic Cancer Center Data and Safety Monitoring Council (DSMC) will review the study's progress at least twice yearly with the understanding the Cancer Protocol Review Committee (CPRC) may require more frequent reporting.
- The PI will comply with at least twice yearly monitoring of the project by the Masonic Cancer Center monitoring services.
- The PI will oversee the submission of all reportable adverse events per the definition of reportable in Section 10.3 to the Masonic Cancer Center's SAE Coordinator, the University of Minnesota IRB, and the FDA.

11.4. Monitoring

The investigator will permit study-related monitoring, audits, and inspections by the investigator and/or investigator designee, IRB, government regulatory bodies, and University of Minnesota compliance groups. The investigator will make available all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data, etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.) will be available for trial related monitoring, audits, or regulatory inspections.

11.5. Record Retention

The investigator will retain study records including source data, copies of case report forms, consent forms, HIPAA authorizations, and all study correspondence in a secured facility for at 6 years after the study file is closed with the IRB.

In addition, the Clinical Trials Office (CTO) will keep a master log of all patients participating in the study with sufficient information to allow retrieval of the medical records for that patient.

Please contact the CTO before destroying any study related records.

12. STATISTICAL CONSIDERATIONS

All analyses of demographics, safety, biological activity, and biomarkers will be descriptive. AEs, SAEs, deaths, and abnormal laboratory values will be summarized by the proportion of patients who experience them. Descriptive statistics of safety will be presented using National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. All on-study AEs, Grade 3-4 AEs, treatment-related AEs, Grade 3-4 treatment-related AEs, SAEs, treatment-related SAEs, and AEs leading to discontinuation will be tabulated using the worst grade per NCI CTCAE v 5.0 criteria by system organ class. On-study lab parameters including hematology, chemistry panel, liver and renal function tests, and thyroid function, and all Grade 3-4 Lab abnormalities will be summarized using worst grade NCI CTCAE v 5.0 criteria.

Each cohort in this phase 2 trial uses a Simon's two-stage design (39). The null hypothesis that the true response rate is 13% will be tested against a one-sided alternative. In the first stage, 16 patients will be accrued. If there are 3 or fewer responses in these 16 patients, the study will be stopped for futility. If there are 4 or more responses in 16 patients, the null hypothesis will be rejected and 6 additional patients will be accrued for a total of 22. The null hypothesis will be rejected if 6 or more responses are observed in 22 patients. This design yields a type I error rate of 0.05 and power of 0.8 when the true response rate is 35%.

The ORR will be presented as the proportion of patients who achieved complete response (CR) or partial response (PR). The disease control rate (DCR) will be presented as the proportion of patients with CR, PR, or stable disease. The best overall response (BOR) will be measured as the maximum change from baseline in the sum of the longest diameter for each of the target lesions over the full 2 year follow-up period. The percentage of responders still in response at different time points (6 and 12 months) will be presented based on the Kaplan-Meier (KM) method.

In general, categorical data measurements will be summarized as counts and percentages (or proportions) and continuous data will be summarized with descriptive statistics such as mean, standard deviation, median, minimum and maximum. Summary statistics will be provided for changes in number of tumor infiltrating lymphocytes and in PD-L1/Tim-3/Lag-3 and other expressed proteins in tumors from baseline to protocol-specified time points during treatment.

Sequential boundaries will be used to monitor excessive toxicity rate as defined in Section 10.3 by treatment cohort (arm). The accrual will be halted and the study re-evaluated if excessive toxicity is seen, that is, if the number of excessive toxicity is equal to or exceeds *8* out of *22* patients in phase 2 with full follow-up (i.e. stop if 2/2, 3/3, 3/4, 4/5, 4/6, 4/7, 4/8, 5/9, 5/10, 5/11, 5/12, 6/13, 6/14, 6/15, 6/16, 6/17, 7/18, 7/19, 7/20, 7/21, or 8/22). This is a Pocock-type (40) stopping boundary that yields the probability of crossing the boundary at most [0.05] when the rate of excessive toxicity is equal to the acceptable rate [0.13].

In addition, the trial would be stopped and re-evaluated if there is ≥ 1 treatment-related mortality event.

13. CONDUCT OF THE STUDY

13.1. Good Clinical Practice

The study will be conducted in accordance with the appropriate regulatory requirement(s). 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.

13.2. Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the patients. The study will only be conducted at sites where IRB approval has been obtained. The protocol, consent, written information given to the patients, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB by the investigator.

13.3. Informed Consent

All potential study participants will be given a copy of the IRB-approved consent to review. The investigator or designee will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, he/she will be asked to sign and date the consent document. Patients who refuse to participate or who withdraw from the study will be treated without prejudice.

APPENDIX 1 – PERFORMANCE STATUS CRITERIA

ECOG Performance Status

These scales and criteria are used by doctors and researchers to assess how a patient's disease is progressing, assess how the disease affects the daily living abilities of the patient, and determine appropriate treatment and prognosis. They are included here for health care professionals to access.

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.

APPENDIX 2 – ALDESLEUKIN (IL-2) TOXICITIES AND CORRECTIVE MEASURES

EXPECTED IL-2 TOXICITIES	CORRECTIVE MEASURES / MANAGEMENT
Arrhythmia	Stop IL-2 (most arrhythmias). Correct electrolytes, minerals, anemia, hypoxia. Use medications as indicated
Anemia	Transfuse PRBC's to achieve Hct>28% during IL-2 dosing
Acidosis	Total CO2 (bicarbonate)<20 mmol/l, give 50meq bicarb <18 mmol/l, give 100 meq bicarb IV
Chills	Warm blankets as first measure IV meperidine if persists
CK elevation	Measure isoenzymes, EKG. If have evidence of myocarditis, must stop IL-2. Will need ECHO before next cycle of IL-2 to rule out myocardial dysfunction. Future IL-2 may be considered if the ECHO is normal.
Dermatitis	Oatmeal baths, lotions (NO steroid or alcohol containing lotions)
Diarrhea	Antidiarrheals. Avoid overuse because of complicating ileus and distention.
Edema	Elevate symptomatic extremity. Use fluids judiciously. AVOID DIURETICS. Notify MD to evaluate if signs of pulmonary edema.
Epigastric pain	Evaluate cause, consider antacids.
Fever breakthrough	Scheduled acetaminophen. Naproxen prn. Consider septic work-up if happens after first 24 hours of therapy.
Hypoalbuminemia	Observe
Hypocalcemia	Maintain above lowest normal at institution (compensate for low albumin).
Hypokalemia	Maintain potassium above 3.6
Hypomagnesemia	Maintain above lowest normal at institution (compensate for low albumin).
Hypotension	Use crystalloids to maintain BPsys>80-90 (1000-1500 cc/day max boluses to avoid pulmonary edema). Auscultate lungs before and after each bolus to identify development of crackles or wheezing.

EXPECTED IL-2 TOXICITIES	CORRECTIVE MEASURES / MANAGEMENT
Infection	Stop IL-2 and treat infection as indicated.
Mucositis/stomatitis	Frequent oral care, mouthwashes, topical anesthetics, nystatin
Oliguria	Fluid boluses to maintain UO 30cc/h based on last 8 hours (240 cc/8h). Limit to 1000-1500 cc/day max boluses to avoid pulmonary edema. Auscultate lungs before and after each bolus to identify development of crackles or wheezing. Stop IL-2 if unable to meet UO criteria for 24 hours since last dose. Consider foley catheter. AVOID DIURETICS!
Nausea/vomiting	Antiemetics (alternate medications and routes if any one not effective)
Nasal congestion	Room humidifier, decongestant (NO topical steroids).
Pruritus	Oatmeal baths, lotions, antipruritics. NO steroids (topical or systemic).
Shortness of breath	Check transcutaneous O2 sat. If <95%, use O2. Use fluids judiciously. Do not use inhalational steroids. Stop IL-2 for airway tightness/reactive airway.
Tachycardia	Correct fever, hypotension, hypoxia, anemia.
Thrombocytopenia	Consider platelet transfusion at appropriate parameters.

APPENDIX 3 – MANAGEMENT ALGORITHMS FOR IPILIMUMAB AND OTHER IMMUNO-ONCOLOGY DRUGS

These general guidelines constitute guidance to the Investigator and may be supplemented by discussions with the principal investigator and Inpatient Attending. The guidance applies to all immuno-oncology (I-O) agents and regimens.

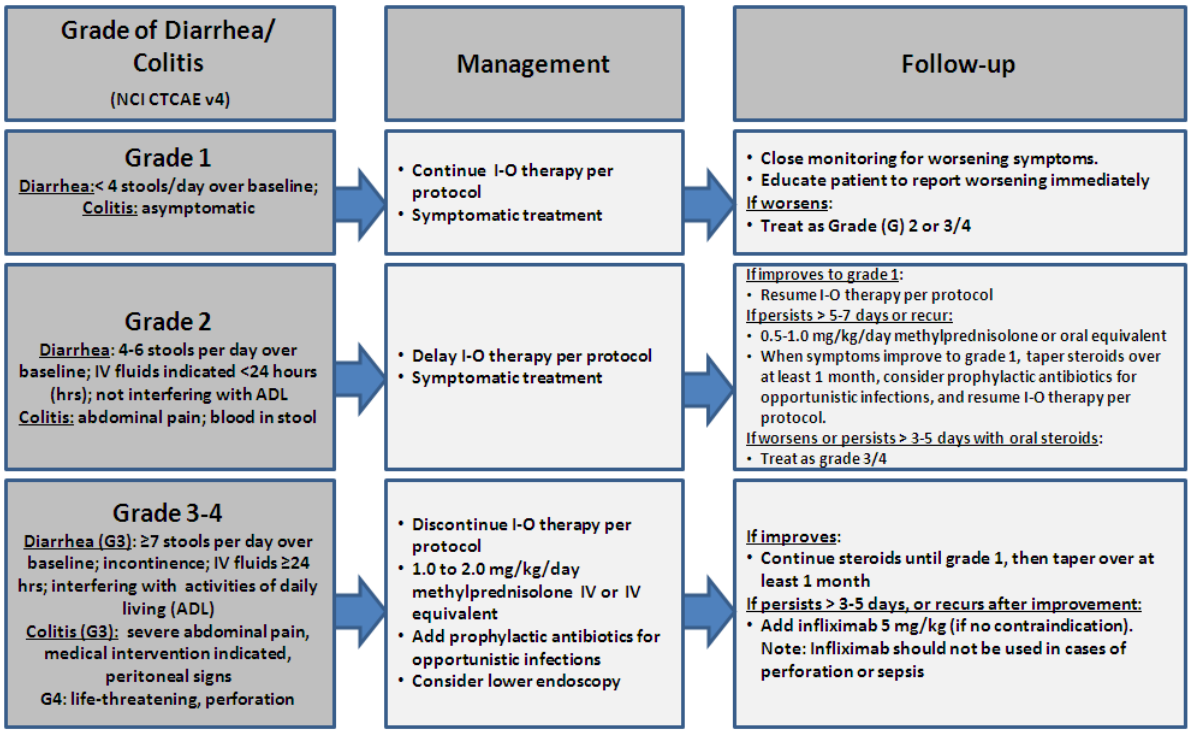
A general principle is that differential diagnoses should be diligently evaluated according to standard medical practice. Non-inflammatory etiologies should be considered and appropriately treated.

Corticosteroids are a primary therapy for immuno-oncology drug-related adverse events. The oral equivalent of the recommended IV doses may be considered for ambulatory patients with low-grade toxicity. The lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Consultation with a medical or surgical specialist, especially prior to an invasive diagnostic or therapeutic procedure, is recommended. The frequency and severity of the related adverse events covered by these algorithms will depend on the immuno-oncology agent or regimen being used.

GI Adverse Event Management Algorithm

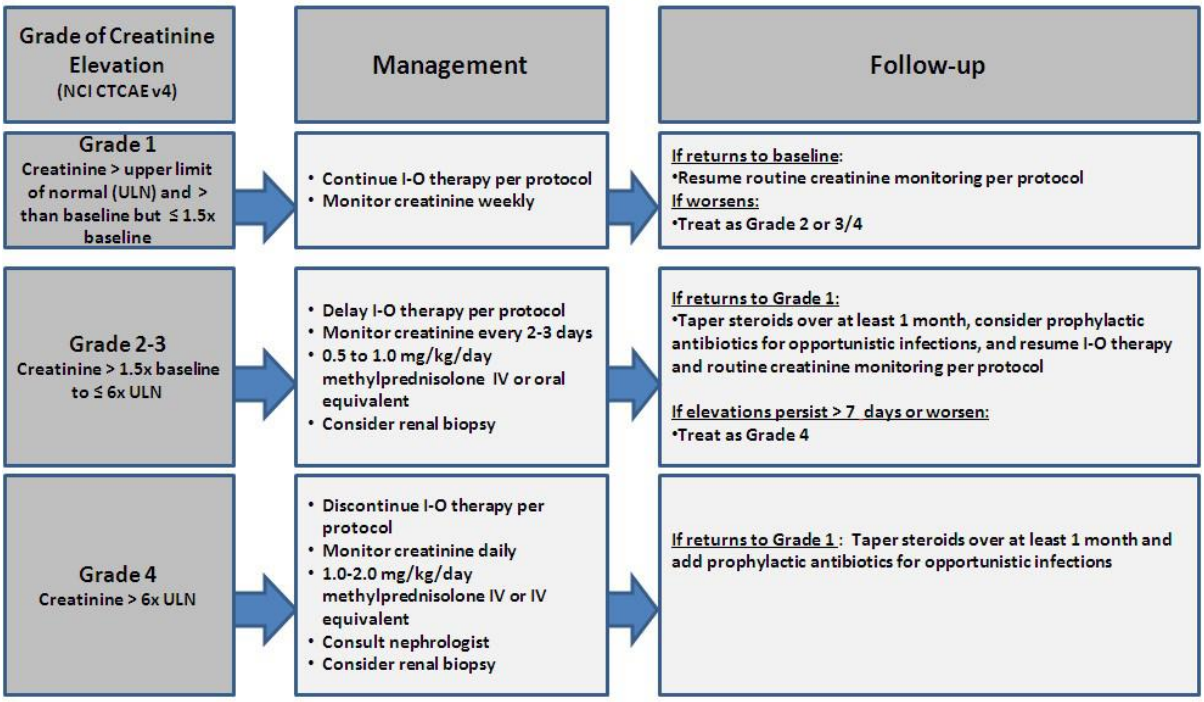
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Renal Adverse Event Management Algorithm

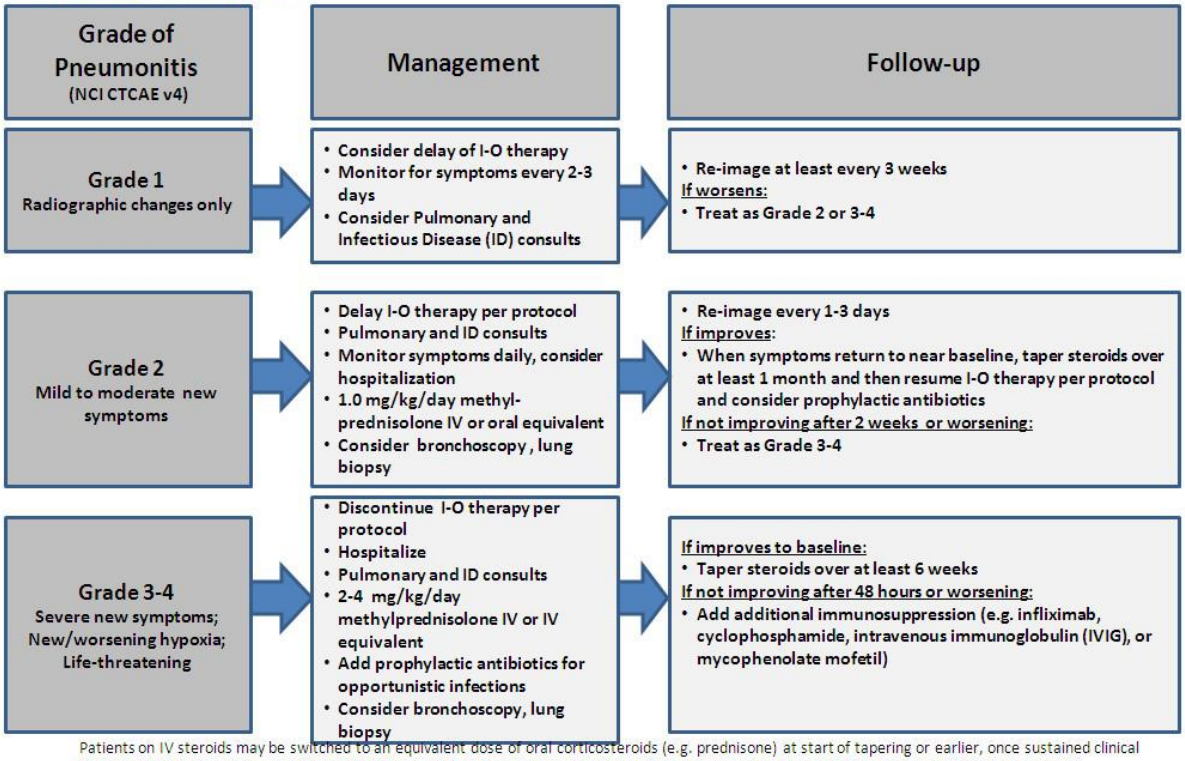
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Pulmonary Adverse Event Management Algorithm

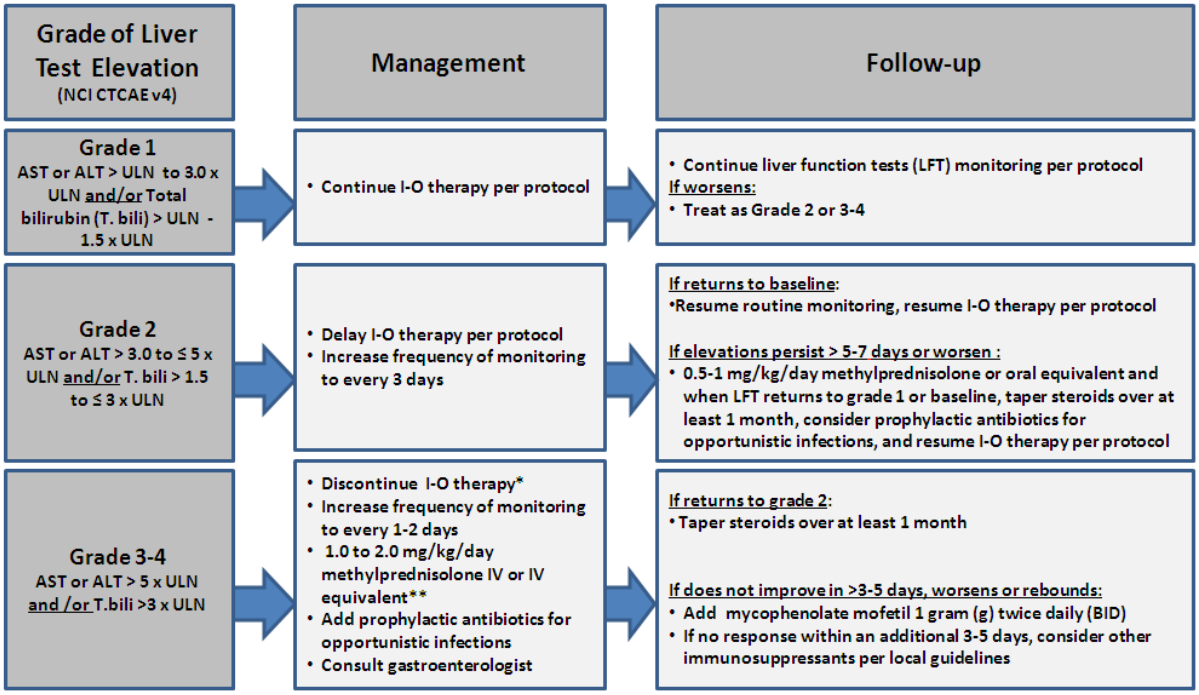
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Hepatic Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider imaging for obstruction.



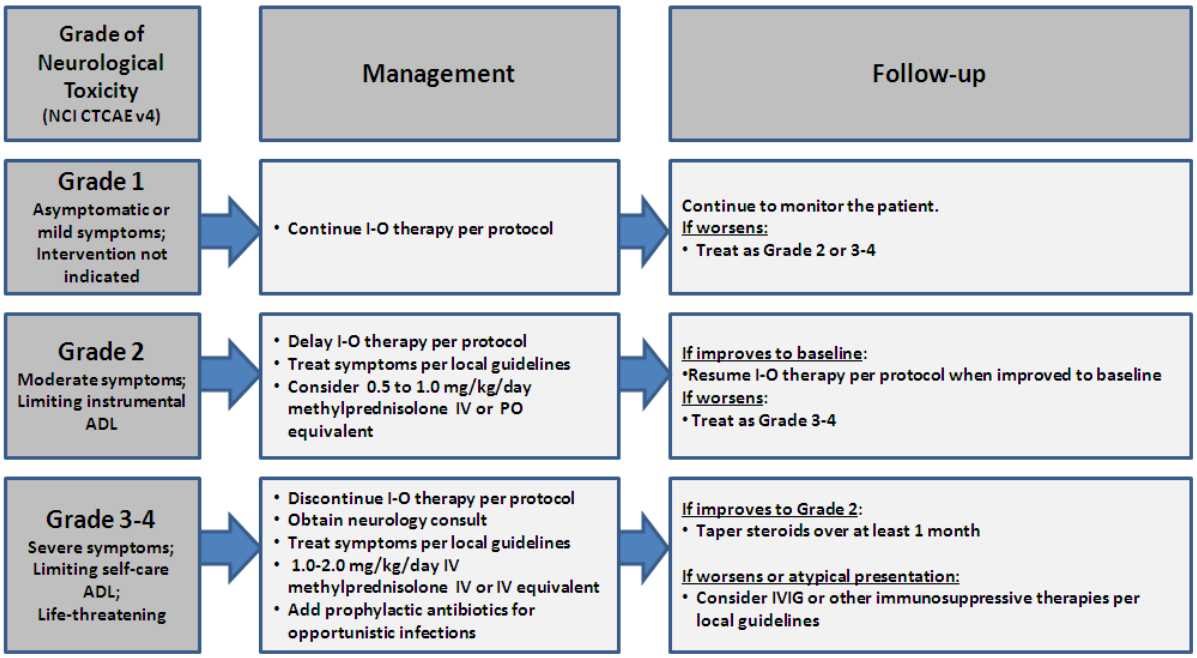
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

***I-O therapy may be delayed rather than discontinued if AST/ALT ≤ 8 x ULN and T.bili ≤ 5 x ULN.**

**The recommended starting dose for grade 4 hepatitis is 2 mg/kg/day methylprednisolone IV.

Neurological Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

APPENDIX 4 – FACT-M (VERSION 4)

http://www.facit.org/LiteratureRetrieve.aspx?ID=42277

FACT-M (Version 4)

Below is a list of statements that other people with your illness have said are important. **Please circle or mark one number per line to indicate your response as it applies to the past 7 days.**

	PHYSICAL WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4

	SOCIAL/FAMILY WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4
Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please mark this box* ☐ *and go to the next section.*					
GS7	I am satisfied with my sex life	0	1	2	3	4

FACT-M (Version 4)

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	EMOTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GE1	I feel sad	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4

	FUNCTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GF1	I am able to work (include work at home)	0	1	2	3	4
GF2	My work (include work at home) is fulfilling	0	1	2	3	4
GF3	I am able to enjoy life	0	1	2	3	4
GF4	I have accepted my illness	0	1	2	3	4
GF5	I am sleeping well	0	1	2	3	4
GF6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF7	I am content with the quality of my life right now	0	1	2	3	4

FACT-M (Version 4)

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	ADDITIONAL CONCERNS	Not at all	A little bit	Some-what	Quite a bit	Very much
M1	I have pain at my melanoma site or surgical site	0	1	2	3	4
M2	I have noticed new changes in my skin (lumps, bumps, color(colour))	0	1	2	3	4
M3	I worry about the appearance of surgical scars	0	1	2	3	4
B1	I have been short of breath	0	1	2	3	4
ITU4	I have to limit my physical activity because of my condition	0	1	2	3	4
An10	I get headaches	0	1	2	3	4
Hep3	I have had fevers (episodes of high body temperature)	0	1	2	3	4
C1	I have swelling or cramps in my stomach area	0	1	2	3	4
C6	I have a good appetite	0	1	2	3	4
M5	I have aches and pains in my bones	0	1	2	3	4
M6	I have noticed blood in my stool	0	1	2	3	4
ITU3	I have to limit my social activity because of my condition	0	1	2	3	4
MS8	I feel overwhelmed by my condition	0	1	2	3	4
M8	I isolate myself from others because of my condition	0	1	2	3	4
M9	I have difficulty thinking clearly (remembering, concentrating)	0	1	2	3	4
HI7	I feel fatigued	0	1	2	3	4

FACT-M (Version 4)

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	At the site of my melanoma surgery:	Not at all	A little bit	Some-what	Quite a bit	Very much
M10	I have swelling at my melanoma site	0	1	2	3	4
M11	I have swelling as a result of surgery	0	1	2	3	4
M12	I am bothered by the amount of swelling	0	1	2	3	4
M13	Movement of my swollen area is painful	0	1	2	3	4
M14	Swelling keeps me from doing the things I want to do	0	1	2	3	4
M15	Swelling keeps me from wearing clothes or shoes I want to wear	0	1	2	3	4
M16	I feel numbness at my surgical site	0	1	2	3	4
M17	I have good range of movement in my arm or leg	0	1	2	3	4

APPENDIX 5 – CORRELATIVE OBJECTIVES AND TESTING

CORRELATIVE OBJECTIVE	LABORATORY	ASSAYS	TISSUE/BODY FLUID TESTED AND TIMING
mRNA expression profiling (immune signature genes)	UMN Genomics Core	Q-RT-PCR NanoString Illumina Arrays	Tumor: Prior to treatment (baseline), prior to Ipilimumab, at EOT, and at time of confirmed progression (if feasible) PBMC: Prior to treatment start, HD-XRT mid-cycle on day 7 (+/-3 days), cycle1 day 1 and day 15 (before 1st dose and 8 hours after last dose), prior to Ipilimumab (same day), cycle 2 day 1 and day 15 (before 1st dose and 8 hours after last dose), and EOT
Tumor Gene expression profiling	UMN Genomics Core	NGS Illumina HiSeq	Tumor: Prior to treatment (baseline), prior to Ipilimumab, at EOT, and at time of confirmed progression (if feasible)
Tumor PD-1 and PD-L1 Expression	BioNet/LaMP	IHC	Tumor: Prior to treatment (baseline), prior to Ipilimumab, at EOT, and at time of confirmed progression (if feasible)
Immune cell infiltrate markers: CD3, CD8, MHC Class I. CD4, CD56, CD16, CD45, CD19, CD68, GITR, TIM-3, FOXP3 and LAG3	BioNet/LaMP	IHC	Tumor: Prior to treatment (baseline), prior to Ipilimumab, at EOT, and at time of confirmed progression (if feasible)
Melanoma specific T cell Immunophenotyping	Flow Cytometry, Mass Cytometry Core	Melanoma peptide specific tetramer isolation, CyTOF	PBMC: Prior to treatment start, HD-XRT mid-cycle on day 7 (+/-3 days), cycle1 day 1 and day 15 (before 1st dose and 8 hours after last dose), prior to Ipilimumab (same day), cycle 2 day 1 and day 15 (before 1st dose and 8 hours after last dose), and EOT
Anti-melanoma T cell gene expression analysis	Flow Cytometry and UMN Genomics Core	mRNA expression (e.g. Nanostring)	PBMC: Prior to treatment start, HD-XRT mid-cycle on day 7 (+/-3 days), cycle1 day 1 and day 15 (before 1st dose and 8 hours after last dose), prior to Ipilimumab (same day), cycle 2 day 1 and day 15 (before 1st dose and 8 hours after last dose), and EOT
Melanoma specific T cell Functional studies	Translational Immunology Laboratory	Pooled CMV and melanoma peptide Antigen profliferation assays IFN-γ ELISpot	PBMC: Prior to treatment start, HD-XRT mid-cycle on day 7 (+/-3 days), cycle1 day 1 and day 15 (before 1st dose and 8 hours after last dose), prior to Ipilimumab (same day), cycle 2 day 1 and day 15 (before 1st dose and 8 hours after last dose), and EOT
PBMC and T cell subset quantification (frequency and	Flow Cytometry, Translational Immunology Laboratory	Multiparameter flow cytometric analysis, CyTOF (Fluidigm)	PBMC: Prior to treatment start, HD-XRT mid-cycle on day 7 (+/-3 days), cycle1 day 1 and day 15 (before 1st

percent), Tregs, NK cells and MDSC.			dose and 8 hours after last dose), prior to Ipilimumab (same day), cycle 2 day 1 and day 15 (before 1st dose and 8 hours after last dose), and EOT
Systemic cytokine levels	Cytokine Reference Laboratory	Luminex	Serum: Prior to treatment start, HD-XRT mid-cycle on day 7 (+/-3 days), cycle1 day 1 and day 15 (before 1st dose and 8 hours after last dose), prior to Ipilimumab (same day), cycle 2 day 1 and day 15 (before 1st dose and 8 hours after last dose), and EOT
Cytomegalovirus (CMV) DNA quantitation and reactivation testing	Nelson Laboratory, Masonic Cancer Center	PCR based assay	Serum: Prior to treatment start, HD-XRT mid-cycle on day 7 (+/-3 days), cycle1 day 1 and day 15 (before 1st dose and 8 hours after last dose), prior to Ipilimumab (same day), cycle 2 day 1 and day 15 (before 1st dose and 8 hours after last dose), and EOT
ctDNA and Neoantigen characterization	UMN Genomics Core	NGS	Serum: Prior to treatment start, HD-XRT mid-cycle on day 7 (+/-3 days), cycle1 day 1 and day 15 (before 1st dose and 8 hours after last dose), prior to Ipilimumab (same day), cycle 2 day 1 and day 15 (before 1st dose and 8 hours after last dose), and EOT

APPENDIX 6 – RECIST 1.1 RESPONSE DEFINITIONS

Baseline selection of lesions

All lesions are measured by long and short axis (perpendicular to long axis). At baseline, all tumor lesions are identified as either target lesions or non-target lesions and will be evaluated at baseline and every post baseline imaging timepoint.

Target lesions: This trial distinguishes between (A) XRT target lesions and (B) non-irradiated target lesions. The minimum size of target lesions is 1.5 cm by long axis. Lymph nodes must measure at least 1.5 cm in short axis. The maximum number of lesions targeted for XRT will be 3, with a maximum of 2 lesions allowed per targeted organ. For response assessment, the maximum number of non-irradiated target lesions followed will be 5.

Non-target lesions: These are radiographically detectable lesions in excess of the number of target lesions already being followed (>8 total or >2 per organ) or that do not meet size criteria for target lesion status. In the event of innumerable metastases, non-target lesions are limited to an additional 8 lesions. Lymph nodes must measure at least 1.0 cm in short axis to qualify as non-target lesions (<1cm considered non-pathologic).

Baseline measurements

The sum of the long diameters (SOD) of A) XRT targeted lesions and B) non-irradiated target lesions is calculated and referred to as SOD-A and SOD-B for the baseline timepoint. All other lesions are considered non-target lesions and also recorded at baseline.

Radiographic response assessments

At each imaging timepoint, the SOD of target lesions is measured and recorded. Response is determined by calculating the SOD-A and SOD-B at each imaging timepoint. Any new lesions (C) are measured and recorded, and the SOD-C calculated for that imaging timepoint. A previously involved lymph node must also measure > 1.0 cm by short axis diameter to continue to qualify as pathologic.

Target lesion response

Complete response (CR): disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 1 cm.

Partial response (PR): Decrease of at least 30% in the sum of diameters (SOD) of target lesions, taking as reference the baseline SOD.

Progressive disease (PD): Increase of at least 20% in the sum of diameters (SOD) of target lesions, taking as reference the smallest prior SOD in the trial (including the baseline sum if it is the smallest).

Stable disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest SOD achieved in the trial.

Non-target lesion response

Complete response (CR): disappearance of all non-target lesions. All lymph nodes must be non-pathological in size (<10 mm short axis).

Non-CR/Non-PD: Persistence of 1 or more non–target lesion(s).

Progressive disease (PD): Unequivocal progression of existing non-target lesions. (Note: appearance of 1 or more new lesions is considered progression).

New lesion assessment

At each imaging timepoint, presence of new lesions is determined, and each new lesion is recorded as a new lesion (i.e. C1, C2, etc.) and they are not considered target or non-target lesions.

Lymph nodes must be a new lesion and measure at least 10 cm by short diameter to qualify as a new lesion overall response assessment.

A lesion identified on a follow-up study in an anatomical location that was not scanned at baseline is considered a new lesion.

Objective Response assessment

An overall response is based on both target and non-target lesion responses, as well as the appearance of any new lesions as listed in the Objective Response table below:

Target Lesions	Non-target Lesions	New Lesions	Objective Response
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

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