SUMMARY OF CHANGES

Protocol Amendment #4

LCCC 1620: Phase 2 Study of Denosumab in Combination with a PD-1 Inhibitor in Subjects with Stage III/IV Melanoma

AMENDMENT INCORPORATES:
__ Editorial, administrative changes
_X_ Scientific changes
__ Therapy changes
__ Eligibility Changes

Rationale for amendment:

The purpose for this amendment is to include the optional collection of tumor tissue from procedures that occurred prior to study enrollment as well as tumor tissue that may be collected after study completion. Often in clinical trials with rigorous translational endpoints where baseline tumor tissue is collected, the material is not sufficient or necrotic, impeding correlative studies. In these cases, any available archived material that is collected prior to study enrollment may be used in lieu of baseline tissue to assist in such studies. In addition, several patients as part of this study went on to undergo therapeutic lymph node dissections following study completion (or progression). Analyzing the tumor following end of study (or progression) is a great opportunity to assess change from baseline (effect of both denosumab plus nivolumab) and from day 21 (effect of denosumab alone).

The reasons for requesting archived tumor blocks from procedures prior to study enrollment and following end of study (or progression) is to:

A. Ensure that pretreatment tumor blocks are available in the event that the selected tumor block does not have sufficient and/ or and high quality tumor tissue available to perform tissue imaging and gene expression profiling studies.
B. Analyze changes of the tumor tissue biomarker(s) under study treatment in earlier stages of melanoma (e.g. primary melanoma).
C. Analyze changes of the tumor tissue biomarker(s) under study treatment from baseline (effect of both denosumab plus nivolumab) and from day 21 (effect of denosumab alone).

The reasons for requesting archived tumor blocks that are from procedures that are conducted after completion of the study (or exit from the study due to tumor progression) are:

A. For the archived tumor tissues that were generated as part of a surgery for tumor progression from the study (e.g. lymphadenectomy), we will assess the effect of treatment following combination with denosumab plus nivolumab.

B. For the archived tumor tissues that were generated following systemic therapies after progression from denosumab plus nivolumab, we will investigate how a tissue biomarker of interest (e.g. RANLK) changes over time due to the clonal evaluation of tumors.

Scientific changes:

Section 1.10.2	Tumor tissue description updated to add optional collection of tumor tissue prior to study enrollment as well as tumor tissue that is collected after study completion.
Section 2.3.1 and Section 3.3.1	[redacted]
Section 2.3.2 and Section 3.3.2	[redacted]
Section 5.1	Treatment Plan updated to include the collection of leftover or excess research tissue from standard of care surgical procedures that occur prior to enrollment, during study participation and after study completion.
Section 5.8	Language in Duration in Follow Up updated to include that subjects who have not progressed on PD-1 inhibitor plus denosumab after a year of treatment will be follow up very 3 months for up to 2 years (i.e., a total of 3 years since study initiation) with phone calls or visits and then annually until death via medical abstraction or phone calls. Language in Duration of Follow Up updated to include that subjects who have progressed on PD-1 inhibitor plus denosumab within the year of the study duration will be followed every 3 months for up to 3 years since study initiation with phone calls or standard of care visits and then annually until death via medical abstraction or phone calls. Survival follow up will continue annually until death via medical abstraction or phone calls.
Section 7.1	Time and Events table and footnote 17 updated to include the collection of optional tumor tissue samples from standard of care surgical procedures that occur prior to enrollment, during study participation and after study completion. Footnote 21 updated that follow up can occur via medical abstraction or phone calls and survival follow up will continue annually until death via medical abstraction or phone calls.

Section 7.3.2.1	Addition of research tumor tissue collection for correlative study procedures.

THE ATTACHED VERSION DATED July 7, 2022 INCORPORATES THE ABOVE REVISIONS

PROTOCOL AMENDMENT 3

LCCC 1620: Phase 2 Study of Denosumab in Combination with a PD-1 Inhibitor in Subjects with Stage III/IV Melanoma

X Editorial, administrative changes
X Scientific changes (IRB approval)
Therapy changes (IRB approval)
X Eligibility Changes (IRB approval)

Rationale for amendment:
This protocol amendment includes changes for consistency, clarifications, and eligibility to enhance patient enrollment. Clarifications were made to visit windows for weeks 17-49, and the use of glucocorticoids during the study was further clarified. Additional administrative updates include the removal of Case Western as a participating site, alignment of the timing of the first dose of nivolumab, and an editorial correction on enrollment numbers in the schema.

Editorial/Administrative
1. Case Western is removed as a participating site
2. Section 1.2: Updated disease related information
3. Section 1.8: Updated information related to denosumab and anti-PD1 agents.
4. Section 4.1: The schema was updated to reflect the potential total enrollment number of n = 28.
5. Section 4.4.1 has been updated to clarify exception for glucocorticoid use during the study.
6. In Section 6.1, the column in the Time and Events table labeled "Wks17-49 (Wks22/28/34/40/46±2wks)" provides a visit window that is inconsistent with Footnote #1 (± 7 days). The correct window is ± 7 days, as stated in Footnote 1, for those study visits. Therefore, the window is changed in Wks17-49 column in the Time and Events table in Section 6.1 from ±2wks to ±7 days.
7. Section 6.1, footnote 1, the footnote contains a 7 week visit that is applicable to the pembrolizumab Time and Events table (Appendix B) but not applicable to nivolumab administration. The third sentence in Footnote 1 has been updated to reflect nivolumab administration.
8. Section 6.1 Time and Events table; Footnote 12 is clarified to reflect the correct time to give the first dose of nivolumab; C2D2.

Scientific Changes
1. Section 6.1 Time and Events Table Footnote 17 and Section 6.3.2.1. In keeping with the change to the Criterion 3.1.16, information related to qualitative aspects of tissues have been removed.

Eligibility changes:

1. Criterion 3.1.1.6: Removed qualitative measures surrounding archival tissue
2. Criterion 3.2.1.2: The criterion is updated to clarify any dose of corticosteroids is unacceptable and subjects with brain metastases should be asymptomatic to participate in the study. Additionally, conditions were added as exceptions related to previously treated brain metastases that would allow for study entry.
3. Criterion 3.2.1.11: Updated to exclude subjects with treatment of active immune disease in the past year rather than 2 years.

THE ATTACHED VERSION DATED October 8, 2021INCORPORATES THE ABOVE REVISIONS

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PROTOCOL AMENDMENT 2

LCCC 1620: Phase 2 Study of Denosumab in Combination with a PD-1 Inhibitor in Subjects with Stage III/IV Melanoma

AMENDMENT INCORPORATES:

X Editorial, administrative changes
Scientific changes (IRB approval)
X Therapy changes (IRB approval)
Eligibility Changes (IRB approval)

Rationale for amendment:
This protocol amendment includes changes for consistency, and clarifications regarding timing of certain testing.

Editorial/Administrative

1. The timing of CBC with differential is listed correctly in Footnote 5 of the Time and Events Table in Section 6.1, but a discrepancy in the Table itself has been corrected by eliminating testing timepoints shown at C3D1 and C5D1.
2. In Protocol Amendment 1, the time window of screening laboratory assessments was increased from 14 to 21 days (see "Summary of Changes"). The timing of screening labs is listed correctly in Footnote 4 of the Time and Events Table in Section 6.1. This change has been made consistent throughout the protocol. The timing has now been corrected to 21 days in the Inclusion Criteria (Section 3.1.1.9), as well as in Footnote 1 of the Time and Events Table in Section 6.1.
3. In Section 3.2.1.5, an exclusion has been amended for consistency with exceptions in the inclusion criteria in Section 3.1.1.16.
4. In the Time and Events Table in Section 6.1, the Archival Tissue/Tumor biopsies row included a reference to Footnote 17 at C2D1. The reference has been corrected to Footnote 18, which correctly clarifies that these samples are mandatory (as per Section 1.10.2).
5. In the Time and Events Table in Section 6.1, the Archival Tissue/Tumor biopsies row included a reference to Footnote 18 at C5D1. The reference has been corrected to Footnote 19, which correctly clarifies that these samples are optional (as per Section 1.10.2).
6. Protocol Amendment 1 simplified treatment by replacing pembrolizumab with nivolumab (see "Rationale"). This change should be reflected throughout the protocol (for subjects enrolled after Amendment 1). While the Time and Events Table in Section 6.1 correctly refers to nivolumab, Footnote 20 of the Time and Events Table referred to pembrolizumab. The references in the footnote and the relevant timepoints in the footnote have been updated.

7. Language in Section 9.6 regarding the process for amendments to the protocol has been updated to reflect the current process.
8. Minor clarifying edits and format changes have been made in the document.

Therapy changes:

1. References to the time period for use of contraception have been corrected to 150 days in multiple sections including 3.1.1 and 4.5.2, in keeping with the longer time period indicated in the package inserts for the two relevant medications.
2. In Section 4.2, the C12D1 Week 44 timepoint for PBMC blood draw is not needed and has been deleted.
3. References to weight based dosage are not relevant and have been deleted from Sections 4.2, 6.1, and 11.2.
4. Reference to an anti-PD-1 agent has been clarified in Section 4.2.
5. The Cycle 2 column in the Time and Events Table in Section 6.1 has been divided into two columns (D1 and D2) to clarify the order of events. Verbiage has also been added to the footnotes for clarification. *Note: This is the change from v1 to v1.1 of this Amendment 2.*
6. A heading in the Time and Events Table in Section 6.1 has been corrected from "C5 D8 – C13D8" to "C6 D1 – C13D1."
7. Regarding the Serum Chemistries row of the Time and Events Table (Section 6.1), footnote 6 has been expanded to indicate that magnesium, calcium and phosphorus testing are required at C1D8.
8. A footnote in the Endocrine row of the Time and Events Table in Section 6.1 has been eliminated to show that this testing is needed at each timepoint.
9. The Urinalysis row in the Time and Events Table in Section 6.1 has been modified to show this testing at every other timepoint.
10. A reference to footnote 19 in the denosumab row of the Time and Events Table in Section 6.1 has been eliminated to indicate that certain timepoints are mandatory.
11. A reference to footnote 19 in the PBMC row of the Time and Events Table in Section 6.1 has been deleted to indicate that certain timepoints are mandatory.

THE ATTACHED VERSION DATED July 26, 2019 INCORPORATES THE ABOVE REVISIONS

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PROTOCOL AMENDMENT 1

LCCC 1620: Phase 2 Study of Denosumab in Combination with a PD-1 Inhibitor in Subjects with Stage III/IV Melanoma

AMENDMENT INCORPORATES:
X Editorial, administrative changes
X Scientific changes (IRB approval)
X Therapy changes (IRB approval)
X Eligibility Changes (IRB approval)

Rationale for amendment: A) Simplify treatment schedule by replacing anti-PD-1 agent pembrolizumab, which is administered every 3 weeks, with nivolumab which was recently approved by the FDA to be administered every 4 weeks, given that denosumab is administered every 4 weeks. Subjects who were enrolled into the study and started their treatment prior to approval of Amendment 1 will continue receiving pembrolizumab administered every 3 weeks. B) Move the Day 29 denosumab injection seven days earlier to allow for three loading doses of denosumab prior to initiating of anti-PD-1 therapy. C) Broaden eligibility criteria to allow for: (i) subjects with earlier than stage IV disease (e.g. bulky stage IIIB or IIIC disease; neoadjuvant administration), (ii) subjects who have progressed to stage IV melanoma following 1 year of adjuvant PD-1 inhibitors, (iii) subjects who have previously responded to PD-1 inhibitors for their stage IV disease by RECIST criteria and have discontinued PD-1 inhibitor due to clinical/subject preference or due to grade 1 or 2 toxicities, but now have progressed D) Update several portions of the protocol to accommodate transition to a multicenter study.

Editorial/Administrative
1. Updated Section 6.1, Section 6.2, Section 6.3 and Section 6.4 by adding bicarbonate (CO_2) to the list of laboratory parameters assessed as part of comprehensive metabolic panel.
2. Updated Section 7.3.3, Section 9.3, Section 9.4, Section 9.5.1, Section 9.5.2 and Section 9.6 to accommodate the transition to multicenter study.
3. Updated Section 5.2 to revise pembrolizumab drug information.
4. Added Section 5.3 with nivolumab drug information.
5. Updated Section 4.2.1, Drug Dosing Schema, to accommodate use of nivolumab as an alternative anti-PD-1 agent following approval of Amendment 1.
6. Added Section 4.3.2, Nivolumab Dose Modifications/Delays.

Scientific changes:

1. Updated Section 6.1 to indicate that mandatory biopsy should performed 24 – 48 hours after third denosumab injection.
2. Updated Section 1.8 to accommodate use of nivolumab as an anti-PD-1 agent following approval of Amendment 1.

Therapy changes:

1. Updated Section 1.1, Section 1.8, Section 1.9.2, Section 2.1.1, Section 4.1, Section 4.2 and Section 5.1.6 to reflect the addition of the third loading dose of denosumab on Day 22, prior to initiation of anti-PD-1 therapy.
2. Updated Section 4.3.4, Pembrolizumab Dose Guidelines.
3. Updated Section 6.6.2.1 to indicate that intrathoracic lesions are allowed to be biopsied, if deemed safe by the Principal Investigator.

Eligibility changes:

1. Revised inclusion criterion 3.1.1.4 to broaden eligibility for the study and indicate that subjects with histologically confirmed melanoma of mucosal primary may be enrolled into the study.
2. Revised inclusion criterion 3.1.1.5 to broaden eligibility for the study and allow for subjects: (i) with earlier than stage IV disease (e.g. bulky stage IIIB or IIIC disease; neoadjuvant administration), (ii) who have progressed to stage IV melanoma following 1 year of adjuvant PD-1 inhibitors, (iii) who have previously responded to PD-1 inhibitors for their stage IV disease by RECIST criteria and have discontinued PD-1 inhibitor due to clinical/subject preference or due to grade 1 or 2 toxicities, but now have progressed) at the discretion of the Principal Investigator.
3. Revised inclusion criterion 3.1.1.9 to increase time window of screening laboratory assessments to 21 days.
4. Revised inclusion criterion 3.1.1.9 to clarify that all but subjects with Gilbert's syndrome must have a serum total bilirubin of $\leq 1.5 \times$ ULN; subjects with Gilbert's syndrome must have a serum total bilirubin of $\leq 2 \times$ ULN to be eligible to participate in the clinical trial.
5. Revised inclusion criterion 3.1.1.14 to broaden eligibility for the study and indicate that subjects who have grade ≤ 2 toxicities after prior radiation therapy that is stable for ≥ 3 months) may be enrolled into the study.

THE ATTACHED VERSION DATED DECEMBER 17, 2018 INCORPORATES THE ABOVE REVISIONS

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LCCC 1620: Phase 2 Study of Denosumab in Combination with a PD-1 Inhibitor in Subjects with Stage III/IV Melanoma

Short Title: Phase 2 Study of Denosumab plus a PD-1 Inhibitor in Subjects with Stage III/IV Melanoma

Principal Investigator
Stergios J. Moschos, MD
Department of Medicine, Division of Hematology/Oncology
University of North Carolina at Chapel Hill
3116 Physicians Office Building
170 Manning Drive, CB# 7305
Chapel Hill, NC 27599-7305
(919) 843-7713 (phone) | (919) 966-6735 (fax)
Email: moschos@med.unc.edu

Biostatistician
Anastasia Ivanova, PhD
3103C McGavran-Greenberg Hall 7420
Email: aivanova@bios.unc.edu
Phone: (919) 843-8086

Clinical Protocol Office
Lineberger Comprehensive Cancer Center
The University of North Carolina at Chapel Hill
450 West Drive, 3rd Floor, CB# 7295
Chapel Hill, NC 27599-7295

Multicenter Project Manager:
CPOMultiCenter@med.unc.edu

Sponsor: Lineberger Comprehensive Cancer Center
Funding Source: Amgen Inc.
Protocol Version Date: July 7, 2022
Protocol Amendment: 4
Version: 1.1

LCCC 1620: Phase 2 Study of Denosumab in Combination with a PD-1 Inhibitor in Subjects with Stage III/IV Melanoma

Short Title: Phase 2 Study of Denosumab and a PD-1 Inhibitor in Subjects with Stage III/IV Melanoma

Principal Investigator
Stergios Moschos, MD
Department of Medicine, Division of Hematology/Oncology
University of North Carolina at Chapel Hill
3116 Physicians Office Building
170 Manning Drive, CB# 7305
Chapel Hill, NC 27599-7305
(919) 843-7713 (phone) | (919) 966-6735 (fax)
Email: moschos@med.unc.edu

Signature Page

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

Principal Investigator (PI) Name:_____

PI Signature: _____

Date:_____

Protocol Version Date: July 7, 2022
Protocol Amendment: 4
Version: 1.1

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	Adverse event
AIRE	Autoimmune regulator
AJCC	American Joint Committee in Cancer
ALC	Absolute lymphocyte count
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
AST	Aspartate aminotransferase
β-HCG	Beta–human chorionic gonadotropin
BRCA1	Breast cancer gene 1
CBC	Complete blood count
CL	Chloride
CLIA	Clinical Laboratory Improvement Amendments
CO2	Bicarbonate
CPO	Clinical protocol office
CR	Complete response
CRA	Clinical Research Associate
CrCl	Creatinine clearance
CRF	Case report form
CT	Computer tomography
CTLA	Cytotoxic T lymphocyte-associated
CYP	Cytochrome P450
D1	Day 1
DC	Dendritic cell
dL	Deciliter
DSMC	Data safety monitoring committee
ECI	Event of clinical interest
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
ELISA	Enzyme-linked immunosorbent assay
FACS	Fluorescence-activated cell sorting
FDA	Food and Drug Administration
FFPE	Formalin-fixed paraffin-embedded
GCP	Good clinical practice
GFR	Glomerular filtration rate
H&E	Hematoxylin & Eosin
Hgb	Hemoglobin
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
Hr	Hour
IB	Investigator's Brochure
ICP	Immune checkpoint proteins
ID	Identification
IDS	Investigational drug service
IF	Immunofluorescence
IHC	Immunohistochemistry
IMGF	Immune Genomics Facility
INR	International normalized ratio
IRB	Institutional Review Board
IU	International unit
IV	Intravenous
K	Potassium
Kg	Kilogram

LDH	Lactate dehydrogenase
MDSC	Myeloid-derived suppressor cell
Mg	Milligram
MHC	Major histocompatibility class
Min	Minute
mL	Milliter
MRI	Magnetic resonance imaging
Na	Sodium
NCI-CTCAE	National Cancer Institute – Common Terminology Criteria for Adverse Events
NE	Nonevaluable
NK	Natural killer
ONJ	Osteonecrosis of the jaw
OPG	Osteoprotegerin
ORR	Overall response rate
OS	Overall survival
OTC	Over the counter
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PD	Progressive disease
PD-1 or PD-L1	Programmed death-1 or Programmed death ligand 1
PE	Physical Exam
PET	Positron Emission Tomography
PFS	Progression-free survival
PFT	Pulmonary function test
Pgp	P-glycoprotein
PR	Partial response
PT	Prothrombin time
PTT	Partial thromboplastin time
Qd	*Quaque die* (once daily)
Q3 weeks	Every 3 weeks
RANK	Receptor activator for NFκB
RANKL	Ligand for Receptor activator for NFκB
RECIST	Response evaluation criteria in solid tumors
RIP3	Receptor interacting protein
RNA	Ribonucleic acid
RTE	Recent thymic integrants
SAE	Serious adverse event
s.c.	subcutaneous
SD	Stable disease
sjTREC	signal joint T-cell receptor excision circles
SmPc	Summary of product characteristics
SNPs	Single nucleotide polymorphism
SUSAR	Serious unexpected adverse reaction
T4	Thyroxine 4
TAM	Tumor-associated macrophages
TCR	T-cell receptor
T1DM	Type 1 diabetes mellitus
TEC	Thymic epithelial cells
TIL	Tumor infiltrating lymphocyte
TNFSFR	Tumor necrosis factor receptor superfamily
TPL	Translational pathology lab
Treg	T regulatory
TRP-1	Tyrosinase related protein-1
TSH	Thyroid stimulating hormone
ULN	Upper limit of normal

UNC	University of North Carolina
Wk	Week
WOCBP	Woman of childbearing potential

1.0 BACKGROUND AND RATIONALE

1.1 Study Synopsis

This is a multicenter open-label, single-arm, phase 2 study designed to investigate the pharmacodynamic and antitumor effects of denosumab alone and in combination with an anti-PD-1 agent (pembrolizumab in subjects enrolled prior to Amendment 1 and nivolumab in subjects enrolled after Amendment 1) in subjects with unresectable PD-1/PD-L1 inhibitor-naïve regional and distant metastatic melanoma (AJCC stage III/IV). The pharmacodynamic and antitumor effects will be investigated by performing translational research on peripheral blood and tumor tissue collected before and during denosumab alone and in combination with anti-PD-1 treatment (details below). Up to 25 subjects will receive denosumab at the FDA approved dose (120 mg s.c. every 4 weeks with additional loading doses of denosumab 120 mg s.c. on day 8 and day 22 following Amendment 1) concurrently with either nivolumab at the recently FDA-approved every 4 week dose (480 mg IV q4wks in subjects enrolled following Amendment 1), or pembrolizumab at the FDA-approved dose (200 mg IV every 4 weeks in subjects enrolled prior to Amendment 1) for up to a year based on clinical benefit. Subjects who continue to have clinical benefit after a year of treatment may continue therapy with PD-1/PD-L1 inhibitor as per standard of care (See Study Schema).

The study has co-primary objectives; one being to assess the pharmacodynamic effects (immunomodulatory and/or antitumor) of denosumab alone and the other being to assess the pharmacodynamic effects following combination therapy with denosumab and an anti-PD-1 agent. To investigate any potential direct antitumor and indirect immunomodulatory effects of denosumab alone, concomitant anti-PD-1 treatments will not start until day 22 of study treatment initiation, after three doses of denosumab have been given on day 1, day 8, and day 22. In fact, mandatory tumor biopsies and peripheral blood will be collected on day 22, immediately prior to the first anti-PD-1 agent infusion. Blood and tumor samples collected on day 22 will allow us to determine the denosumab-alone effect compared to peripheral blood samples and archived or fresh tumor tissue collected at baseline. Multi-parameter flow cytometry of peripheral blood mononuclear cells (PBMCs), ELISA assays of serum, and immunohistochemistry (IHC) and immunofluorescence (IF) analyses of tumor tissue are the methods that will be utilized for correlative studies.

The secondary co-primary endpoint of the study includes assessment of the pharmacodynamic effects of denosumab and anti-PD-1 agent combination therapy in peripheral blood samples collected during the study (i.e., at weeks 16, 28, and 40) for comparison with denosumab alone. Moreover, an optional tumor biopsy may be obtained at week 16. Secondary endpoints include assessments to determine the safety of the combination in subjects with metastatic melanoma, plus measurements of the clinical benefit of denosumab in combination with an anti-PD-1 agent based on response rate, per Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1) criteria at week 16, progression-free survival

(PFS) rate at 6 months of combination therapy and overall survival (OS) rate at 1-year of study treatment. The safety of the combination will also be evaluated per NCI Common Terminology Criteria for Adverse Events (NCI-CTCAE). Pre-defined Pocock-stopping criteria will dictate early trial termination, if excessive toxicity or other adverse effects (AE) of clinical interest are observed with this combination.

1.2 Incidence and Current Standard of Care for Metastatic Melanoma

The American Cancer Society estimates that approximately 106,110 new cases of melanoma and 7,180 melanoma-related deaths will occur in the United States in 2021[1]. Overall, the lifetime risk for melanoma is about 2.5%. While the average age of patients diagnosed with melanoma is 63 years, around the time that immune system may be compromised due to aging, a concerning statistic is that melanoma is also a common cancer in young adults. Early detection and treatment significantly impacts long-term prognosis as OS drops precipitously for patients diagnosed with melanomas that are locally advanced and/or distant metastatic. The 5-year OS rate is only about 15% to 20% for patients diagnosed with stage IV melanoma in comparison to patients with early stage tumors (Stage IA to IIB) with 5-year survival rates in the 87% to 99% range[2]. Unfortunately, metastatic cutaneous melanoma remains difficult to treat despite the introduction of novel therapies for this disease in recent years.

Significant advances have occurred in the treatment of metastatic cutaneous melanoma with ten new treatments having been approved by the US FDA over the last 10 years. These novel treatments include small molecule inhibitors that target BRAF and MEK signaling pathways and monoclonal antibodies directed against the immune checkpoint proteins (ICP) PD-1 and CTLA-4 [3,4]. _ENREF_3Targeted therapies with BRAF inhibitors are well studied in patients with *BRAFV600E*-mutant melanoma, For example, the 3-year OS rate of patients with *BRAFV600*-mutant melanoma who received dabrafenib or vemurafenib (both are BRAF inhibitors) alone was 41% and 21-31%, respectively whereas the median OS of concurrent inhibition of BRAF and MEK, using dabrafenib and trametinib or encorafenib (another BRAF inhibitor) plus binimetinib (a MEK inhibitor) was 25.9 months and 33.6 months, respectively [5-7]. However, only ~50% of melanoma patients harbor this mutation. More recently, treatment of patients with *NRASQ61*-mutant melanoma with binimetinib, another MEK inhibitor, significantly prolonged PFS compared to standard chemotherapy; however the clinical benefit was relatively small[8]._ENREF_7

Immunotherapies targeting co-inhibitory ICP CTLA-4 (ipilimumab) and PD-1 (nivolumab, pembrolizumab) have demonstrated considerable clinical benefit measured by durable PFS and OS. Clinical benefit from single-agent PD-1 inhibitors has been significantly greater compared to that conferred by CTLA-4 inhibitors (ipilimumab), based on randomized phase III studies [9,10]. Furthermore, PD-1 inhibitors are better tolerated than ipilimumab alone and with a lower incidence of serious adverse events (SAEs). Interestingly, concurrent blockade of

PD-1 and CTLA-4 has been associated with overall response rates (RR) up to 58%, as measured by RECIST v1.1 criteria; however, this is counterbalanced by increased risks of up to 55% in treatment-related SAEs [9] and lack of overall survival clinical benefit compared to nivolumab alone_ENREF_11.[11] A clinical trial using a lower dose of ipilimumab administered on a less frequent dosing schedule, suggests that the major driver of SAEs seen with concurrent PD-1/CTLA-4 blockade regimens is due to ipilimumab_ENREF_12.[12] Overall, the high frequency of serious and life-threatening AEs observed with concurrent PD-1 and CTLA-4 blockade has limited the use of this regimen in community oncology settings despite its indisputable superior clinical benefit. Nevertheless, the superiority of concurrent dual CTLA-4 and PD-1 blockade over single target blockade suggests that multiple etiologies may simultaneously impair host immune response. Identification of novel immune-based targets that are dysregulated in cancer and are amenable to drug manipulation may potentially identify combination immunotherapeutic strategies that match the clinical benefit conferred by concurrent CTLA-4/PD-1 blockade without the intolerable side effects.

1.2.1 Future Directions for Treating Metastatic Melanoma

Further advances in the treatment of metastatic melanoma are focusing upon: (a) understanding the optimal sequence of targeted therapies versus immunotherapies in patients with *BRAFV600* and *NRASQ61* mutations (NCT02224781, EA6134), (b) identifying patient subgroups that benefit from combination CTLA-4/PD-1 blockade as opposed to single-agent PD-1 blockade, (c) administering targeted therapies with PD-1/PD-L1 pathway inhibitors simultaneously, in particular for patients with *BRAFV600*-mutant melanoma (e.g. NCT02130466, KEYNOTE-022), (d) combining PD-1/PD-L1 pathway inhibitors with immunotherapies other than CTLA-4 or cytokines (e.g. IL-2, interferons) based on better understanding of novel mechanisms of immune dysfunction, (e) testing novel targeted therapies for patients with non *BRAFV600*-mutant melanoma. Approach (d) is crucial given the high degree of toxicity of combined nivolumab and ipilimumab and the lack of predictive biomarkers of response, despite the indisputable durable clinical benefit. Therefore, PD-1/PD-L1-based treatments with comparable efficacy to nivolumab-ipilimumab, and a better toxicity profile may hold greater promise than concurrent nivolumab-ipilimumab.

Our proposed study is in line with priority (d) listed above. We have identified that RANKL-mediated central immune tolerance is clinically relevant in melanoma, based on both preclinical observations and clinical findings (see Sections 1.4 and 1.5)[13]. Thus, we propose to conduct this exploratory phase 2 study of anti-PD-1 agent (nivolumab administered at the FDA-approved dose 480 mg every 4 weeks in subjects enrolled after Amendment 1, or pembrolizumab administered at the FDA-approved dose 200 mg every 3 weeks in subjects enrolled prior to Amendment 1) in combination with denosumab administered at the FDA-approved dose (120 mg s.c. every 4 weeks with the addition of a loading dose of 120 mg administered on day 8 and day 21) in subjects with unresectable regional or distant metastatic melanoma (AJCC stage III/IV). We predict that this treatment

combination will be safe, well tolerated and may lead to antitumor responses that are significantly higher than those observed with a single anti-PD-1 agent.

To better understand the translational approach taken in our proposed study of denosumab-anti-PD-1 agent combination therapy in subjects with metastatic melanoma, it is important to know how immune function and dysregulation relate to immunotherapeutic treatment strategies for this disease. The following sections provide background on the salient topics of immune tolerance, normal T cell development, thymus function, and the role of RANK-RANKL-AIRE in thymus function with a particular emphasis on how these phenomena may influence the development and treatment of melanoma.

1.3 Immune Tolerance

Immune tolerance is a state of immune system unresponsiveness to antigens that may otherwise enable immune response [14]. This state of tolerance is contrasted with the conventional immune-mediated elimination of 'non-self'/foreign antigens important for normal physiology (e.g. pregnancy). The balance between tolerance and immune-mediated elimination of 'non-self'/foreign antigens comes with some crucial negative tradeoffs. For example, deficits in immune tolerance can lead to autoimmune diseases, infections, and cancer. Immune tolerance is classified as central or peripheral, depending on the anatomic location where tolerance is originally induced (e.g. thymus/bone marrow versus peripheral tissues/lymph nodes). The sections below elaborate on the role of immune tolerance in cancer, with particular emphasis on the most well studied peripheral type, and how recent advances in understanding of the mechanisms of central immune tolerance may be clinically relevant for cancer and even provide opportunities for novel anticancer therapies.

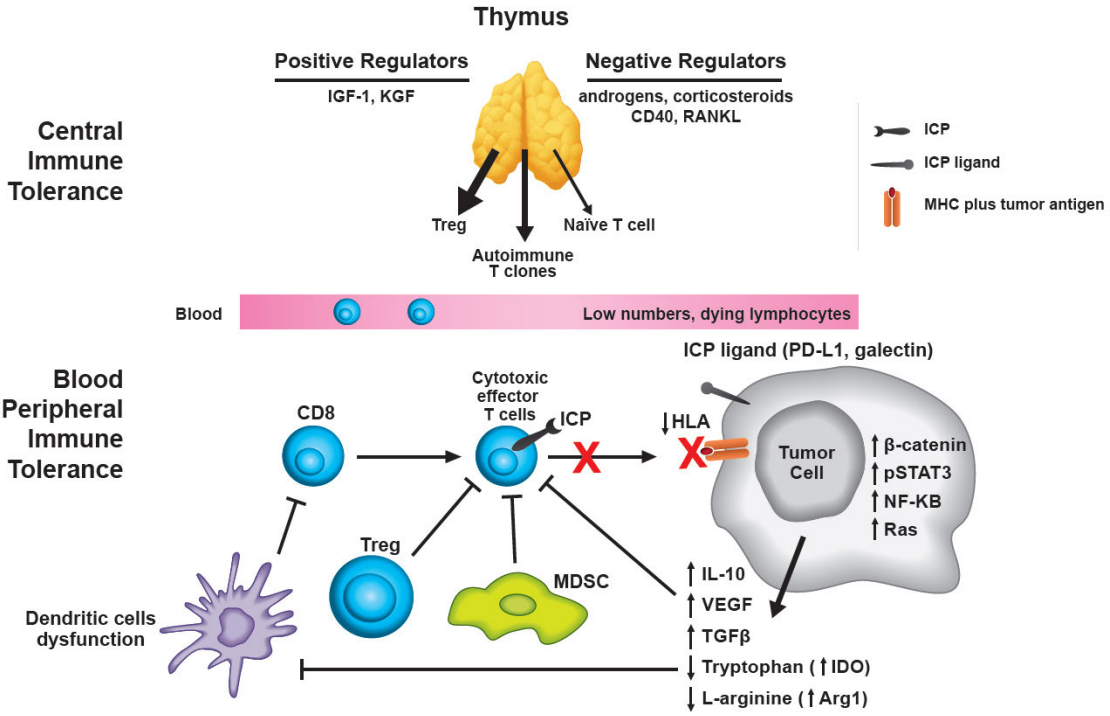

Figure 1. Central and peripheral immune tolerance in cancer. The complexity and the various immune cell subset involved, underlies multiple levels of regulation that can be potentially exploited for therapeutic intent. It also implies why single immunotherapy approaches may not be as effective as combination strategies, such as concurrent PD-1 and CTLA-4 blockade. Adapted from [4].

1.3.1 Peripheral Immune Tolerance is the Most Well Studied and Clinically Applicable Immunoregulatory Mechanism

Peripheral immune tolerance is key in preventing over-reactivity of the immune system to various environmental antigens (allergens, gut microbes, etc.). Mechanisms of peripheral immune tolerance in cancer are complicated and diverse, but have been extensively studied. Large-scale analysis of cutaneous melanoma tumor samples shows that in approximately 50% of melanomas, tumor-infiltrating lymphocytes (TIL) express both co-inhibitory and co-activating signals, a sign of an inability of immune cells to clear non-self-antigens. More specifically, as long as the 'non-self' antigen cannot be effectively eliminated, early upregulation of co-activating ICP that regulate cell proliferation, survival, cytotoxicity, type I interferon production, and activation is progressively followed by compensatory upregulation of co-inhibitory ICP that render immune cells more prone to apoptosis and anergy. In advanced stages of peripheral immune tolerance, TILs become tolerant of the nearby tumor cells in a symbiotic state that favors the latter [15]. As shown in **Figure 1** peripheral immune tolerance involves expression of various co-inhibitory ICP ligands and/or their cognate receptors by cancer cells themselves and/or other immunoregulatory populations, such as T regulatory cells (Treg), myeloid-derived suppressor cells (MDSC), and dendritic cells (DC). CTLA-4 and PD-1 are two of the most extensively studied co-inhibitory ICP targets in melanoma

and other cancers and their targeted inhibition is now an FDA-approved treatment strategy for various cancers. A number of clinical trials are underway that simultaneously target ICPs other than CTLA-4 and PD-1[4] _ENREF_5as a strategy for effectively inhibiting peripheral immune tolerance while keeping toxicity to a minimum.

1.3.2 Central Immune Tolerance is Insufficiently Explored in Cancer

Central tolerance is the main mechanism used by the immune system for discriminating 'self' from 'non-self'. Central tolerance relies on the continuous production of early precursor T cells by the bone marrow, followed by their maturation within the thymus. Multiparameter flow cytometry applied to PBMC allows us to follow the distinct immune cell subsets that are prevalent during each maturation stage without need for invasive biopsies to the thymus, which are nearly impossible in the adult life due to thymus involution. Nevertheless, few studies have investigated the abundance and functional status of these and other relevant immune cell subsets during physiologic (e.g. aging) and pathologic conditions (e.g. cancer) and the potential role that changes in these immune cell subsets may play in the etiopathogenesis of various diseases, including cancer. To understand the role of central immune tolerance in cancer, it is important to review the sequential steps and the corresponding regulatory feedback loops involved in the generation of mature T cells (**Figure 2**; also [16]).

1.3.2.1 *Normal T Cell Development*

Precursor T cells are derived from bone marrow stem cells. They migrate from the bone marrow to the thymus cortex as CD3-CD4-CD8- (double negative) cells for further processing. In the steps following T cell maturation within the thymus, the physical interaction between precursor T cells with cortical and medullary thymic epithelial cells (TEC) is crucial; therefore, regulation of TEC development, differentiation, and self-tolerance by various endocrine signals as well as other cytokines may significantly affect the type and repertoire of both effector immune response as well as peripheral tolerance. These double negative cells first undergo T-cell receptor (TCR) re-arrangement, which is an important maturation step towards diversification of T cells to different clones that will later on recognize distinct peptide epitopes. The extent of TCR re-arrangement among T cells, the surrogate for T-cell clonality repertoire and therefore epitope diversity, can be assayed by measuring signal joint TCR excision circles (sjTREC), namely small circles of DNA generated by T cells during their passage through the thymus as they rearrange their TCR genes [17,18]. In fact, sophisticated laboratory assays that precisely quantify TREC in the peripheral blood can be performed in clinical laboratory improvement amendments (CLIA) certified laboratories (e.g. Mayo Laboratories, Duke) and have been infrequently used as a diagnostic tool to monitor thymic output in certain physiologic states (e.g. aging) and diseases (e.g. T-cell reconstitution following bone marrow transplantation, autoimmune diseases).

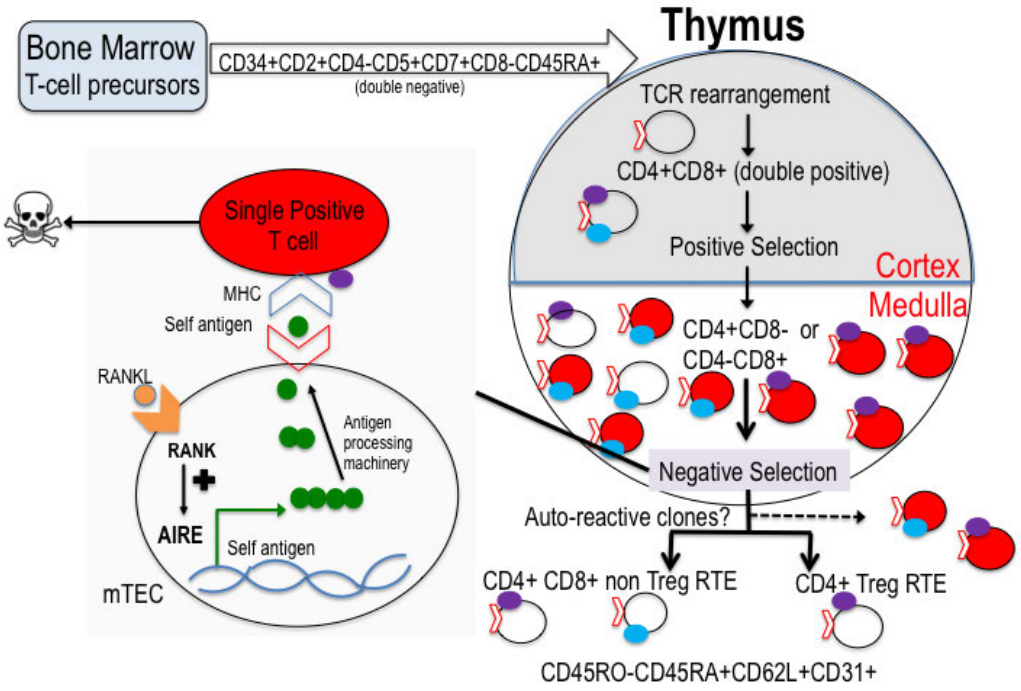

Figure 2. Schematic presentation of thymic input, thymic output, and intrathymic T cell development. Emphasis on negative selection (red-filled cells) and the role of RANK-RANKL-AIRE axis. Abbreviations: TCR, T-cell receptor; mTEC, medullary thymic epithelial cells; RTE, recent thymic emigrants; Treg, T regulatory cells.

Following TCR rearrangement, double negative precursor T cells become CD4+CD8+ (double positive) cells and undergo positive selection within the thymus cortex, only if they bear a TCR self-peptide complex that can interact with major histocompatibility class (MHC) class I or MHC class II receptors on the surface of TEC. Cells that are unable to bind to MHC receptors undergo apoptosis. At this point, T cells are either CD4+ or CD8+ (single negative), depending on which MHC class they are able to bind to during positive selection, and enter the thymic medulla for further processing. Of the T cell clones that undergo positive selection, those bearing TCR:self-peptide complexes that bind too strongly with self MHC are clonally deleted whereas the remaining are released from the thymus as single positive (CD4-CD8+ or CD4+CD8-) recent thymic emigrants (RTE). RTE are essentially the naïve T-cells (CD45RA+CD45RO-) that travel to secondary lymphoid organs (i.e. spleen, lymph nodes) and become effector T cells capable of proliferation and activation following encounter with distinct antigens. Phenotypically, they express the following membrane markers: CD4+ or CD8+ CD45RO-CD45RA+CD62L+CD31+ [19] and their clonal diversity can be measured by TREC, as mentioned above. TREC are diluted as T cells divide, so high levels indicate that a cell is a RTE (reviewed in [20,21]).

1.3.2.2 *Regulation of Thymic Function; the Particular Role of RANKL and AIRE*

It is important to emphasize that the process of T-cell development and maturation is highly regulated by various endocrine and paracrine signals. Understanding these

positive and negative feedback loops and the precise anatomic locations within the thymus where they exert their effect (cortex versus medulla) is important, because various normal physiologic and pathologic states may affect thymic function via changes in the abundance of these signals. For example, keratinocyte growth factor and growth hormone enhance proliferation of both cortical and medullary TEC. Negative regulators include glucocorticoids that cause decreased proliferation in cortical TEC (cTEC) and increase apoptosis in medullary TEC (mTEC) with the immature double positive thymocytes being the most susceptible [22]. Androgens and estrogens at high levels (e.g. pregnancy) suppress thymic output of naïve T cells via effects on both TEC subsets as well as bone marrow stromal cells. In contrast, physiologic levels of estrogens are fundamental for thymus development. Identification of these factors as regulators of thymic development is clinically relevant, because it may in part explain several long-standing clinical observations, such as:

(a) why females usually have better prognosis than males across various cancer types after adjusting for age, stage, and duration of follow-up [23]?

(b) why advanced age may be an adverse prognostic factor across various cancers (e.g. small-cell lung cancer, melanoma, bladder, ovarian), after adjusting for sex, cancer stage, performance status, and various other comorbid factors[24]?

(c) why females are more prone to autoimmune diseases than men [25]?

(d) why chemical castration in patients with prostate cancer increases circulating naïve T-cells [26]?

(e) why obese males have greater clinical benefit to both targeted therapies and immune checkpoint inhibitors compared to all other groups[27]?

In addition to hormonal factors, various members of the tumor necrosis factor (TNF) receptor superfamily (TNFSFR) play a significant role in regulating mTEC development and function. More specifically, mTEC express three principal TNFSFR members, the receptor activator for NFκB (RANK), CD40, and the lymphotoxin beta receptor (LTβR). Ligands for the three corresponding receptors (TNFSFRL), RANKL, CD40L, and lymphotoxin α/β are normally expressed by thymocytes and bind to their respective receptors on mTEC to initiate signaling and activation of classical and non-classical NFκB pathways [28]. In the case of RANKL-RANK, this pathway positively regulates expression of the autoimmune regulator (AIRE) in mTEC. AIRE is a transcription factor that regulates expression of a large set of downstream genes; 45% encode peripheral tissue antigens, which largely represent auto-antigens expressed by diverse tissues and organs. Therefore, exposure of differentiating thymocytes to a large variety of peripheral tissue antigens ensures negative selection of highly self-reactive nascent T-cell clones and prevents autoimmune diseases. As expected, loss-of-function mutations in the human *Aire* gene causes a recessive autoimmune syndrome, termed autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy [29].

1.4 Preclinical Evidence on the Role of the RANKL-RANK-AIRE Axis in Melanoma

Pioneering research by Dr. Maureen Su and colleagues from our institution has shown that molecular targeting of AIRE in mTEC can induce antitumor responses in melanoma syngeneic mouse models. More specifically, Dr. Su and colleagues have shown that the mechanism is immune-mediated and tumor antigen-specific; *Aire*-deficient mice have increased escape of specific T cells against melanoma-associated antigens (e.g. tyrosinase-related protein-1, TRP-1) from negative selection. This results in increased numbers of melanoma antigen-specific CD8+ cells infiltrating tumors, which in turn suppresses melanoma growth and improves survival [30]. Dr. Su and colleagues went on to show that pharmacologic blockade using an antibody targeting RANKL in melanoma-bearing syngeneic mice confers an antitumor effect that is similar to what was seen with *Aire*-deficient melanoma-bearing mice [31]. Given the availability of RANKL inhibitors in the clinic, these results point towards the potential role of RANKL blockade as a novel immunotherapeutic strategy in melanoma.

More recently, Dr. Su and colleagues from our institution have shown that simultaneous targeting of CTLA-4 and RANKL in syngeneic melanoma mouse models results in antitumor responses that are superior compared to either treatment alone (**Figure 3,** and [13]). The mechanism of action is novel, because it does not involve significant changes in the activation status of tumor-infiltrating CD8+ T cells, but rather upregulation of tumor-infiltrating, proliferating (Ki67+), killer-like receptor subfamily G member 1 positive (KLRG1+) –a unique CD4+ T cell population that expresses high levels of cytotoxicity-associated genes [32]– and cytolytic (granzyme B+) 'effector' CD4+ T cells. Further analysis of the immune-mediated changes in the *Aire*-deficient or loss-of-function *AireG228W* mice shows that the expression of the ICP, CTLA-4, PD-1, and OX-40, is increased in CD4+ T effector cells, whereas CTLA-4 was not significantly changed in CD4+FoxP3+ Tregs (**Figure 4** and [13]). Furthermore, Dr. Su and colleagues have shown that *Aire* deficiency and CTLA-4 blockade have additive effects by increasing the frequency of T cells that are reactive against the self/melanoma antigen TRP-1 that includes a trend towards increased frequency of T cells reactive against other self/melanoma antigen, TRP-2 and gp100, but not towards the irrelevant foreign antigen, ovalbumin [13]. In summary, *Aire* deficiency upregulates a unique population of melanoma-associated cytotoxic CD4+ T cells that are activated and express various co-inhibitory and co-stimulatory ICP, including CTLA-4 and PD-1. It is perhaps the upregulation of co-inhibitory ICP in those unique CD4+ cells, including CTLA-4 that, if inhibited by CTLA-4 antibodies, induces antitumor responses that are superior to *Aire* deficiency alone or CTLA-4 blockade alone.

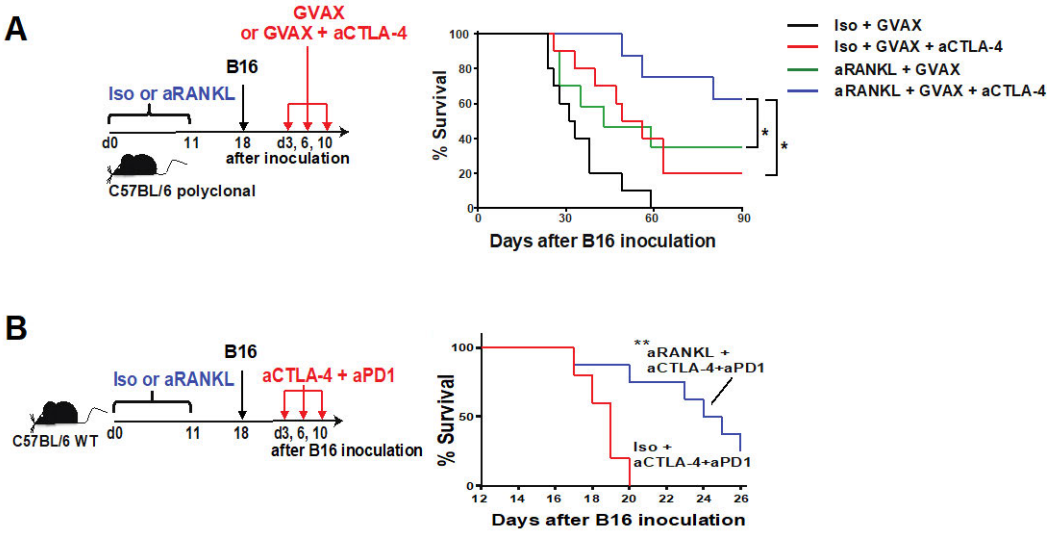

Figure 3. Anti-RANKL antibody enhances the immunotherapeutic effects of aCTLA-4 antibody monotherapy (A) or combination aCTLA-4 and aPD-1 therapy (B) in melanoma-bearing mice. Left panel. Schema of combination treatment strategy to block central and peripheral T cell tolerance mechanisms. Anti-RANKL antibody or isotype control antibody was injected every other day for 11 days, followed by subcutaneous B16 melanoma injection (1×10^5 cells) on Day 18. GVAX with or without anti-CTLA-4 antibody (A) or combination anti-CTLA-4 plus anti-PD-l1 antibody was given on days 3, 6, and 10 following melanoma cell injection. Host survival curves in GVAX treated mice receiving indicated combinations of antibody(ies) therapy. n=5 for each group. *p<0.05. Tumor-infiltrating lymphocytes (TIL) were harvested on Day 19 following B16 melanoma inoculation.

1.5 Is the RANKL-RANK-AIRE Axis Clinically Relevant in Melanoma?

Several lines of evidence suggest that AIRE is clinically relevant in melanoma. Firstly, analysis of the cutaneous melanoma TCGA dataset [33] suggests increased expression of various melanoma-associated and cancer testis antigens across all melanoma subsets (**Figure 5**). Secondly, AIRE expression and peripheral tissue-specific antigen expression are highly variable among individuals [34]. This may be explained by the fact that several single nucleotide polymorphisms (SNPs) can be found in humans. Although the impact of most of these SNP on *Aire* RNA stability and protein function is unknown, a handful of SNPs have been associated with decreased AIRE mRNA stability, and presumably decreased AIRE protein expression [35]. We postulate that differences in AIRE expression among humans may explain why certain individuals may be more prone to autoimmune diseases than others. Interestingly, distinct SNPs in the *Aire* gene were more frequent in healthy subjects as opposed to patients with melanoma diagnosis, presumably due to decreased stability of the AIRE mRNA [35].

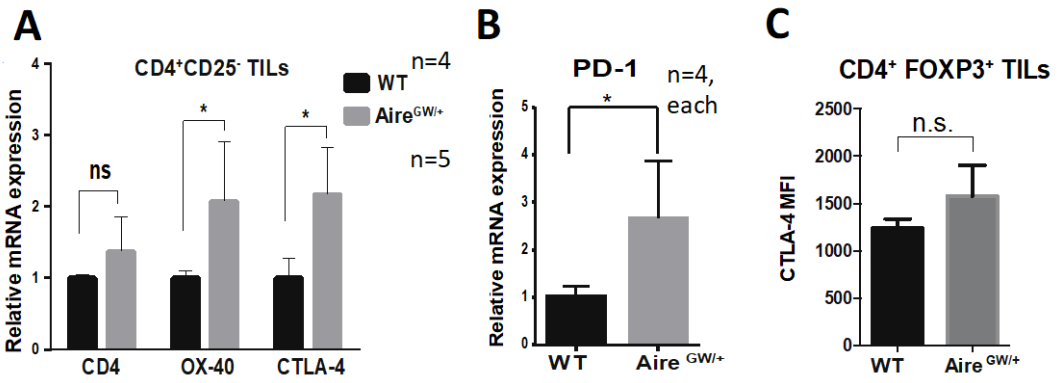

Figure 4. Various immune checkpoint proteins are upregulated in melanoma-infiltrating CD4+CD25- (panel A, B), but not CD4+CD25+ from Aire-loss-of-function (G228W/+) mice (panel C). Relative CTLA-4, OX-40, PD-1 mRNA expression by quantitative RT-PCR analysis was performed on flow-sorted cells on day 21 (panel A), or day 14 (panel B, C) post B16 melanoma inoculation. mRNA expression for PD-1 was not tested for CD4+CD25+ cells. Significance level was set at p=0.05 (*). N.s, non-significant.

To assess whether certain *Aire* polymorphisms, which presumably lead to decreased levels of *Aire,* and therefore increases in the frequency of autoreactive clones, predict response to ipilimumab, Dr. Su and colleagues genotyped 79 patients with metastatic cutaneous melanoma who participated in the E1608 study[36] for 5 *Aire* polymorphisms (rs1800522, rs2075876, rs56393821, rs1800520, and rs1055311). All 79 patients received ipilimumab alone. The rs1055311 TT polymorphism, which is associated with protection against melanoma development [35], was present in 6/79 patients (7.6%). Interestingly, patients with the TT SNP had an increased incidence (50%) of complete or partial responses (CR, PR) to ipilimumab[13], which is approximately 3 times higher than the antitumor responses seen with ipilimumab in large phase III studies [9]. These results suggest that although the incidence of patients with metastatic melanoma with *Aire* SNPs that may lead to a higher frequency of autoreactive clones is low, it is those patients who perhaps derive the greatest benefit from ipilimumab treatment. It is therefore tempting to hypothesize that targeting the pathway that regulates AIRE expression in mTEC, RANKL-RANK, may further increase the number of patients with metastatic melanoma who express autoreactive T cell clones, presumably against melanoma-associated and cancer testis antigens, which in combination with CTLA-4 blockade may increase antitumor response. In fact, durable responses following treatment with ipilimumab plus denosumab have already been reported in patients with metastatic melanoma [37].

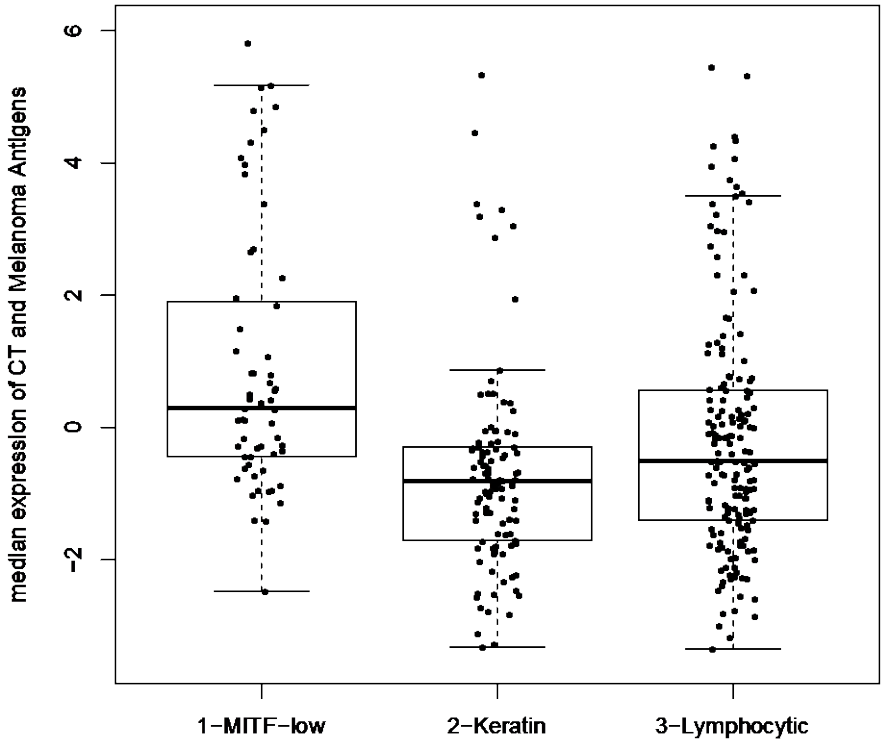

Figure 5. mRNA expression of various self-antigens (cancer testis, CT; and melanoma associated antigens) in cutaneous melanoma samples as part of the RNAseq analysis of the TCGA 329 tumor dataset[33]. Antigens included are of the MAGE family (A1, A4, A5, A9B, A10, A11, B1, B2, C1, C2, D4, D4B, L2,), PAGE family (1, 2, 2B, 5), cancer testis (CT) family (45A1, AG1B, AG2, AGE9), VCX family (VCX, 3A, 3B), FAM133A, XAGE1D, TPTE, CXORF48. Median expression was calculated after rank ordering (with 1 being the highest expression) in relation to the median expression across all three subtypes. Courtesy of Dr. Katherine Hoadley, Ph.D.

1.6 Peripheral Effects of the RANK-RANKL System

RANKL (also known as TRANCE/TNFSF11/ODF) along with its signaling receptor, RANK, and the soluble decoy receptor, osteoprotegerin (OPG), were discovered in the late 1990s as an important TNF superfamily system primarily involved in DC viability and function as well as in bone homeostasis, via regulation of osteoclasts. In its simplified form, RANKL is upregulated in T cells upon TCR activation (more in memory CD4 (Th1>Th2) than in memory CD8 cells) with kinetics much slower than the CD40-CD40L system (membrane expression of RANKL peaks around 48 hours and is present until 96 hours) and can be induced by various cytokines, such as IL-4 and transforming growth factor beta (TGFβ)[38]. RANKL can be membrane-bound on the surface of T cells, but also as a soluble receptor [39]. Interestingly, serum levels of RANKL are increased in patients with various cancers [40]. The receptor for RANKL, RANK, is physiologically expressed on the surface of various types of innate immunity cells, such as mature DC [41], natural killer (NK) cells [42], and monocytes/macrophages [43]. In these immune cell subsets RANK signaling promotes survival and/or function. In the case of

osteoclasts, which are bone marrow-derived cells or MDSC, RANKL induces differentiation and bone loss.

A recent study in syngeneic melanoma mouse models showed that the superior antitumor activity of concurrent CTLA-4 and RANKL blockade was dependent on T-cell and NK cell activity [37]. There is accumulating evidence that the RANKL-RANK-OPG system also plays an even more important role in tumor immunology. Among T cells that express RANKL, Tregs (CD4+Foxp3+) cells are the most interesting, because it has been shown that RANKL expression by Tregs may promote metastasis in RANK+ breast cancer cells [44]. The influence of the RANKL-RANK-OPG system on immune cell subsets that play an immunomodulatory role in cancers, such as MDSC and the alternatively activated macrophages (M2), is unknown.

In the case of melanoma, both receptors for RANKL, RANK and OPG, were overexpressed by melanoma cells whereas RANKL was not. More specifically, RANK is expressed by melanoma-initiating cells (CD133+ or ABCB5+) and metastatic melanoma, implying that RANK may be involved in the development and maintenance of melanoma [45]. OPG was upregulated by metastatic melanomas in response to TNFα [42]. Given that the cell survival effect is conferred by RANK signaling through anti-apoptotic proteins (e.g. bcl-2 and bcl-x) in DC [46], it is reasonable to speculate that similar RANKL-mediated receptor pathways exist for melanoma cell survival. Thus, blocking RANKL with anti-RANKL antibody may, in addition to its effect in central immune tolerance, potentially have both direct antitumor (e.g. melanoma cell death), and indirect-immunomodulatory effects, such as: 1) blocking the suppressive effects of Tregs and, 2) blocking survival signals in MDSC and alternatively activated macrophages (M2).

1.7 Function of Thymus in Relation to Aging and Cancer; Facts and Unanswered Questions

The thymus is an extremely sensitive organ to factors/conditions that are frequently prevalent in cancer, in particular conditions related to cancer treatment, such as irradiation, corticosteroids (either stress-induced or exogenously administered), and chemotherapy. It appears that both T-cell progenitors (most double positive thymocytes) and TEC can be adversely affected. Nevertheless, thymus can be regenerated, based on studies following allogeneic stem cell transplantation. Aging also causes involution of the thymus, which leads to defects in innate and adaptive immunity as well as immune dysregulation that may lead to autoimmune diseases [47]. However, using signal-joint TREC as a measurement of thymic function, studies have shown that the thymus continues to generate T cells, albeit in much lower numbers [48]. Interestingly, our meta-analysis on patients who have been treated in large randomized controlled trials using ICP inhibitors (ipilimumab, tremelimumab, nivolumab, and pembrolizumab) and were dichotomized into younger and older patients with an age cutoff of 65-70 years showed that the clinical benefit in terms of prolongation of OS was similar in both younger and older patients, when all trials testing ICP inhibitors were grouped together.

However, the subgroup of older patients who were treated in 4 trials testing PD-1 antibody therapies did not have clinical benefit compared to younger patients, suggesting either defects in adaptive immunity or different melanoma biology [49]. Targeting pathways that regulate thymic growth and enhance thymic recovery is an active area of research [47]. However, the impact of cancer on thymic function has not been systematically investigated beyond a handful of preclinical models (reviewed in [50]) and only a few clinical trials in solid tumors have assessed the impact of systemic treatments in thymic function [51].

It is important to emphasize that all preclinical experiments that Dr. Su and colleagues have performed on RANKL inhibition in melanoma were generated in young syngeneic melanoma mouse models. The clinical relevance of RANKL inhibition in patients with metastatic melanoma, 50% of whom are > 50 years old is therefore unknown. In this clinical trial we not only aim to assess the efficacy of denosumab in combination with ICP, but we also aim to systematically investigate the effect of this treatment on the abundance of various T-cell populations in peripheral blood (e.g. bone marrow T-cell precursors, thymocytes, recent thymic emigrants) as surrogate markers of influx and efflux from the thumus.

As summarized above, we have identified that RANKL-mediated central immune tolerance is clinically relevant in cutaneous melanoma, based on preclinical observations and clinical findings. Thus, we will investigate the pharmacodynamic and antitumor effects of anti-PD-1 therapy in combination with denosumab administered at 120 mg s.c. q4 weeks in subjects with distant metastatic melanoma. Our hypothesis is that denosumab-anti-PD-1 drug combination therapy will be safe, well tolerated and may lead to antitumor responses that are significantly higher than those observed with a single anti-PD-1 agent.

1.8 Investigational Treatment – Denosumab Alone and with an Anti-PD-1 Agent

Denosumab

Denosumab is a human IgG2 monoclonal antibody that binds to soluble RANKL and is indicated for prevention of skeletal complications from bone metastases of any solid tumor malignancy [52]. The standard dose is 120 mg administered s.c. every 4 weeks. Although denosumab is generally well tolerated, common side effects include nausea (31%), diarrhea (20%), electrolyte abnormalities (hypocalcemia 18%, hypophosphatemia 32%), fatigue (45%), and dyspnea (21%). Denosumab has also been associated with osteonecrosis of the jaw (1.8%) and atypical femur fractures [see denosumab package insert]. We will administer additional extra "loading" doses of denosumab on day 8 and day 22 in our trial, prior to the initiation of an anti-PD-1 agent, in order to assess the pharmacodynamic and immunomodulatory effects of denosumab alone on central immune tolerance as well as assess any potential direct antitumor effects of denosumab.

Nivolumab

Nivolumab is a potent and fully humanized monoclonal antibody of the immunoglobulin IgG4 that directly blocks the interaction between PD-1 and its ligands, PD-L1 and PD-L2. This blockade enhances functional activity of the target lymphocytes to facilitate an antitumor immune response, leading to tumor regression and immune rejection of the tumor. PD-L1 is expressed on approximately 40–50% of melanomas and has limited expression otherwise in most visceral organs with the exception of respiratory epithelium and placental tissue [53]. The FDA has approved nivolumab as an adjuvant treatment for patients with completely resected melanoma with lymph node involvement or metastatic disease, based on findings from the phase III CheckMate-238 trial. In this trial in patients with stage IIIB/C or IV melanoma, the most commonly reported treatment-related adverse events with nivolumab were fatigue (34.5%), diarrhea (24.3%), pruritus (23.2%), rash (19.9%), and nausea (15% vs 20.1%). The most common grade 3/4 treatment-related adverse events were diarrhea (1.5%), rash (1.1%), and increased ALT (1.1%). More recently, the FDA approved nivolumab dosing 480mg every 4 weeks on the basis of similar time-averaged concentration, 16^ lower trough levels and 45% higher peak levels at steady-state[54]. The concordance of dosing for both nivolumab and denosumab and the lack of data suggesting that pembrolizumab and nivolumab are significantly different in terms of side effect profile and/or efficacy prompted us to allow for use of nivolumab in combination with denosumab in our study.

Pembrolizumab

Pembrolizumab is FDA-approved for upfront treatment of unresectable stage III or distant metastatic melanoma (AJCC stage III/IV) [10]. Pembrolizumab is an IgG4 kappa immunoglobulin that blocks the interaction between PD-1 and PD-L1, which decreases proliferation of T cells and production of cytokines. The standard dose for melanoma is 200 mg every 3 weeks. Side effects are immune-mediated and include pneumonitis (2%, 0.4% grade 3), colitis (2%, 1.2% grade 3-4), hepatitis (1%, 0.8% grade 3-4), hypophysitis (0.8%, 0.4% grade 3-4), hyperthyroidism (3.3%, 0.1% grade 3), and hypothyroidism (8.1%, 0.1% grade 3) [see pembrolizumab package insert].

Combination of Denosumab and anti-PD1 Agents

We do not feel that a phase 1 study of the denosumab-anti-PD-1 agent combination is required for two reasons: first, denosumab is usually well tolerated and, second, a significant number of patients with metastatic melanoma to the bone are already receiving denosumab concomitantly with anti PD-1 therapy without reported incidents in peer-reviewed literature per standard of care. However, use of denosumab in combination with PD-1 inhibitors administered to a broader group of patients with metastatic melanoma, irrespective of bone metastases, has not been systematically investigated, short of retrospective case series [55,56]. Admittedly, there is at least a theoretical premise for increased risk of colitis with the combination, based on preclinical experimental mouse models of colitis. In these models it was shown that the RANKL-RANK system is one of the important mediators of colitis. More specifically, RANKL was found to be expressed in

mucosal Treg cells and in subepithelial stromal cells within the domes of Peyer's patches whereas RANK was expressed on CD11c+ (presumably dendritic) cells in the lamina propria ([57]; [58]). In fact, inhibition of RANKL may result in a reduction of Treg cells in inflamed mucosa, and thus may worsen colitis [58]. However, Dr. Su and colleagues have shown that *Aire* deficiency does not exacerbate colitis severity in mice that have been treated with CTLA-4 blocking antibody[13].

1.9 Rationale for the LCCC1620 Study Design

There is currently an early indication that denosumab may have effects beyond its well-described osteo-immunologic mechanism in cancer. For example, retrospective analysis of 811 patients with non-small cell lung cancer who have received denosumab versus zolendronic acid showed that denosumab prolonged median OS [8.9 months versus 7.7 months, hazard ratio (HR) 0.80, 95%CI 0.67-0.95, p=0.01] [59]. A retrospective analysis of patients with metastatic melanoma who had bone metastases, and therefore received denosumab in combination with immune checkpoint inhibitor(s), showed a trend towards longer OS in patients who received immune checkpoint inhibitor(s) plus denosumab, in particular for patients with visceral metastases (M1c) [56]. RANKL was found to be a key paracrine effector of progesterone signaling in a subset of BRCA1 mutation carriers. In fact, denosumab treatment of patients with premenopausal BRCA1 mutation carriers resulted in substantial reduction of Ki67 staining seen in histologically normal tissue that was collected during the luteal phase [60], suggesting that denosumab may have chemopreventative effects in premenopausal RANK+ BRCA1-mutation carriers.

1.9.1 Why Denosumab is Combined with PD-1 Blockade and Not CTLA-4 Blockade?

Dr. Su and colleagues have demonstrated that RANKL blockade in combination with CTLA-4 inhibition leads to higher anti-tumor responses via a mechanism that involves increased melanoma antigen-specific, tumor-infiltrating T cells (both CD4+ and CD8+), and, in particular, activated cytotoxic effector CD4+ cells (**Figure 3** and **Figure 4**). While to date there is preclinical and clinical evidence (anecdotal case reports, [37]) that RANKL blockade synergizes with CTLA-4 blockade, there is only a single retrospective single-institution analysis of patients who received denosumab in combination with PD-1 and/or CTLA-4 inhibition[56]. Dr. Su and colleagues have shown in syngeneic B16-bearing mice that addition of RANKL inhibition to the combination with concurrent CTLA-4 and PD-1 inhibition is more effective that CTLA-4/PD-1 inhibitor blockade alone (**Figure 3B**). Furthermore, RANKL expression identifies tumor-infiltrating CD4+ and CD8+ T cells that expressing higher levels of PD-1 which can be modulated by anti-PD-1[61]. In addition, the target, PD-1, is highly expressed in preclinical models of *Aire* deficiency **(Figure 4)**. An additional, perhaps more important, reason is a clinical one; randomized phase 3 studies have consistently shown that PD-1 blockade is far superior compared to CTLA-4 blockade [10]. A triple combination (concurrent CTLA-4, PD-1, and RANKL blockade) may potentially be more effective than dual blockade (PD-1 and RANKL); however unforeseen side effects

may be observed with the triplet combination that are worse than ipilimumab-nivolumab, which is already highly toxic. It is best to determine at this time if denosumab is safe and effective in combination with anti-PD-1 agent first before exploring the triplet combination, which may require a dedicated phase I study.

1.9.2 Why Three "Loading" Doses of Denosumab Prior to Anti-PD-1 Agent Administration?

Dr. Su and colleagues have shown that in order for concurrent RANKL and CTLA-4 blockade to be effective, RANKL blockade must precede treatment with CTLA-4 blockade [13]. In addition, in the single-group, open-label, multicenter study of denosumab in patients with giant-cell tumor of the bone a total of three loading doses (day 1, day 8, and day 15) during the first 4 weeks was not associated with any new or worse side effects [62]. Therefore, we believe that two additional loading doses of denosumab during the first 3 weeks will be safe, and would therefore mitigate the need for a dedicated phase I study.

1.9.3 Primary Endpoints are Translational Investigations and Not Clinical Efficacy?

While this study aims to investigate the clinical benefit of the denosumab-anti-PD-1 agent combination, this is not the primary objective, and as such the study is underpowered for efficacy. Our study will investigate the pharmacodynamic effects of denosumab alone and in combination with an anti-PD-1 agent on central immune tolerance as well as denosumab's direct effect on tumor tissues collected on day 22 after three loading doses of denosumab are given on day 1, day 8 and day 22. As described in the Correlative Studies section (Section 1.10), we plan to perform analysis in peripheral blood and tumor tissue to investigate thymic input and output (see **Figure 2**) using multi-parameter flow cytometry and sjTREC analysis as well as the effect of the treatment combination in tumor tissue. The results from these three translational analyses will provide insights into the impact of RANKL inhibition and its role in abrogating central immune tolerance as well as highlight any potential direct effect RANKL inhibition may have on melanoma and the abrogation of peripheral immune tolerance. If there is a signal of clinical activity and benefit that is supported by correlative studies, future phase 1-3 studies may be conducted to combine denosumab with CTLA-4 and/or PD-1 inhibitors, and therefore expand the label of use of denosumab beyond the current one.

1.10 Correlative Studies

The co-primary endpoints of this study require collection of PBMCs, serum samples, and tumor tissue biopsies to understand the immunomodulatory and potentially direct antitumor effects of denosumab alone and in combination with an anti-PD-1 agent. The ability to collect tumor tissues at baseline and after three weeks of denosumab monotherapy will allow us to investigate the immunomodulary role of denosumab-alone treatment. Anti-PD-1 therapy will be initiated after samples are collected on day 22 (at the start of week 4) to explore the effects of combination therapy. The ability to collect peripheral blood at baseline; after three weeks of denosumab and then at weeks 16, 28, and 40, during

combination therapy coupled with disease restaging studies will allow us to assess the early effects of denosumab alone versus the early and late effects of the combination of denosumab and an anti-PD-1 agent.

1.10.1 Peripheral Blood

1.10.1.1 *Serum levels of RANKL (free and OPG-bound) and OPG*

These will be measured by ELISA (Su's lab). We predict serum OPG will be low, serum-free RANKL will be considerably high whereas the serum OPG/RANKL ratio will be low in a significant number of patients with metastatic melanoma at baseline. In fact, we predict that patients with high serum-free RANKL and/or low serum OPG levels will receive the greatest benefit from anti-PD-1-denosumab combination therapy. Following treatment with denosumab, we project that free RANKL will significantly decrease. If denosumab administered at the FDA-approved dose is able to bind free RANKL, then undetectable levels of free RANKL are expected.

1.10.1.2 *Peripheral Blood Mononuclear Cell Immune Monitoring*

The following cell populations will be ennumerated using multiparameter flow cytometry with the following decreasing order of importance:

(1) *Bone marrow-derived double negative early thymocytes* (baseline, week 4, week 16, 28, and 40; Su's lab) CD34+CD2+CD4-CD5+CD7+CD8-CD45RA+ (see **Figure 2**). We anticipate no changes following treatment with denosumab plus anti-PD-1 agent., although overall numbers may be significantly lower compared to same-age individuals who do not have cancer (i.e. patients' relatives).

(2) RTE by flow cytometric analysis (baseline, week 4, week 16, 28, and 40; Su's lab) CD4+CD45RO-CD45RA+CD62L+CD31+, CD4+Foxp3+ CD45RO-CD45RA+CD62L+CD31+, and CD8+CD45RO-CD45RA+CD62L+CD31+ (see **Figure 2**). We anticipate that denosumab alone will slightly increase total output of CD8+ and CD4+ non-Treg RTE, as well as CD4+ Tregs, although changes may be borderline. However, TCR repertoire analysis on fluorescence activated cell sorting (FACS)-sorted CD8+ RTE, CD4+ non-Tregs RTE, and CD4+ Tregs RTE will likely show increase of distinct clones for CD4+ non-Tregs and CD8+, and decrease of distinct clones for CD4+ Tregs.

(3) *RTE by sjTREC analysis on FACS-sorted CD4+ and CD8+ PBMCs* (baseline, week 4, week 16, 28, and 40; Duke Human Vaccine Institute, Dr. Gregory D Sempowski). The reason why we propose two independent assays for assessment of RTE is because TRECs do not solely reflect recently emigrated cells from the thymus, but also long-lived naïve cells in the periphery. In fact, proliferation of naïve T-cells in the periphery is the dominant homeostatic mechanism of T cell numbers in the elderly[48].

(4) *Immune cell receptor repertoire analysis on FACS-sorted CD4+ and CD8+ PBMCs* (baseline, week 4, week 16, 28, and 40; Immune Genomics Facility, UNC-CH, Dr. Benjamin Vincent). Treatment with PD-1 inhibitors alone leads to significant increases in the size of distinct immune receptor clones (both TCR and B-cell receptor) without changes in diversity [63]. We postulate that treatment with denosumab alone will increase thymic output of autoreactive clones, and therefore will increase clonal diversity. When denosumab is co-administered with an anti-PD-1 agent, we will observe a combination of increase size in diversity as well as clonality.

(5) *Effector T cell subtypes* (baseline, week 4, week 16, 28, and 40; Su's lab), such as cytotoxic (CD3+CD4-CD8+Granzyme B+) and exhausted (CD3+CD4-CD8+PD1+TIM3+LAG3+). RANKL is upregulated on activated T cells within 48 hours and are sustained until 96 hours [38]. There is little knowledge about the effect of RANKL inhibition in effector T cells in cancer, which may well bear the exhausted phenotype. We anticipate that denosumab alone will not affect numbers of activated effector T cells, whereas the effect on exhausted phenotype T cells is unknown, but important to explore.

(6) *Treg cells* (baseline, week 4, week 16, 28, and 40; Su's lab) CD4+CD25high+FoxP3+(RANKL)
Assuming that RANKL transmits a survival effect on RANK+ Tregs [44], treatment with denosumab alone will decrease the number of RANK+ Tregs. Pembrolizumab did not increase the number of intratumoral Tregs [64]. Therefore, we do not anticipate further reduction of peripheral Tregs, including RANK+ ones, when PD-1 inhibitors are concurrently administered with denosumab.

(7) *MDSC* (baseline, week 4, week 16, 28, and 40; Su's lab) monocytic (HLA-DRlowCD14+), other monocytic (lin1⁻HLA-DR⁻CD33+CD11b+), lymphoid (lin1⁻HLA-DR⁻CD33+CD11b+)
We cannot predict the outcome of distinct MDSC numbers and types when denosumab will be administered in combination with an anti-PD-1 agent, but the result will be important to explore. It is possible that RANKL transmits survival signals to alternatively activated MDSC, and therefore RANKL inhibition will suppress distinct subtypes of MDSC. Pembrolizumab increased number of intratumoral monocytic MDSC [64]. Suppression of all MDSC by denosumab in combination with anti-PD-1 agent will be beneficial.

1.10.2 Tumor Tissue

The goal of tumor tissue collection is to investigate the effect of denosumab-alone in tumor biopsies, both in terms of mechanistic (immunomodulatory and/or direct-antitumor) as well as pharmacodynamic effects (i.e. saturation of the denosumab target within tumor tissue, as we have previously shown with other antibody therapies in melanoma[65]).

Optional archived tumor tissue will be collected from any procdures that took place prior to study enrollment. Biopsies on day 22 will be mandatory because investigation of denosumab-alone effect in tumor tissue is a co-primary endpoint. As a co-primary aim, we would like to perform tumor biopsies at week 16 (i.e. 12 weeks after both anti-PD-1 agent and denosumab are concurrently administered) to assess the combined effect of these agents. Tumor biopsies at the end of week 16 will be optional, because we believe that a second mandatory biopsy within a clinical trial may impair subject accrual. Optional archived tumor tissue will also be collected on any procedures that occur after study completion or study removal due to tumor progression. Given the small size of excisional biopsies, it is impossible to perform flow cytometry to assess melanoma-infiltrating immune cell subsets on these samples. Rather, excised tumors will be fixed in formalin and paraffin-embedded (FFPE). Hematoxylin and eosin (H&E)-stained tissue sections will be generated to assess the quality (necrosis) and amount of tumor cells present. In addition, the amount of TILs will be scored, as previously described [33]. Furthermore, 5-μm thick tissue sections will be prepared to perform tumor-imaging analysis (all in Translational Pathology Laboratory, TPL, University of North Carolina at Chapel Hill, UNC-CH) in the following decreasing level of importance.

1.10.2.1 *Immunohistochemistry; single-stain* (baseline, week 4, and/or week 16)

(1) CD8 stain; we anticipate that denosumab alone will increase tumor-infiltrating CD8+ cells (baseline →day 22), as Dr. Su has shown previously in *Aire*-deficient syngeneic melanoma-bearing mouse models [30]. This increase will be further augmented when anti-PD-1 agent is added [64].

(2) RIP3 and Apoptag® stain. The reason for this analysis is because both RANK and OPG are expressed by melanoma cells and transmit survival signals [42,45]. We postulate that denosumab alone will increase cell death (necroptosis and/or apoptosis).

(3) RANKL stain using denosumab as a primary antibody. To investigate whether denosumab administered at 120 mg s.c. on day 1 and day 8 sufficiently binds ("saturates") all RANKL sites on the cell membrane of cells within tumor tissue (presumably Treg, other T cells) or stromal cells, we will use denosumab as a primary antibody for IHC. Tissue saturation studies of the antibody target in tumor tissue have been previously used to assess whether the dose/schedule of antibody therapy is adequate[65]. We anticipate that if denosumab fully saturates tissue-associated RANKL, no IHC stain will be observed on day 22 compared to baseline, similar to our previously published pharmacodynamic studies using monoclonal antibody therapies [65].

1.10.2.2 *3-color IF (baseline)*

(1) Sox10 (melanoma-specific transcription factor), RANK, OPG (baseline only). We would like to investigate expression of receptors for RANKL in

melanoma cells. We postulate that expression of any of the two RANKL receptors correlates with clinical benefit from denosumab-anti-PD-1 agent.

(2) CD8, RANKL, PD1 (baseline only). We would like to investigate expression of RANKL in total CD8+ and exhausted (PD-1+) effector T cells to assess degree of colocalization of RANKL and PD-1 in immune cells at baseline.

(3) CD68, CD163 (baseline, day 22, and/or week 16). RANK is known to be expressed in macrophages [43]. The role of macrophages in cancer is controversial, but there are reports suggesting a tumor-promoting role. We postulate that denosumab will disrupt RANK-dependent survival signals in tumor-associated macrophages (TAM). Therefore, we anticipate that the number of TAM will decrease on day 22 and, even more so, on week 16.

(4) CD33, CD11b, HLA-DR (baseline, day 22, week 16). RANK is also expressed in MDSC [46], which are precursors for osteoclast progenitors. MDSC may suppress effector T-cell functions. We postulate that, similar to TAM, RANK transmits survival signals to MDSC. We therefore postulate the number of tumor-infiltrating MDSC will decrease on day 22 and, even more so, at week 16.

2.0 STUDY OBJECTIVES

2.1 Co-primary Objectives

2.1.1 Assess the mechanistic (immune-mediated and/or direct antitumor effect) and pharmacodynamics effect (tissue saturation studies) of denosumab alone (i.e., after three loading doses of denosumab are given on day 1, 8 and day 22) in patients with unresectable (or resectable) stage III or distant metastatic PD-1/PD-L1 inhibitor-naïve* (*, see exceptions to this rule in sections 3.1 and 3.2 Inclusion/Exclusion criteria) melanoma (stage III/IV) by performing translational research on peripheral blood and tumor biopsy samples collected at baseline and after third loading dose of denosumab.

2.1.2 Assess the immune-mediated and direct antitumor effect of denosumab in combination with anti-PD-1 agent in patients with unresectable (or resectable) stage III or distant metastatic PD-1/PD-L1 inhibitor-naïve* (*, see exceptions to this rule in sections 3.1 and 3.2 Inclusion/Exclusion criteria) melanoma (AJCC stage III/IV) by performing translational research on peripheral blood and tumor biopsy samples collected at weeks 16, 28 and 40 of the study and comparing the results with those from baseline and after third loading dose of denosumab.

2.2 Secondary Objectives

2.2.1 Assess the safety of the denosumab-anti-PD-1 agent combination in unresectable (unresectable) stage III or distant metastatic PD-1/PD-L1 inhibitor-naïve* (*, see exceptions to this rule in sections 3.1 and 3.2 Inclusion/Exclusion criteria) melanoma (AJCC stage III/IV) by NCI-CTCAE version 5.0.

2.2.2 Determine antitumor response by RECIST v1.1 criteria of the denosumab-anti-PD-1 agent combination at 16 weeks in patients with unresectable (resectable) stage III or distant metastatic PD-1/PD-L1 inhibitor-naïve* (*, see exceptions to this rule in sections 3.1 and 3.2 Inclusion/Exclusion criteria) melanoma (AJCC stage III/IV).

2.2.3 Determine the 1-year OS rate of the denosumab-anti-PD-1 agent combination in patients with unresectable (or resectable) stage III or distant metastatic PD-1/PD-L1 inhibitor-naïve* (*, see exceptions to this rule in sections 3.1 and 3.2 Inclusion/Exclusion criteria) melanoma (AJCC stage III/IV).

2.2.4 Determine the 6-month PFS rate of the denosumab-anti-PD-1 agent combination in patients with unresectable (or resectable) stage III or distant metastatic PD-1/PD-L1 inhibitor-naïve* (*, see exceptions to this rule in sections 3.1 and 3.2 Inclusion/Exclusion criteria) melanoma (AJCC stage III/IV).

2.3 Exploratory Objectives

2.3.1 [redacted]

[redacted]

3.0 STUDY ENPOINTS

3.1 Co-primary Endpoints

3.1.1 The immune-mediated mechanism of action of denosumab alone will be evaluated in blood and tumor samples collected at baseline and after third loading dose of denosumab. Multiparameter flow cytometry and ELISA assays will be performed on peripheral blood/serum samples as outlined above in section 1.10.1 Tumor biopsy samples will be evaluated by IHC and IF studies as outlined above in section 1.10.2 (See referenced sections for assay details. We will estimate differences after 3 weeks of denosumab treatment versus baseline).

3.1.2 The immune-mediated mechanism of action of denosumab combined with anti-PD-1 agent will be evaluated in blood and tumor samples collected at weeks 16, 28 and 40 of the study. Multi-parameter flow cytometry and ELISA assays will be performed on peripheral blood/serum samples collected at weeks 16, 28 and 40 as outlined above in section 1.10.1. Tumor biopsy samples obtained at week 16 will be evaluated by IHC and IF studies as outlined above in section 1.10.2 (See referenced sections for assay details. We will describe differences in immunomodulatory/antitumor effects observed with denosumab therapy with later immunomodulatory/antitumor effects observed after the addition of an anti-PD-1 agent to denosumab).

3.2 Secondary Endpoints

3.2.1 AEs experienced by patients receiving denosumab-anti-PD-1 agent will be assessed per NCI-CTCAE v.5.0.

3.2.2 The overall RR (CR + PR) at 16 weeks will assessed based on RECIST v1.1 criteria.

3.2.3 OS rate at 1-year is defined as the time from day 1 of study treatment until death as a result of any cause within one year of initiating study treatment.

3.2.4 PFS rate at 6 months is defined as the time from day 1 of treatment until disease progression or death status measured 6 months after initiating study treatment. Progression events will be defined per RECIST v1.1 criteria.

3.3 Exploratory Endpoints

3.3.1 [redacted]

4.0 SUBJECT ELIGIBILITY

Subjects must meet all of the following inclusion and exclusion criteria to participate in this study.

4.1 Inclusion Criteria

4.1.1.1 Signed written informed consent and HIPAA authorization for release of personal health information.

4.1.1.2 Age ≥ 18 years at the time of consent.

4.1.1.3 ECOG Performance Status of 0 – 2.

4.1.1.4 Histologically confirmed melanoma of cutaneous or mucosal primary (e.g. sinus, vagina, anus, gastrointestinal tract); metastatic melanomas from unknown primary are allowed because melanoma of unknown primary is biologically similar to cutaneous melanomas[66].

4.1.1.5 AJCC stage III/IV unresectable (or resectable) disease. Both should be measurable by RECIST v1.1 criteria. Subjects with resectable bulky stage IIIB, stage IIIC or stage IIID melanoma (≥ 2-cm in shortest diameter for lymph nodes infiltrated by tumor and ≥ 2-cm in longest diameter for non-lymph nodes infiltrated by tumor) can also be entered into the study at the discretion of the Principal Investigator.

4.1.1.6 Must have available and consent to collect archived tumor blocks from previous surgeries confirming or treating metastatic disease (e.g. radical lymph node dissection); if not available they can be enrolled into the trial, if they consent to have a tumor biopsy before treatment initiation.

4.1.1.7 Must agree to undergo one on-treatment biopsy on week 4 of the study; the biopsy at week 16 is optional.

4.1.1.8 Must agree to have 100 mL blood drawn for study purposes on week 1, week 4, week 16, week 28, week 40 and end of treatment.

4.1.1.9 Demonstrate adequate organ function, as defined in the table below; all screening labs to be obtained within 21 days prior to registration.

System	Laboratory Value
Hematological	
Hemoglobin (Hgb)	≥ 10 g/dL without transfusion or erythropoietin dependency (within 7 days of assessment)
Absolute Neutrophil Count (ANC)	≥ 1,500/mm^3
Absolute Lymphocyte Count	≥ 1,000/mm^3

(ALC)	
Platelets	≥ 100,000/mm^3
Renal	
Serum Creatinine **OR** Measured or calculated creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤ 1.5 × upper limits of normal (ULN) **OR** ≥ 60 mL/min using the Cockcroft-Gault formula for subject with creatinine levels > 1.5 × ULN
Hepatic	
Serum Total Bilirubin	≤ 1.5 × ULN or ≤ 2 × ULN for subjects with Gilbert's Syndrome
Aspartate aminotransferase (AST)	≤ 2.5 × ULN OR < 5 × ULN for subjects with liver metastases
Alanine aminotransferase (ALT)	≤ 2.5 × ULN OR < 5 × ULN for subjects with liver metastases
Albumin	≥ 2.5 mg/dL
Other	
Serum calcium	≥ 2.0 mmol/L (8.0 mg/dL)

4.1.1.10 Females of childbearing potential must have a negative serum pregnancy test within 72 hours prior to study treatment. NOTE: Females are considered of child bearing potential unless they are surgically sterile (have undergone a hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or they are naturally postmenopausal for at least 12 consecutive months.

4.1.1.11 Females of childbearing potential must be willing to use adequate method of contraception, as outlined in Section 4.5.2 – *Oral contraception* is required 14 days prior to initiation of study medications until 150 days after treatment discontinuation. NOTE: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

4.1.1.12 Male subjects with female partners must have had a prior vasectomy or agree to use an adequate method of contraception as outlined in Section 4.5.2 – Contraception, starting with the first dose of study therapy through 150 days after the last dose of study therapy.

Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

4.1.1.13 As determined by the enrolling physician or protocol designee, willingness and ability of the subject to understand and comply with study procedures.

4.1.1.14 Previous radiation therapy is allowed, provided it is completed ≥14 days prior to starting therapy and subject has recovered adequately from any related toxicities (grade ≤1, or grade ≤2 that is stable for ≥3 months).

4.1.1.15 If subject has received adjuvant treatments, in particular ipilimumab and high dose interferon, any toxicities must have resolved to grade 1 or less. Grade 2 toxicities attributed to ipilimumab from autoimmune endocrinopathies that require permanent hormone replacement therapy are allowed as long as they are adequately treated. This implies that subjects should be off systemic steroids for treatment of any of these or other autoimmune toxicities (e.g. colitis, rash).

4.1.1.16 Subjects who have previously received PD-1 inhibitors in stage III (adjuvant) or stage IV are allowed as long as:

(a) the interval between the last dose of the adjuvant PD-1 inhibitor and the date of relapse (clinical or radiographic) is at least 1 year,

(b) if subjects who received treatment for stage IV had antitumor response (partial response or complete response) by RECIST criteria version 1.1 but they stopped due to subject/investigator preference for at least a year between the last dose of the PD-1 inhibitor and the date of relapse (clinical or radiographic). Allowing for these subjects who have previously received PD-1 inhibitors in the adjuvant setting (i.e. no knowledge about clinical benefit) or following definite antitumor response in the metastatic setting is based on a recent case series of subjects who responded to PD-1 inhibitor rechallenge, if they had previously responded to PD-1 inhibitors[67]. This implies that waning antitumor immunity in the absence (i.e. >1 year) of costimulation with PD-1 inhibitors may be the reason for cancer recurrence and NOT primary resistance of PD-1 inhibitors.

(c) any side effects that may have occurred during the previous exposure to PD-1 inhibitors are not serious (i.e. grade 1 or 2 by CTCAE version 5.0 criteria).

4.2 Exclusion Criteria

4.2.1.1 History of prior malignancy, with the exception of the following:

- Non-melanoma skin cancers, non-invasive bladder cancer, and carcinoma *in situ* of the cervix,
- Prior history of prostate, provided that subject is not under active systemic treatment other than hormonal therapy and with documented undetectable prostate specific antigen (PSA < 0.2 ng/mL),

- Chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL) provided subject has isolated lymphocytosis (Rai stage O), and does not require systemic treatment [for "B" symptoms, Richter's transformation, lymphocyte doubling time (< 6 months), lymphadenopathy or hepatosplenomegaly],
- Lymphoma or any type of hairy-cell leukemia, provided subject is not on an active systemic treatment and is in complete remission, as evidenced by PET/CT scans and bone marrow biopsies for at least 3 months,
- History of other malignancy, provided subject has completed therapy, or does not require therapy, and is free of disease for ≥ 2 years. If subject has had other malignancy within the last 2 years from which he/she may have been completely cured by surgery alone, or does not require any treatment other than observation at the specialist's discretion, he/she may considered to be enrolled on condition that the risk of development of recurrent or distant metastatic disease based on the American Joint Committee in Cancer (AJCC) staging system is less than 30% in 3 years from the original diagnosis of other malignancy.

4.2.1.2 Has known active central nervous system (CNS) metastases that are symptomatic and require antiepileptic drugs or corticosteroids (any dose). Subjects with previously treated brain metastases may participate provided they are asymptomatic (i.e., no neurologic symptoms) for at least 2 weeks prior to the first dose of trial treatment and are not using steroids for at least 7 days prior to trial treatment. Patients with previously treated brain metastases should have evidence of stable brain metastases (without evidence of progression by imaging) for at least 2 weeks prior to the first dose of trial treatment. Patients with previously treated but grown brain metastases (or new brain metastases if there is no prior history of brain metastases) are still allowed in the study as long as the net growth of pre-existing brain lesions (or new brain lesions if there is no prior history of brain metastases) does not exceed 1-cm in largest diameter as measured by brain MRI with IV contrast (for example, a patient with pre-existing brain lesion(s) that has grown by 6-mm in largest diameter and also has a new lesion that measures 4-mm in largest diameter can still be enrolled in the study; alternatively, a patient that has a new brain lesion that measures 1-cm in largest diameter and no growth of pre-existing brain lesions can still be enrolled in the study). This exception does not include carcinomatous meningitis, which is excluded regardless of clinical stability. Subjects with leptomeningeal disease, detected either by brain MRI or by cytology (e.g. lumbar puncture) are also excluded.

4.2.1.3 Treatment with any investigational drug, immunotherapy or chemotherapy within 28 days prior to study treatment (i.e., initiation of denosumab). Treatment with any targeted

therapy (e.g. MAPK inhibitors) is allowed as long as at least 15 days have elapsed since last dose of drug.

4.2.1.4 Subjects discontinuing prior therapy with tyrosine kinase inhibitors for melanoma should be off these medications for at least 15 days before starting study treatment.

4.2.1.5 Prior PD-1/PD-L1 therapies in the adjuvant setting (with exceptions noted in inclusion Section 3.1.1.16); targeted therapies or prior ipilimumab in the adjuvant setting are allowed.

4.2.1.6 Any condition, including laboratory abnormalities, that in the opinion of the investigator places the subject at unacceptable risk, if he/she were to participate in the study. This includes, but is not limited, to serious medical conditions or psychiatric illness likely to interfere with participation in this clinical study.

4.2.1.7 Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy equivalent to daily doses of prednisone of 10 mg or greater (or an equivalent dose of other corticosteroids) or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment.

4.2.1.8 Has a known history of active tuberculosis (Mycobacterium Tuberculosis).

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

4.2.1.10 Hypersensitivity to nivolumab, pembrolizumab or denosumab or any of their excipients.

4.2.1.11 Has active autoimmune disease that has required systemic treatment in the past 1 year (i.e. with use of disease-modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

4.2.1.12 Has a history of non-infectious pneumonitis that required systemic corticosteroids or evidence of interstitial lung disease or current active, non-infectious pneumonitis.

Episodic, brief (< 7 day) exposure to systemic corticosteroids (e.g. steroid taper for poison ivy or COPD exacerbation) is allowed.

4.2.1.13 Has a history of an acute coronary event (e.g. myocardial infarction) within 3 months since study entry, uncontrolled and symptomatic coronary artery disease, or congestive heart failure New York Heart Association class III/IV.

4.2.1.14 Has an active infection requiring systemic therapy within 7 days prior to treatment initiation.

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

4.2.1.16 Known serologic status reflecting active hepatitis B or C infection. Subjects that are hepatitis B core antibody positive, but antigen negative, will need a negative polymerase chain reaction (PCR) prior to enrollment. [NOTE: Hepatitis B antigen or PCR positive subjects will be excluded].

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

4.2.1.18 Known active metabolic bone disease such as Paget's disease, Cushing's disease, hyperprolactinemia, over the last year 12 months, known history of osteoporosis that is symptomatic (e.g. history of fractures, bone pain), or hypercalcemia/hypocalcemia of any type (serum free calcium being more than 1.1 × ULN and less than 0.9 x, lower limits normal. LLN) over the last 2 weeks since study initiation that requires treatment beyond calcium and vitamin D supplementation.

4.2.1.19 Prior treatment with denosumab. Use of bisphosphonates for treatment of metastatic bone disease, but not for hypercalcemia of malignancy, is allowed.

4.2.1.20 History of current evidence of osteonecrosis or osteomyelitis of the jaw. *Note*: Subject should be referred to dentist before study treatment initiation for poor dentition or other dental issues that, in the opinion of the treating physician, may increase the risk of osteonecrosis of the jaw.

5.0 TREATMENT PLAN

5.1 Schema

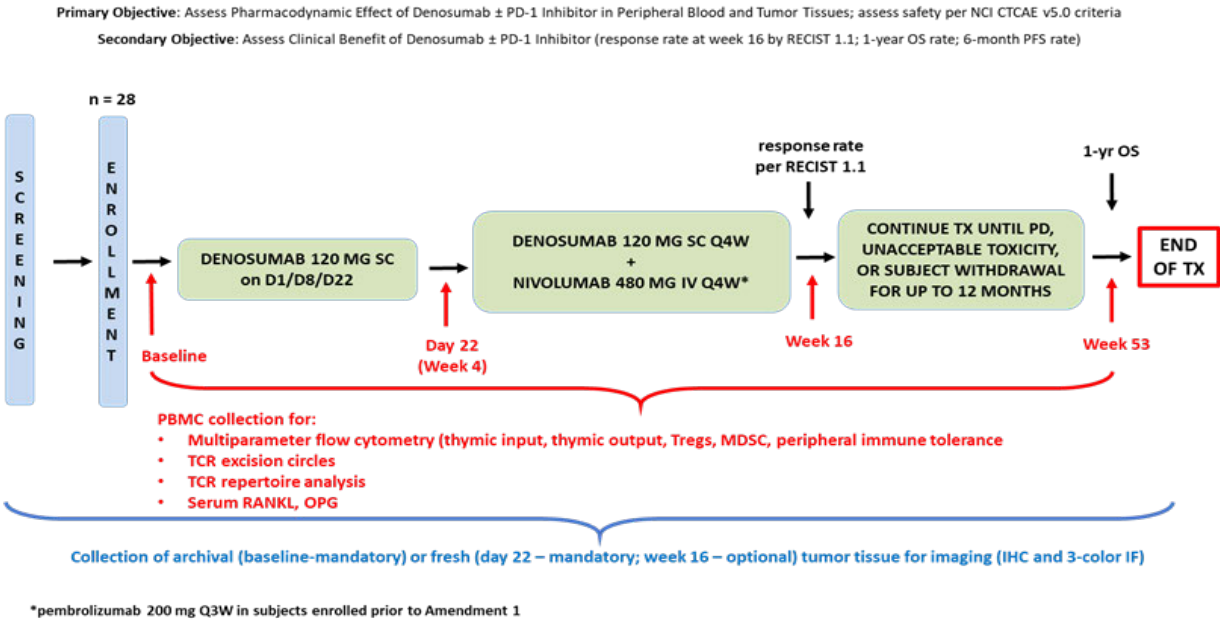

Figure 6. LCCC1620 Study Schema. Abbreviations: SC, subcutaneous; D, day; Wk, week; RECIST, Response Evaluation Criteria in Solid Tumors; OS, overall survival; Tx, treatment; AE, adverse events; pt, patient; PBMC, peripheral blood mononuclear cells; Tregs, T regulatory cells; MDSC, myeloid-derived suppressor cells; TCR, T-cell receptor; OPG, osteoprotegerin; IHC, immunohistochemistry; IF, immunofluorescence.

Treatment schema is shown in Figure 6. Eligible subjects must have AJCC stage III/IV melanoma of cutaneous or mucosal (or unknown) primary without any prior treatment with a PD-1 inhibitor or denosumab and available archived tumor blocks from prior melanoma surgeries. Please see Section 3.1.1.16 for exceptions to the PD-1 inhibitor naïve rule. Alternatively, if subjects do not have tumor tissue blocks from metastatic melanoma, they can be enrolled into the study if they undergo an excisional or CT-guided biopsy. At baseline, peripheral blood and archived (or fresh) tumor blocks will be collected for correlative studies. Leftover or excess research tumor tissue may be collected during all standard of care surgical procedures prior to enrollment, during study participation and will continue during follow up until death. To investigate denosumab-induced changes in peripheral blood and tumor tissue, denosumab will be administered first on day 1, day 8 and day 22 (day 29 prior to Amendment 1) and concomitant anti-PD-1 agent will be initiated on day 22. Immediately prior to the first infusion of anti-PD-1 agent (nivolumab or pembrolizumab), peripheral blood and a mandatory tumor biopsy will be collected. Following the day 22 time point, pembrolizumab will be administered every 3 weeks (in subjects enrolled prior to Amendment 1), nivolumab (in subjects enrolled after Amendment 1) and denosumab will be administered every 4 weeks as combination therapy for up to 1 year. Restaging radiographic studies will be performed at week 16 and then every 12 weeks thereafter, as per standard of care; peripheral blood will be collected at the same time points and an optional biopsy will be performed at week 16 to investigate denosumab plus anti-

PD-1 therapy effects on tumor tissue. Subjects will be considered to have completed all trial requirements if:

(a) their disease has progressed by RECIST version 1.1 criteria within less than a year from study initiation,

(b) they develop toxicity related to any of the study drugs, but still complete sample collection (peripheral blood and tumor tissue) and tumor assessments by at least week 16,

(c) complete the entire year of combination therapy and study-related assessments without experiencing disease progression. Those subjects experiencing clinical benefit after 1 year may continue anti-PD-1 therapy per standard of care.

5.2 Treatment Dosage and Administration

Subjects in this trial will be given denosumab, 120 mg s.c. every 4 weeks, starting on day 1 of study treatment. Additional loading doses of denosumab will be administered on day 8 and day 22 (after Amendment 1). Nivolumab, 480 mg will be administered intravenously (IV) every 4 weeks and initiated 21 days after the first dose of denosumab is given. In subjects enrolled prior to Amendment 1 pembrolizumab, 200 mg will be administered intravenously (IV) every 3 weeks and initiated 21 days after the first dose of denosumab is given. Combination therapy with both agents will continue as long as subjects benefit from therapy for up to 1 year. Study therapy will be discontinued for intolerable toxicity, disease progression or for other reasons at the discretion of the investigator. If subjects are not withdrawn prematurely then their last dose of study medications will be administered approximately 49 weeks after denosumab was initiated.

Agent	Required Premedications; Precautions	Dose	Route	Schedule
Denosumab	Correct hypocalcemia prior to initiating denosumab therapy. Monitor calcium levels during therapy, especially during the first 8 weeks of therapy, and adequately supplement all subjects with calcium (≥ 500mg qd) and vitamin D (≥ 400 IU qd). Monitor calcium, magnesium and phosphorus.	120 mg	s.c.; Administer in upper arm, upper thigh or abdomen	Every 28 days. Additional loading doses of 120mg s.c. will be administered on days 8 and 22
Nivolumab	Risk for immune-mediated and, infusion-related reactions	480 mg	IV; Administer over 30 min	Every 28 days
Pembrolizumab	Risk for immune-mediated[1] and, infusion-related[2] reactions	200 mg[3]	IV; Administer over 30 min	Every 21 days

1. See Section 4.3.4 for management guidelines for immune-mediated AEs
2. See Section 4.5.1 for management guidelines for infusion-related AEs
3. **Note:** On days when denosumab is administered on the same day as anti-PD-1 agent, the s.c. injection should be given after the infusion of the anti-PD-1 agent is completed.

5.2.1 Drug Dosing Schema

	C1D1	C1D8	C2D1	C3D1	C4D1	C5D1	C6D1
	Wk 1	Wk 2	Wk 4	Wk 8	Wk 12	Wk16	Wk20
Denosumab	×	×	×	×	×	×	×
Nivolumab			×	×	×	×	×
PBMC blood draw	×		×			×	
Tumor biopsy	×		×			×*	

*optional

	C7D1	C8D1	C9D1	C10D1	C11D1	C12D1	C13D1	EOT
	Wk24	Wk28	Wk32	Wk36	Wk40	Wk44	Wk48	Wk52
Denosumab	×	×	×	×	×	×	×	
Nivolumab	×	×	×	×	×	×	×	
PBMC blood draw		×			×			×
Tumor biopsy								

Figure 7. Study medications should be administered during the cycles denoted above. See the schedule of assessments in Time and Events table for further details. On days when denosumab is administered on the same day as nivolumab, the s.c. injection should be given after the infusion of nivolumab is completed.

Dosing Schema for Subjects Enrolled Prior to Amendment 1

	C1D1	C1D8	C2D1	C3D1	C3D15	C4D1	C5D1	C6D1
	Wk 1	Wk 2	Wk 4	Wk 7	Wk 9	Wk 10	Wk13	Wk16
Denosumab	×	×	×		×		×	
Pembrolizumab			×	×	×	×	×	×
PBMC blood draw	×		×					×
Tumor biopsy	×		×					×*

*optional

	C6D8	C7D1	C7D15	C8D1	C9D1	C10D1	C10D8	C11D1	C11D15
	Wk16	Wk19	Wk21	Wk22	Wk25	Wk28	Wk29	Wk31	Wk33
Denosumab	×		×		×		×		×
Pembrolizumab		×		×	×	×		×	
PBMC blood draw						×			
Tumor biopsy									

	C12D1	C13D1	C14D1	C15D1	C15D15	C16D1	C17D1	EOT
	Wk34	Wk37	Wk40	Wk43	Wk45	Wk46	Wk49	Wk52
Denosumab		×			×		×	
Pembrolizumab	×	×	×	×		×	×	
PBMC blood draw			×					×
Tumor biopsy								

Figure 8. Study medications should be administered during the cycles denoted above. See the schedule of assessments in Time and Events table for further details. On days when denosumab is administered on the same day as pembrolizumab, the s.c. injection should be given after the infusion of pembrolizumab is completed.

5.3 Toxicities and Dosing Delays/Dose Modifications

Any subject who receives treatment on this protocol will be evaluable for toxicity. Each subject will be assessed periodically for the development of any toxicity according to the Time and Events table. Toxicity will be assessed according to the NCI-CTCAE version 5.0. No dosing adjustments will be made for any of the two drugs; instead and depending on the side effects, there would be either treatment delays (up to 3 days for days between 22 and 43; up to 7 days between 64 and 106; up to 15 days for infusions beyond day 105), or drug(s) discontinuation. If drug delays go beyond what is allowed they will skip the particular dose and a note will be made regarding the reason.

5.3.1 Denosumab Dosing Guidelines

Dosing with denosumab should continue without treatment interruption as long as subjects benefit from therapy. Denosumab should be permanently discontinued if a severe toxicity occurs related to its use; for example, clinically significant allergic reaction to denosumab, serious infections attributed to denosumab (e.g. osteomyelitis of the jaw, appendicitis, etc.) or a subject becomes pregnant during the study. Denosumab therapy should be interrupted pending risk/benefit assessment (e.g. consideration for jaw surgery or tooth extraction) on an individual basis in cases where a subject develops osteonecrosis of the jaw (ONJ) or presents with an atypical femur fracture. See section 5.1 for additional information on denosumab. See also Section 8.1 for rules regarding continuous toxicity monitoring for SAEs attributed to either anti-PD-1 agent alone or denosumab alone or their treatment combination along with stopping rules for denosumab.

Monitor serum calcium levels during denosumab therapy, especially during the first 8 weeks of therapy, and adequately supplement all subjects with calcium (≥ 500mg qd) and vitamin D (≥ 400 IU qd). In addition to calcium, be sure to monitor magnesium and phosphorus in subjects receiving denosumab.

5.3.2 Nivolumab Dose Modifications/Delays

Nivolumab is used in this study per standard of care. *Suggestions* for toxicity management are provided below, but the final decision regarding management of nivolumab toxicity should be made by clinical investigator, consistent with standard of care judgements.

Toxicity grades per NCI-CTCAE Criteria v 5.0

Adverse Reaction	Severity	Dose Modification
Colitis	Grade 2 diarrhea or colitis	Withhold dose[a]
	Grade 3 diarrhea or colitis	Withhold dose[a]
	Grade 4 diarrhea or colitis	Permanently discontinue
Pneumonitis	Grade 2 pneumonitis	Withhold dose[a]
	Grade 3 or 4 pneumonitis	Permanently discontinue
Hepatitis/non-HCC[b]	Aspartate aminotransferase (AST)/or alanine aminotransferase (ALT) more than 3 and up to 5 × the ULN or total bilirubin > 1.5 and up to 3× ULN	Withhold dose[a]

Adverse Reaction	Severity	Dose Modification
	AST or ALT more than 5× ULN or total bilirubin > 3× ULN	Permanently discontinue
Hepatitis/HCC[b]	If AST/SLT is within normal limits at baseline and increases to more than 3 and up to 5 times the ULN or total bilirubin more than 1.5 and up to 3 times the ULN If AST/ALT is more than 1 and up to 3 times ULN at baseline and increases to more than 5 and up to 10 times the ULN If AST/ALT is more than 3 and up to 5 times ULN at baseline and increases to more than 8 and up to 10 times ULN	Withhold dose[c]
	If AST/ALT is more than 3 and up to 5 times ULN at baseline and increases to more than 8 and up to 10 times the ULN	Permanently discontinue
Hypophysitis	Grade 2 or 3 hypophysitis	Withhold dose[a]
	Grade 4 hypophysitis	Permanently discontinue
Adrenal insufficiency	Grade 2 adrenal insufficiency	Withhold dose[a]
	Grade 3 or 4 adrenal insufficiency	Permanently discontinue
Type 1 Diabetes Mellitus	Grade 3 hyperglycemia	Withhold dose[a]
	Grade 4 hyperglycemia	Permanently discontinue
Nephritis and Renal Dysfunction	Serum creatinine more than 1.5 and up to 6x ULN	Withhold dose[a]
	Serum creatinine more than 6x ULN	Permanently discontinue
Skin	Grade 3 rash or suspected Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN)	Withhold dose[a]
	Grade 4 rash or confirmed SJS or TEN	Permanently discontinue
Encephalitis	New-onset moderate or severe neurologic signs or symptoms	Withhold dose[a]
	Immune-mediated encephalitis	Permanently discontinue
Other	Other Grade 3 adverse reaction First occurrence Recurrence of same Grade 3 adverse reaction	Withhold dose[a] Permanently discontinue
	Life-threatening or Grade 4 adverse reaction	Permanently discontinue
	Grade 3 myocarditis	Permanently discontinue
	Requirement for 10 mg per day or greater prednisone or equivalent for more than 12 weeks	Permanently discontinue
	Persistent Grade 2 or 3 adverse reactions lasting 12 weeks or longer	Permanently discontinue

a) Resume treatment when adverse reaction returns to Grade 0 or 1.
b) HCC: hepatocellular carcinoma
c) Resume treatment when AST/ALT returns to baseline

5.3.3 Management of Nivolumab Infusion Reactions

Nivolumab can cause severe infusion reactions which have been reported in less than 1.0% of subjects. If such a reaction were to occur, symptoms would manifest as fever, chills, rigors, headache, rash, pruritis, arthralgias, hypo- or hypertension, bronchospasm, or other symptoms of allergic-like reactions.

All Grade 3 or 4 infusion reactions should be reported as an SAE if criteria are met. Infusion reactions should be graded according to NCI CTCAE v5.0 guidelines.

Treatment recommendations are provided below and may be modified based on local treatment standards and guidelines as appropriate.

For Grade 1 symptoms during nivolumab infusion (mild reaction; infusion interruption not indicated; intervention not indicated). Remain at bedside and monitor subject until recovery from symptoms. The following prophylactic pre-medications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen), at least 30 minutes before additional nivolumab administrations.

For Grade 2 symptoms during nivolumab infusion (moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, non-steroidal anti- inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids); prophylactic medications indicated for 24 hours).

Stop the nivolumab infusion, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen); remain at bedside and monitor subject until resolution of symptoms. Corticosteroid or bronchodilator therapy may also be administered as appropriate. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor subject closely. If symptoms recur, then no further nivolumab will be administered at that visit. Administer diphenhydramine 50 mg IV, remain at bedside, and monitor the subject until resolution of symptoms. The amount of study drug infused must be recorded on the electronic case report form (eCRF). The following prophylactic pre-medications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) should be administered at least 30 minutes before additional nivolumab administrations. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.

For Grade 3 or Grade 4 symptoms during nivolumab infusion: (Severe reaction, Grade 3: prolonged [i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae [e.g., renal impairment, pulmonary infiltrates]). Grade 4: (life threatening; vasoactive agents for hemodynamic support or mechanical ventilation indicated).

Immediately discontinue infusion of the therapeutic antibody. Begin an IV infusion of normal saline, and treat the subject as follows. Recommend bronchodilators, epinephrine 0.2 to 1 mg of a 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. Subject should be monitored until the investigator is comfortable that the symptoms will not recur. Nivolumab should be permanently discontinued.

Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery from symptoms.

In the case of late-occurring hypersensitivity symptoms (e.g., appearance of a localized or generalized pruritis within 1 week after treatment), symptomatic treatment may be given (e.g., oral antihistamine, or corticosteroids).

5.3.4 Pembrolizumab Dose Guidelines

Adverse events (AE, both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. It is quite possible that their frequency may be augmented by concurrent denosumab treatment, based on the presumed mechanism of action. These AE may occur shortly after the first dose or several months after the last dose of pembrolizumab. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per the table provided below. See Section 4.5 for supportive care guidelines, including use of corticosteroids.

General instructions:

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

Immune-related AEs	Toxicity grade or conditions (CTCAEv4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		

				• Add prophylactic antibiotics for opportunistic infections
Diarrhea / Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (i.e., diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus). • Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.
	Grade 4	Permanently discontinue		
AST / ALT elevation or Increased bilirubin	Grade 2	Withhold	• Administer corticosteroids (initial dose of 0.5- 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable
	Grade 3 or 4	Permanently discontinue	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold	• Initiate insulin replacement therapy for participants with T1DM • Administer anti-hyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated.	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue[1]		

Hyperthyroidism	Grade 2	Continue	• Treat with non-selective beta-blockers (e.g., propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders.
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hypothyroidism	Grade 2-4	Continue	• Initiate thyroid replacement hormones (e.g., levothyroxine or liothyroinine) per standard of care	• Monitor for signs and symptoms of thyroid disorders.
Nephritis and Renal dysfunction	Grade 2	Withhold	• Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper.	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1 or 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
All other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	• Based on type and severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Guillain-Barre Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		

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

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

Dosing interruptions are permitted in the case of medical/surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, subject vacation, and/or holidays). Subjects should be placed back on study therapy within 4 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the subject's study record.

5.3.5 Combined Denosumab and a PD-1 Agent Dosing Guidelines

Given that denosumab's mechanism of action presumably increases thymic output of autoreactive clones, it is quite possible that concurrent administration of denosumab and anti-PD-1 agent may increase frequency of immune-mediated adverse events caused by anti-PD-1 therapy (e.g. thyroiditis, rash, nephritis, pneumonitis) or, unexpectedly anti-PD-1 agent may somehow increase frequency of AE attributed to denosumab (e.g. infections, ONJ, rash). It is also a possibility that the denosumab-anti-PD-1 treatment regimen may result in SAE that have never been previously described with any of the two drugs alone (e.g. stroke, myocardial infarction, anaphylactic reactions during administration of nivolumab or pembrolizumab). As we will describe in the Statistical Considerations Section (Section 8.1), we will apply continuous toxicity monitoring throughout the study focusing on the frequency of AE attributed to anti-PD-1 agent alone and denosumab alone, as well as previously undescribed AE that are definitely attributed to study drug. Depending on the type, frequency, and severity of AE, we may end up with decision regarding permanently discontinuing denosumab for either a particular subject or across the entire trial (and therefore the entire study) if the denosumab-anti-PD-1 agent combination is considered too toxic.

5.4 Concomitant Medications/Treatments

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

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered 30 days after the last dose of trial treatment should be recorded for SAEs and Events of Clinical Interest (ECIs) as defined in Section 7.3.3.1.

Note: Pre-existing hypocalcemia must be corrected prior to initiating therapy with denosumab. Additionally, serum calcium, magnesium and phosphorus levels should be monitored during denosumab therapy, especially during the first 8 weeks of therapy. All subjects must be adequately supplemented with calcium (≥ 500mg qd) and vitamin D (≥ 400 IU qd).

5.4.1 Prohibited Concomitant Medications

Subjects with pre-existing hypersensitivity to denosumab, nivolumab or pembrolizumab disqualifies a subject from participation in the trial.

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

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol

- Investigational agents other than nivolumab or pembrolizumab
- Radiation therapy
  - Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed at the investigator's discretion.
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids for any purpose other than replacement/physiologic doses for autoimmune-related adrenal insufficiency, the management of immune-related events, adverse events, and as supportive care as outlined in the protocol. Study treatment must be held during the period of steroid administration. Subjects must be off steroids, if the modification allows, to resume study treatment.

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

The Exclusion Criteria describe other medications that are prohibited in this trial. Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the Amgen Clinical team. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician.

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

5.5 Rescue Medications and Supportive Care

Subjects should receive appropriate supportive care measures, as deemed necessary by the treating investigator. Suggested supportive care measures for the management of AEs with potential immunologic etiology are outlined below. Where appropriate, these guidelines include the use of oral or IV treatment with corticosteroids as well as additional anti-inflammatory agents, if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes, such as metastatic disease, bacterial or viral infection, which might require additional supportive care. The treatment guidelines will be applied when the investigator determines the events to be related to anti-PD-1 agent.

NOTE: if after the evaluation the event is determined unrelated, the investigator does not need to follow the treatment guidance (as outlined below). Refer to Section 4.3.4 for dose modification guidelines.

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

5.5.1 Management of Infusion Reactions from Pembrolizumab

Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. The table below outlines guidelines for subjects who experience an infusion-related reaction to pembrolizumab administration.

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for ≤ 24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to IV fluids, antihistamines, NSAIDS, acetaminophen, narcotics. Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be pre-medicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be pre-medicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab with: -Diphenhydramine 50 mg po (or equivalent dose of antihistamine). -Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to IV fluids, antihistamines, NSAIDS, acetaminophen, narcotics, oxygen, pressors, corticosteroids, epinephrine. Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

5.5.2 Contraception

For this trial, male subjects will be considered to be of non-reproductive potential if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

Female subjects will be considered of non-reproductive potential if they are either:

(1) postmenopausal (defined as at least 12 months with no menses without an alternative medical cause; in women < 45 years of age a high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient);

OR

(2) have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation/occlusion, at least 6 weeks prior to screening;

OR

(3) has a congenital or acquired condition that prevents childbearing.

Female and male subjects of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving study drug and for 150 days after the last dose of study drug by complying with one of the following:

(1) practice abstinence† from heterosexual activity;

OR

(2) use (or have their partner use) acceptable contraception during heterosexual activity.

Acceptable methods of contraception are‡:

Single method (one of the following is acceptable):

- intrauterine device (IUD)
- vasectomy of a female subject's male partner
- contraceptive rod implanted into the skin

Combination method (requires use of two of the following):

diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)

- cervical cap with spermicide (nulliparous women only)
- contraceptive sponge (nulliparous women only)
- male condom or female condom (cannot be used together)
- hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

† Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the subject's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and ERCs/IRBs. Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.

‡ If a contraceptive method listed above is restricted by local regulations/guidelines, then it does not qualify as an acceptable method of contraception for subjects participating at sites in this country/region.

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. To participate in the study, subjects of childbearing potential must adhere to the contraception requirement (described above) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 150 days after the last dose of trial therapy. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

5.5.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with denosumab-anti-PD-1 agent, the subject will immediately be removed from the study. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to Amgen without delay and within 24 hours to the Sponsor and within 2 working days to Amgen if the outcome is a SAE experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn).

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor. If a male subject impregnates his female partner, the study personnel at the site must be informed immediately and the pregnancy reported to the Sponsor and to Amgen, as described above and in Section 7.3.3.

5.5.4 Overdose of Pembrolizumab

There is no information on overdosage of pembrolizumab.

5.5.5 Overdose of Nivolumab

There is no information on overdosage of nivolumab.

5.5.6 Overdose of Denosumab

There is no experience with overdosage of denosumab.

5.6 Other Modalities or Procedures

A mandatory tumor biopsy will be performed on day 22 of the study, after 3 injection of denosumab and immediately prior to the administration of the first dose of PD-1 inhibitor. Depending on the location of the tumor, the biopsy can be excisional or punch and performed as an outpatient procedure at the bedside (if palpable; e.g. subcutaneous, cutaneous, or palpable lymph node or CT-guided by interventional radiology). In addition, an optional biopsy can be offered to subjects on week 16 (i.e., should be performed within 1 week of scheduled visit at week 16).

Up to 100 mL of peripheral blood will be collected for the purpose of analyzing the translational endpoints outlined in section 1.10.1.2. The reasons for the high amount of

blood to be collected are many-fold: (a) the immune cell subsets to be analyzed and enumerated are rare events, especially in adults (thymic input and output, MDSC), (b) peripheral blood lymphopenia is frequent in subjects with metastatic melanoma and an adverse prognostic factor [68], (c) assays will be performed in batched samples on the same day to avoid artifacts associated with assay variability; this implies that for each time point PBMCs will be isolated and cryopreserved, a procedure which is inherently associated with at least 30% loss of PMBCs upon thawing and reconstitution. Therefore, to compensate for these challenges, and on condition that subjects meet certain inclusion criteria [hemoglobin is ≥10g/dL, absolute lymphocyte count (ALC) is ≥1,000 cells/mm^3, absent coronary artery disease], a higher amount of peripheral blood should be collected to be able to measure the primary translational endpoints of the study.

A frequent scenario that we may encounter with respect to isolating adequate numbers of PBMCs is as follows. Subjects may have ALC approximately 1,000 cells/mm^3. Under the optimal conditions of Ficoll separation, processing of 100 mL of peripheral blood will yield approximately 100 million PMBCs. Of these cells, approximately 50-70 million will be suitable for downstream analysis following thawing and reconstitution on the day of the assay(s). The following are the minimum-optimal requirements (in millions of cells) for each assay:

(a) items 1, 2, 5, 6, 7, and 8 of section 1.10.1.2 15 million cells; no further processing is required.

(b) item 3 of section 1.10.2.1 (CD4+ and CD8+ sjTREC). The assay requires approximately 3 μg of genomic DNA in duplicates (at least). An adequate DNA extraction of genomic DNA from PBMCs yields approximately 4-6 μg of DNA per one million cells. We will perform isolation of CD4+ and CD8+ positive cells; they are roughly 5-30% of CD8+ T cells and 25-60% of CD4+ T cells in PBMCs. In addition, up to 20% of reconstituted PBMCs following thawing will not be reliably sorted into CD4+ and CD8+ compartments by FACS analysis. This means that for every 20 million reconstituted cells we will be able to isolate approximately 0.8 million CD8+ cells (worst case scenario) and 4 million CD4+ cells (worst case scenario). Thus, approximately 3.2 μg of DNA can be isolated for CD8+ sjTREC analysis (worst case scenario) and 16 μg of DNA can be isolated from CD4+ sjTREC. Therefore, 38 million PBMCs are required to ensure enough CD8+ sorted cells to perform the sjTREC analysis in duplicates.

(c) item 4 (CD4+ and CD8+ selected cells for immune cell receptor repertoire analysis). The assay requires 100 ng of RNA. Given that abundance of RNA in μg is approximately 5 times lower than that of DNA, one million PBMCs yields approximately 0.75-1 μg of RNA. CD4+ and CD8+ cells will also be sorted following thawing and reconstitution. Thus, 5 million cells will yield 200 ng of RNA from CD4+ sorted PBMCs and 1,000 ng of RNA from CD8+ sorted PBMCs. Given that DNA/RNA extraction will occur sequentially on the same batch of PBMCs sorted for CD4+ and CD8+ cells, we will isolate more than enough RNA using the 38 million PBMC batch for DNA extraction.

In summary, for a subject whose absolute lymphocyte count is approximately 1,000 cells/mm^3, approximately 53 million cells are necessary to perform the proposed downstream analyses.

5.7 Duration of Therapy

In the absence of treatment delays due to AEs, treatment with study medications may continue for up to 1 year or until:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Pregnancy,
- Subject decides to withdraw from study treatment, or
- General or specific changes in the subject's condition render the subject unacceptable for further treatment in the judgment of the investigator.

Subjects receiving clinical benefit at the conclusion of the study assessment period (after 1 year) may continue anti-PD-1 agent as per standard of care.

5.8 Duration of Follow Up

Subjects who have not progressed on PD-1 inhibitor plus denosumab after a year of treatment will be followed every 3 months for up to 2 years (i.e., a total of 3 years since study initiation) with phone calls or visits and then annually until death via medical record abstraction or phone calls. The following information will be recorded: (a) details regarding systemic treatment for their melanoma that they are currently on (type, response if any, duration on treatment), (b) whether they have progressed (and when) from current PD-1 inhibitor treatment, (c) whether treatment-related AEs that subjects may have developed during the year of denosumab- PD-1 inhibitor treatment have resolved, and when, (d) whether they are alive or not; if dead, precise date of death.

Subjects who have progressed on PD-1 inhibitor plus denosumab within the year of the study duration will be followed every 3 months for up to 3 years since study initiation with phone calls or standard of care visits and then annually until death via medical record abstraction or phone calls. The following information will be recorded: (a) systemic treatment for their melanoma they are currently on (type, response if any, duration on treatment, treatment-related AEs), (b) whether treatment-related AEs that subjects may have developed during the year of denosumab plus PD-1 inhibitor treatment have resolved, and when, (c) whether they are alive or not; if dead, precise date of death.

Subjects removed from study treatment for unacceptable AEs will be followed for resolution or stabilization of the adverse event(s). All subjects (including those withdrawn for AEs) should be followed after removal from study treatment as stipulated in the protocol.

Survival follow up will continue annually until death via medical record abstraction or phone calls.

5.9 Removal of Subjects from Protocol Therapy

Subjects will be removed from protocol therapy and the PI notified when any of the criteria listed in section 4.7 apply. The reason for discontinuation of protocol therapy will be documented on the electronic CRF (eCRF).

In case a subject decides to prematurely discontinue protocol therapy ("refuses treatment"), the subject should be asked if she or he may still be contacted for further scheduled study assessments. The outcome of that discussion should be documented in both the medical records and in the eCRF.

High number of subject withdrawals from study can render the study un-interpretable; therefore, unnecessary withdrawal of subjects will be avoided.

5.10 Study Withdrawal

If a subject decides to withdraw from the study (and not just from protocol therapy) an effort should be made to complete and report study assessments as thoroughly as possible. At the time of withdrawal, the investigator should attempt to establish the reason for the study withdrawal as completely as possible. The option of contacting the next of kin should be available.

- The subject should be asked if they are willing to allow for the abstraction of relevant information from their medical record in order to meet the long term follow up (e.g., survival) objectives outlined in the protocol.
- A complete final evaluation at the time of the subject's study withdrawal should be obtained with an explanation of why the subject is withdrawing from the study.
- If the subject is noncompliant and does not return for an end-of-study follow-up assessment, this should be documented in the eCRF.
- If the reason for removal of a subject from the study is an AE, the principal specific event will be recorded on the eCRF.

Excessive subject withdrawals from protocol therapy or from the study can render the study un-interpretable; therefore, unnecessary withdrawal of subjects should be avoided.

6.0 DRUG INFORMATION

6.1 Denosumab

6.1.1 Indications

- Prevention of skeletal-related events in subjects with bone metastases from solid tumors.
- Treatment of adults and skeletally mature adolescents with giant cell tumor of bone that is unresectable, or where surgical resection is likely to result in severe morbidity.
- Treatment of hypercalcemia of malignancy refractory to bisphosphonate therapy.

6.1.2 Mechanism of action

Denosumab binds to RANKL, a transmembrane or soluble protein essential for the formation, function and survival of osteoclasts, the cells responsible for bone resorption, thereby modulating calcium release from bone. Increased osteoclast activity, stimulated by RANKL, is a mediator of bone pathology in solid tumors with osseous metastases. Denosumab prevents RANKL from activating its receptor, RANK, on the surface of osteoclasts, their precursors, and osteoclast-like giant cells.

6.1.3 Product description

Denosumab is a human IgG2 monoclonal antibody that binds to human RANKL. Denosumab has an approximate molecular weight of 147 kDa and is produced in genetically engineered mammalian (Chinese hamster ovary) cells.

6.1.4 Supplier/How Supplied

Denosumab will be supplied as a sterile, clear, colorless to slightly yellow, preservative-free liquid, in single-use 3.0 mL glass vials containing a deliverable dose of 1.7 mL.

6.1.5 Storage and Handling

Store denosumab in a refrigerator at 2°C to 8°C (36°F to 46°F) in the original carton. Do not freeze. Once removed from the refrigerator, denosumab must not be exposed to temperatures above 25°C/77°F or direct light and must be used within 24 hours. Protect from direct light and heat. Avoid vigorous shaking of denosumab.

6.1.6 Dose and route of administration

Denosumab is intended for s.c. route only and should not be administered IV, intramuscularly, or intradermally.

The recommended dose is 120 mg administered as a s.c. injection every 4 weeks in the upper arm, upper thigh, or abdomen. In this study we will administer additional loading doses of 120 mg denosumab on day 8 and day 22 (after Amendment 1).

6.1.7 Possible side effects

- *Hypersensitivity.* Clinically significant hypersensitivity has been reported with denosumab. If an anaphylactic or other clinically significant allergic reaction occurs, initiate appropriate therapy and discontinue denosumab therapy permanently.

- *Hypocalcemia.* Denosumab can cause severe symptomatic hypocalcemia, and fatal cases have been reported. Monitor calcium levels through denosumab therapy, especially in the first 8 weeks of initiating therapy. We will advise all subjects to take daily supplements containing 1,000 mg calcium, or more, and 400 IU of vitamin D, or more.

- *Osteonecrosis of the Jaw (ONJ)*. ONJ manifesting as jaw pain, osteomyelitis, bone erosion, tooth or periodontal infection, tooth ache, gingival ulceration, or gingival erosion. Consider temporary discontinuation of denosumab therapy if an invasive dental procedure must be performed.

- *Atypical subtrochanteric and diaphyseal femoral fracture.* These fractures can occur anywhere in the femoral shaft. A number of reports note that subjects were also receiving treatment with glucocorticoids at the time of fracture. Subjects should be advised to report new or unusual thigh, hip, or groin pain.

- *Hypercalcemia* following treatment discontinuation in subjects with growing skeletons.

- *Embryo-fetal toxicity.* Denosumab can cause fetal harm when administered to a pregnant woman. Advise females of reproductive potential to use highly effective contraception during therapy and for at least 150 days thereafter.

6.1.8 Handling and Disposal

Please see policy on hazardous drugs:
http://intranet.unchealthcare.org/intranet/hospitaldepartments/safetynet/policies/hazardousdrugs.pdf

Local requirements for disposal of hazardous drugs should be followed at each participating clinical site.

6.1.9 Precautions

Pre-existing hypocalcemia must be corrected prior to initiating therapy with denosumab. See section above possible side effects Hypocalcemia for details.

For additional information, please refer to the full prescribing information on denosumab is available at: http://pi.amgen.com/united_states/xgeva/xgeva_pi.pdf

6.2 Pembrolizumab

Pembrolizumab will be provided by subject's health insurance. Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

6.2.1 Description

Pembrolizumab solution for infusion is a sterile, non-pyrogenic aqueous solution supplied in single-use type I glass vial containing 100 mg/4 mL of pembrolizumab (manufactured using the fully formulated drug substance with L-histidine as a buffering agent, polysorbate 80 as a surfactant, and sucrose as a stabilizer/tonicity modifier). The product is preservative-free solution which is essentially free of extraneous particulates.

6.2.2 Supplier/How Supplied

Pembrolizumab is supplied as single-use 100 mg vials containing a sterile, non-pyrogenic, clear to opalescent aqueous solution (25 mg/mL), or as single-use 50 mg vials containing lyophilized powder in a single-dose vial for reconstitution). Pembrolizumab solution for infusion is formulated in 10mM histidine buffer, pH 5.2-5.8, containing 7% sucrose and 0.02% polysorbate 80, supplied in Type I glass vials with a cap color of red, salmon, or blue.

6.2.3 Storage and Handling Requirements and Dispensing

Clinical supplies must be stored in a secure, limited-access location under refrigerated conditions (2°C to 8°C).
Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site.
Clinical supplies may not be used for any purpose other than that stated in the protocol.

6.2.4 Preparation of Infusion Solution

Aseptic technique must be strictly observed throughout the preparation procedure, preferably in a biologic safety cabinet or hood since no anti-microbial preservative is present in the solutions.
Equilibrate required number of pembrolizumab vials to room temperature.
The preferred method of dose preparation is the volumetric method, gravimetric method is not permitted.
Choose a suitable infusion bag size so that the following conditions are met:

- Concentration of pembrolizumab is between 1 mg/mL and 10 mg/mL
- The infusion volume to bag capacity ratio should not be less than 0.3. In other words, the bag must be filled to at least 30% of its capacity.

Choose a suitable infusion bag material. The bag may be empty, or it may contain normal saline. The following infusion bag materials are compatible with pembrolizumab:

- PVC plasticized with DEHP
- Non-PVC (polyolefin)
- EVA
- PE lined polyolefin

*Contact Sponsor for materials not listed above

Calculate the volume of pembrolizumab and normal saline required to prepare the infusion (admixture) bag

Volume of pembrolizumab (mL) = required dose amount (mg) / 25 (mg/mL)

Volume of normal saline = total infusion volume – volume of pembrolizumab from above

If a bag pre-filled with normal saline is being used, remove the excess volume of normal saline using a sterile syringe (Polypropylene, latex-free) attached to a suitable needle. Keep in consideration the excess bag fill volume as well as the volume of reconstituted pembrolizumab to be added to the bag to prepare the infusion solution.
If an empty bag is being used, withdraw the necessary volume of normal saline from another appropriate bag and inject into the empty bag. Keep in consideration the volume of reconstituted pembrolizumab to be added to the bag to prepare the infusion solution.

Withdraw the required volume of pembrolizumab from the vial(s) (up to 4 mL from each vial) using a sterile syringe attached to a suitable needle. The vial(s) may need to be inverted to remove solution.

Volume of pembrolizumab (mL) = required dose amount (mg) / 25 (mg/mL)

Note: If it is necessary to use several vials, it is advisable to withdraw from several vials into a suitable size single use syringe using a new needle for each vial.

Add the required pembrolizumab into the infusion IV bag containing normal saline and gently invert the bag 10-15 times to mix the solution.

Pembrolizumab solutions may be stored at room temperature for a cumulative time of up to 6 hours. This includes room temperature storage of admixture solutions in the IV bags and the duration of infusion.

In addition, IV bags may be stored under refrigeration at 2 °C to 8 °C (36 °F to 46 °F) for up to 20 hours. If refrigerated, allow the IV bags to come to room temperature prior to use. Do not freeze the pembrolizumab infusion solution.
Discard any unused portion left in the vial as the product contains no preservative.

6.2.5 Method of Administration

Pembrolizumab infusions should be administered in 30 minutes, with a window of -5 and +10 minutes, using an infusion pump. A central catheter is not required for infusion; however, if a subject has a central venous catheter in place, it is recommended that it be used for the infusion. The following infusion set materials are compatible with pembrolizumab:

- PVC and tri-(2-ethylhexyl) trimellitate (TOTM) infusion set
- Polyethylene lined PVC infusion set
- PVC Infusion set that is plasticized using Di-2-ethylhexyl Terephthalate (DEHT)

- Polyurethane set

A sterile, non-pyrogenic, low-protein binding 0.2 to 5 µm in-line filter made of polyethersulfone (PES) must be used during administration to remove any adventitious particles. If the infusion set does not contain 0.2 to 5 µm in-line filter, it is recommended to use 0.2 to 5 µm add-on filter which may contain an extension line (Note: the materials of the extension line and filter should be as mentioned above).
Attach the infusion line to the pump and prime the line, either with normal saline (at least 25 mL) or with infusion solution as per local SOP, before starting the infusion.
Infuse pembrolizumab over approximately 30 minutes, with a window of -5 and +10 minutes, through a peripheral line or indwelling catheter.
Ensure the entire contents of the bag are dosed and all remaining drug solution in the line is administered according to institutional guidelines for saline flushing.
Document volume administered according to data entry guidelines.

In case of infusion reactions, infusion rate may differ; refer to protocol for specific instructions.

Whenever possible, the lowest infusion rate should be used that will allow completion of the infusion within the 30 minutes. Maximum rate of infusion should not exceed 6.7 mL/min. through a peripheral line or indwelling catheter. However, when it is necessary to infuse a larger volume (i.e. 250 mL), the flow rate may go as high as 10 mL/min (maximum) in order to keep the infusion within the window as defined above.

Do not co-administer other drugs through the same infusion line.

Unused infusion solution for injection should not be used for another infusion of the same subject or different subject.

6.2.6 Storage and Stability

Pembrolizumab (MK-3475) Solution for Infusion, 100 mg/ 4 mL vial: pembrolizumab solution for Infusion vials should be stored at refrigerated conditions (2 – 8 °C) and protected from light.

Note: vials should be stored in the original box to ensure the drug product is protected from light.

Pembrolizumab infusion solutions should be prepared in 0.9% Sodium Chloride Injection, USP (normal saline) or regional equivalent or 5% Dextrose Injection, USP (5% dextrose) or regional equivalent and the final concentration of pembrolizumab in the infusion solutions should be between 1 mg/mL and 10 mg/mL.
Please note, the preferred diluent is 0.9% sodium chloride; 5% dextrose is only permissible if normal saline is not available.
Pembrolizumab should not be mixed with other diluents.
Pembrolizumab solutions may be stored at room temperature for a cumulative time of up to 4 hours. This includes room temperature storage of admixture solutions in the IV bags and the duration of infusion

In addition, IV bags may be stored under refrigeration at 2°C to 8°C (36°F to 46°F) for up to a cumulative time of 20 hours. If refrigerated, allow the IV bags to come to room temperature prior to use.
Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. Discard the drug product vial if extraneous particulate matter other than translucent to white proteinaceous particles is observed.
Sites should follow their SOPs for drug transport and delivery, with all possible effort to minimize agitation of the drug product between the pharmacy and the clinic.
Do not use pembrolizumab if discoloration is observed.
Do not shake or freeze the vial(s).
Do not administer the product as an intravenous (IV) push or bolus.
Do not combine, dilute or administer it as an infusion with other medicinal products.
Any deviation from this guidance must be discussed with sponsor

6.2.7 Return and Retention

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

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

6.3 Return and Retention of Study Medications

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

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

6.4 Nivolumab

Nivolumab will be administered per standard of care following the institutional guidelines. Please consult the prescribing information for nivolumab available at:
https://packageinserts.bms.com/pi/pi_opdivo.pdf

6.4.1 Description

Nivolumab injection drug product is a sterile, non-pyrogenic, single-use, isotonic aqueous solution formulated at 10 mg/10 mL. The vials supplied contain 100 mg. Nivolumab is a sterile, preservative-free, non-pyrogenic clear to opalescent, colorless to pale-yellow liquid that may contain light (few) particles for IV administration.

6.4.2 Supplier/How Supplied

Nivolumab is supplied as single-use 240 mg/24 mL (10 mg/mL) vials containing a sterile, non-pyrogenic, clear to opalescent, colorless to pale-yellow solution in a single-dose vial.

6.4.3 Packaging and Labeling

Primary Packaging (Volume)/Label type: Carton of 5 or 10 vials.
Secondary Packaging (Qty)/Label type: 10-mL Type 1 flint glass vials stoppered with butyl stoppers and sealed with aluminum seals.

6.4.4 Storage and Handling

Store nivolumab under refrigeration at 2°C to 8°C (36°F-46°F) in the original package until time of use. Protect from light and freezing. Do not shake the vial.

If stored in a glass front refrigerator, vials should be stored in the carton. Recommended safety measures for preparation and handling of nivolumab include laboratory coats and gloves.

Nivolumab solution for injection can be stored after preparation at room temperature for no more than 4 hours. This includes room temperature storage of the infusion in the IV container and time for administration of the infusion OR under refrigeration at 2°C to 8°C (36°F-46°F) for nor more than 24 hours from the time of infusion preparation.

6.4.5 Dose, Schedule and Administration

Nivolumab will be given every four weeks at a dose of 480 mg to be administered as an IV infusion per institutional guidelines.

There are no premedications recommended for nivolumab on the first cycle.

Subjects should be carefully monitored for infusion reactions during nivolumab administration. If an acute infusion reaction is noted, subjects should be managed according to institutional guidelines.

Doses of nivolumab may be interrupted, delayed, or discontinued depending on how well the subject tolerates the treatment.

6.4.6 Preparation

Follow institutional guidelines for nivolumab preparation or see the nivolumab prescribing information for preparation instructions. Nivolumab is to be administered as an IV infusion through a 0.2-micron to 1.2-micron pore size, low-protein binding polyether sulfone (PES) membrane in-line filter at the protocol-specified dose. It is not to be administered as an IV

push or bolus injection. Nivolumab injection can be infused undiluted (10 mg/mL) or diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to protein concentrations as low as 1 mg/mL. Care must be taken to assure sterility of the prepared solution as the product does not contain any antimicrobial preservative or bacteriostatic agent.

6.4.7 Handling and Disposal

Please see UNC policy on hazardous drugs:
http://intranet.unchealthcare.org/intranet/hospitaldepartments/safetynet/policies/hazardousdrugs.pdf

6.4.8 Adverse Events Associated with Nivolumab

The adverse events listed below have been reported in subjects receiving nivolumab.

- *Immune-mediated pneumonitis*: Defined as requiring use of corticosteroids and no clear alternate etiology, including fatal cases have occurred with nivolumab treatment.
- *Immune-mediated colitis*: Defined as requiring use of corticosteroids and no clear alternate etiology, including fatal cases have occurred with nivolumab treatment.
- *Immune-mediated hepatitis*: Defined as requiring use of corticosteroids and no clear alternate etiology have occurred with nivolumab treatment.
- *Immune-mediated endocrinopathies*: Hypophysitis, adrenal insufficiency, hypo- and hyper-thyroidism, and type I diabetes mellitus have occurred with nivolumab treatment.
- *Immune-mediated nephritis and renal dysfunction*: Defined as renal dysfunction or grade ≥2 increased creatinine, requirement for corticosteroids, and no clear alternate etiology, can occur with nivolumab treatment.
- *Immune-mediated rash*: Severe rash (including rare cases of fatal toxic epidermal necrolysis) can occur with nivolumab treatment.
- *Immune-mediated encephalitis*: Withhold nivolumab in subjects with new-onset moderate to severe neurologic signs or symptoms.
- *Complications of allogeneic human stem cell transplant after nivolumab:* Monitor for hyper acute graft-versus-host disease (GVHD). Transplant related mortality has occurred.
- *Infusion reactions*: Severe infusion reactions have been reported in <1.0% of subjects in clinical trials of nivolumab.

7.0 EVALUATIONS AND ASSESSMENTS

7.1 Time and Events Table

Assessments[1]	Pre-Study[1] Day-21 to -0	Study Treatment (cycle 1 = 21 days, cycle 2+n = 28 days)								End of Tx	Long-term follow up[21]
		C1D1	C1D8	C2D1	C2D2	C3D1	C4D1	C5D1	C6D1 – C13D1		
		Wk1 D1±1d	Wk2 D8±1d	Wk4 D22±2d	Wk4 D23-2 days through +1 day	Wk8 D50±3d	Wk12 D78±7d	Wk16 D106±7d	Wks17-49[20] (Wks22/28/34/ 40/46±7 days)	Wk52	
Informed consent	×										
History, PE[2]	×	×		×		×	×	×	×	×	×
Serum pregnancy test	×[3]										
ECOG Performance status	×	×[4]		×		×	×	×	×	×	
Concomitant medications review	×	×	×	×		×	×	×	×	×	
CBC with differential[5]	×	×[4]		×			×		×	×	
Serum chemistries[6]	×	×[4,6]	×[6]	×		×	×	×	×	×	
Endocrine tests[7]	×	×		×		×	×	×	×	×	
Urinalysis	×	×[4]		×			×		×	×	
Toxicity assessment[8]	×	×	×	×		×	×	×	×	×	
Brain MRI[9]	×										
Whole body CT[10]	×							×	×[10]	×[10]	
Denosumab[11]		×	×	×		×	×	×	×		
Nivolumab[12]					×[12]	×	×	×	×[12, 20]	×[13]	
Peripheral blood mononuclear cells[15]		×			×			×	×	×	
Hydration prior to blood draw[14]		×			×				×	×	
Archival tissue[16, 17]	×[16, 17]				×[18]			×[19]			×[17]
Tumor Biopsies[17]	×[17]										
Dental examination	×[22]										

Footnotes to Time and Events Table

1. All screening labs to be obtained within 21 days prior to registration. All study visits should be performed within ±1 day for the first two weeks of study therapy. For the C2D1 visit the window is ±2 days, then ±3 days for assessments scheduled on Day 50 (Week 8) and then ±7 days for all subsequent visits.
2. Complete history/physical at baseline only, thereafter focused history/physical exam on symptoms/toxicity.
3. Serum pregnancy test should be performed within 72 hours prior to the first dose of denosumab.
4. All screening labs to be obtained within 21 days prior to registration.
5. Will be performed at baseline and every other nivolumab infusion starting from C2D1, as per standard of care.
6. Include sodium, potassium, chloride, CO_2, BUN, creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase, magnesium, phosphorus, LDH, amylase and lipase. All listed serum chemistries will be performed before each nivolumab infusion as per standard of care. At Cycle 1 Day 8, magnesium, calcium, and phosphorus only.
7. Include cortisol levels, free T_4 (fT_4)/TSH, prolactin (women only), and testosterone (males only).
8. Toxicity assessments per NCI-CTCAE version 5.0.
9. Is performed at baseline; on future visits only if clinically indicated, as per standard of care.
10. Includes CT of the neck (if applicable), chest, abdomen, pelvis with IV contrast. Scans are performed at baseline, week 16, and then every 12 weeks thereafter (i.e., weeks 28, 40, and 52). Scans may be performed within ±7 days of the scheduled visit.
11. Denosumab will be administered s.c. as a flat dose, 120 mg every 4 weeks, as per FDA-approved dose, for up to a year until intolerable toxicity or disease progression occurs. Extra 120 mg loading doses of denosumab will be administered on C1D8 and C2D1. See Dosing schema in section 4.2.1. Denosumab will not be continued beyond the one-year duration of the trial, irrespective of treatment response.
12. Nivolumab will be administered every 4 weeks, 480 mg, for up to a year, starting on C2D2, until intolerable toxicity or disease progression occurs. See Dosing schema in section 4.2.1. Note that first dose of nivolumab may be given on day of biopsy depending on recovery from biopsy.
13. Following completion of the trial at 1 year and on condition that there is no disease progression, nivolumab can be continued at the opinion of the investigator, as per standard of care guidelines.
14. Subjects should receive hydration with 500 mL of normal saline per discretion of treating investigator prior to, in between or, following blood draw for correlative studies on week 1, week 4, week 16, week 28, week 40 and at the end of treatment visit.
15. PBMCs will be isolated from peripheral blood (approximately 100 mL at each time point) using the Ficoll isolation technique; an aliquot will be used for multiparameter flow cytometry, another for immune receptor repertoire analysis, and another for sjTCR excision cycle analysis. Samples will be collected at baseline on D1/wk1 and on D22/wk4, D106/wk16, wk28, wk40 and end of treatment. 500 mL of normal saline will be administered through IV either prior to collection, in between collection of study tubes, or following collection of all tubes.
16. Archival tissue or fresh tumor will be collected at baseline and a mandatory excisional biopsy will be performed on D22 of the study, after the third denosumab injection and before the first PD-1 inhibitor infusion. An optional excisional biopsy should be obtained on D106 (wk16). Tumor tissue will be submitted to the Tissue Pathology Laboratory (TPL) for IHC and multicolor IF. If archived tumor blocks

are not available, subject can still be enrolled on condition that he/she has a tumor biopsy prior to study enrollment.

17. Optional tumor tissue samples from standard of care surgical procedures may be collected for research purposes while the subjects are on study. These include samples that may have been collected after study enrollment, but prior to study biopsies, from any surgical procedure that occurs during study enrollment, and those procedures that may occur after study treatment completion as long as the subject is still on study. NOTE: For subjects who are withdrawn from LCCC1620, subjects must be re-consented and placed back on the study for attainment of optional tumor tissue samples from standard of care surgical procedures. These subjects will then remain on study for long term follow up.
18. Mandatory (excisional, punch or CT-guided), performed 24 – 48 hours after third denosumab injection and prior to nivolumab infusion.
19. Optional
20. Following radiographic disease assessment on week 16, subjects will be seen by the investigator on every other nivolumab infusion (i.e., every 8 weeks on D1 of weeks 24, 32, 40, 48 and at the end of treatment visit at week 52) for safety assessment and on every fourth nivolumab infusion (i.e., 16 weeks) for response assessment for up to a year. See sections identified below for details of scheduled assessments.
21. Subjects who have not progressed on nivolumab plus denosumab after a year of treatment will be followed every 3 months for up to 2 years (i.e. a total of 3 years since study initiation) with at least phone calls and then annually until death via medical record abstraction or phone calls. The following information will be recorded: (a) details regarding systemic treatment for their melanoma that they are currently on (type, response if any, duration on treatment, treatment-related side effects), (b) whether they have progressed (and when) from current PD-1 inhibitor treatment, (c) whether treatment-related adverse events that subjects may have developed during the year of denosumab plus nivolumab treatment have resolved, and when, (d) whether they are alive or not; if dead, precise date of death. Subjects who have progressed on nivolumab plus denosumab within the year of the study duration will be followed every 3 months for up to 3 years since study initiation with at least phone calls and then annually until death via medical record abstraction or phone calls. The following information will be recorded: (a) systemic treatment for their melanoma they are currently on (type, response if any, duration on treatment, treatment-related side effects), (b) whether treatment-related AEs that subjects may have developed during the year of denosumab plus nivolumab treatment have resolved, and when, (c) whether they are alive or not; if dead, precise date of death. Survival follow up will continue annually until death via medical record abstraction or phone calls.
22. Subjects with poor dentition or other dental issues that, in the opinion of the treating physician, may increase the risk of osteonecrosis of the jaw, should be examined and cleared by dentist prior to treatment initiation.

7.2 Follow-up Assessments

Subjects who have not progressed on PD-1 inhibitor plus denosumab after a year of treatment will be followed every 3 months for up to 2 years (i.e. a total of 3 years since study initiation) with phone calls or standard of care visits. The following information will be recorded: (a) details regarding systemic treatment for their melanoma that they are currently on (type, response if any, duration on treatment, treatment-related side effects), (b) whether they have progressed (and when) from current PD-1 inhibitor treatment, (c) whether treatment-related AEs that subjects may have developed during the year of denosumab plus PD-1 inhibitor treatment have resolved, and when, (d) whether they are alive or not; if dead, precise date of

death. Subjects who have progressed on PD-1 inhibitor plus denosumab within the year of the study duration will be followed every 3 months for up to 3 years since study initiation with phone calls or standard of care visits. The following information will be recorded: (a) systemic treatment for their melanoma they are currently on (type, response if any, duration on treatment, treatment-related side effects), (b) whether treatment-related AEs that subjects may have developed during the year of denosumab plus PD-1 inhibitor treatment have resolved, and when, (c) whether they are alive or not; if dead, precise date of death.

7.3 Correlative Studies Procedures

As part of this protocol, peripheral blood will be collected for quantification of certain hormones using ELISA and enumeration of immune cell subsets using multiparameter flow cytometry whereas tumor tissue will be collected for assessment of TILs, both effector and immunoregulatory, and treatment effects of the denosumab and PD-1 inhibitor combination at the tumor site. Detailed description of correlative studies in peripheral blood and tumor tissues is available in section 1.10. Rationale regarding the amount of blood requested per each time point is available in section 4.6. No dedicated genetic analysis will be performed beyond the standard of care. The sections below describe details regarding time points for specimen collection, amount of tissue to be collected at each time point and procedures for specimen collection, handling, and storage.

7.3.1 Peripheral Blood

Peripheral blood will be collected at baseline (day 1, week 1), day 22 (week 4), day 105 (week 16), week 28, week 40 and end of treatment for multiparameter flow cytometric analysis of immune cell subsets, signal joint TCR excision circle analysis (sjTREC) of CD4+ and CD8+ sorted T cells, immune cell receptor (TCR and B-cell receptor) repertoire analysis of CD4+ and CD8+ sorted T cells, and ELISA studies (RANKL and OPG serum levels). 100 mL of peripheral blood will be collected for non-ELISA studies and 5 mL will be collected for ELISA studies.

7.3.1.1 Peripheral blood Mononuclear Cell Isolation

- Collect peripheral blood in ten 10mL green-top (sodium heparin) tubes (BD Vacutainer, Catalog no. 366480). Mix the blood by gently converting the tube few times right after the blood is drawn.
- Transport green top tubes at ambient temperature on the same day of collection to the Tissue Procurement Facility, Lineberger Comprehensive Cancer Center (LCCC), for PBMC isolation and cryopreservation storage. Peripheral blood collection will be limited to Monday-Thursday.
- Ficoll-enriched PBMC isolation will be conducted per UNC institutional standard procedure.
- Refer to the LCCC Study Laboratory Manual for details on sample collection and processing procedures.

7.3.2 Tumor Tissue

Tumor tissue will be collected at baseline and during study treatment at two time points, day 22 and week 16. Collection of tumor tissue at baseline and on day 22 is mandatory whereas tumor tissue at week 16 is optional. Tumor tissue specimens as well as unstained-blank slides will be stored at UNC-CH TPF and will be sent to UNC-CH TPL) for correlative analysis. Details about specific stains are described in Section 1.10.2. Refer to the LCCC1620 Study Laboratory Manual for details pertaining to the collection, processing, and storage of fresh tumor biopsies, plus the procedure for quality control analysis of both archived and freshly isolated tumor biopsies.

7.3.2.1 Research Tissue Collection

Optional tumor tissue samples from standard of care surgical procedures may be collected for research purposes while the subjects are on study. These include samples that may have been collected after study enrollment, but prior to study biopsies, from any surgical procedure that occurs during study enrollment, and those procedures that may occur after study treatment completion as long as the subject is still on study.

7.3.2.2 Freshly Collected Tumor Tissues

If archived tumor blocks are not available, subject can still be enrolled to the trial on condition that they have fresh tumor biopsy prior to study enrollment.

Subjects must consent to undergo fresh tumor biopsy collection (baseline; day 22 biopsy mandatory; week 16 is optional). Both tumor biopsies are not standard of care and are for research purposes. If palpable tumors from a metastatic site are available, then excisional, punch, or core biopsies can be performed. If, however, palpable tumors are not available then a core biopsy procedure using an 18-gauge needle will be performed by interventional radiology. Intrathoracic lesions may be subjected to core biopsy if deemed safe by clinical investigator. The number of cores obtained is dependent on the subject's clinical condition at the time of biopsy and the health care professional who is performing the procedure. Therefore, it is possible that more than one core (up to 5) may be procured for this research project and make cores to the one FFPE block.

Freshly collected biopsy cores will be transferred to the UNC-CH TPF and will be handled as described in the LCCC1620 Study Laboratory Manual.

7.3.3 Handling and Storage of Biospecimens Collected for Correlative Research

Biospecimens collected as part of this study will be stored in the LCCC TPF (tumor tissue) or if needed, in a secure off-site storage facility. All biospecimen samples will be obtained in accordance with procedures outlined in the LCCC 1620 Study Laboratory Manual and stored in containers with controlled access. Each sample will be assigned a unique code number and no identifiable PHI will be on the

specimen label. Information about the subject's disease will be linked to the specimens stored in the repository database. TPF-associated research staff, LCCC Bioinformatics staff who support the TPF database and the LCCC Data Warehouse, and study researchers with IRB-approval for access to PHI for each subject in this study will be able to link specimens to relevant medical information. Some results from laboratory analyses that occurred during the subject's participation in the clinical study may also be included. This information may be important for understanding how the subject's cancer developed and responded to treatment.

Storage Time

- The biospecimen will be used first and foremost for research purposes outlined within the confines of this protocol. Samples will be discarded/destroyed after relevant data are collected for this study, unless consent was obtained from the subject to use tissue for other research purposes (e.g., TPF consent form was signed by the subject). In this circumstance, there is no time limit on how long biospecimens may be stored.
    - The investigator must agree to abide by policies and procedures of the TPF facility and sign a letter of research agreement for ethical and appropriate conduct of their research that utilizes specimens obtained from the TPF facility (e.g., use of leftover specimens will require a protocol outlining the research plan for biospecimen use).

Compliance Statement

Biospecimen collection for this study will be conducted in full accordance to all applicable UNC-CH Research Policies and Procedures and all applicable Federal and state laws and regulations including 45 CFR 46, and the Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule. Any episode of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain consent (unless a waiver is granted) and will report unexpected problems in accordance with The UNC IRB Policies and Procedures and all federal requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

7.4 Assessment of Safety

Any subject who receives treatment on this protocol will be evaluable for toxicity. Each subject will be assessed periodically for the development of any toxicity according to the Time and Events table. Toxicity will be assessed according to the NCI CTCAE version 5.0. Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

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

7.5 Assessment of Efficacy

Subjects who have received at least 1 dose of a PD-1 inhibitor and the first two doses of denosumab will be evaluable for assessment of response and progression. Subjects who drop out of the study for any reason (e.g., toxicity of treatment, decide to withdraw) will still be followed for PFS and OS.

7.5.1 Assessment of Disease-Tumor Measurement Based on RECIST v1.1

Disease assessment according to RECIST v1.1 criteria will include imaging and physical examination. Refer to the RECIST v1.1 for additional details [69]. To ensure comparability between baseline and subsequent disease assessments, the same method(s) of assessment (e.g., CT scan, MRI, etc.) will be used throughout the study for determining response. Tumor assessment will be completed as outlined in the Time and Events table.

Measurable disease will be defined as the presence of at least one measurable lesion that can be accurately measured in at least one dimension with the longest diameter a minimum size of:

- ≥10-mm by CT scan (CT scan slice thickness no greater than 5-mm).
- 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable).
- 20 mm by chest X-ray.

For lymph nodes to be considered pathologically enlarged and measurable, a lymph node must be ≥15mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5-mm). At baseline and in follow-up, only the short axis will be measured and followed.

All other lesions, including small lesions (longest diameter <10-mm or pathological lymph nodes with ≥10 to <15-mm short axis) as well as truly non-measurable lesions, will be considered non-measurable. Lesions considered truly non-measurable include: leptomeningeal disease; ascites; pleural/pericardial effusion; inflammatory breast disease; lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

All measurements should be recorded in metric notation, using calipers, if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment. The same method of assessment and the same technique should be used to characterize each identified lesion at baseline and during follow-up. Imaging-based evaluation should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam. Clinical lesions will only be considered measurable when they are superficial and ≥10-mm diameter as assessed using calipers (e.g. skin nodules). In the case of skin

lesions, documentation by color photography, including a ruler to estimate the size of the lesions, is recommended.

7.5.2 Baseline Documentation of Target and Non-Target Lesions

All measurable lesions up to a maximum of 5 (and a maximum of two lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline.

Target lesions should be selected on the basis of their size (lesions with the longer diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements.

A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

All other lesions (or sites of disease), including pathological lymph nodes, should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as "present" or "absent", or in rare cases "unequivocal progression".

7.5.3 Evaluation of Target Lesions using RECIST 1.1 Criteria

Note: In addition to the information below, also see section 4.3.2 in the international criteria proposed by the RECIST v1.1 for special notes on the assessment of target lesions[69].

Complete Response (CR) − Disappearance of all target lesions. Any pathological lymph node (LN) (whether target or non-target) must have decreased in short axis to <10-mm.

Partial Response (PR) − At least a 30% decrease in the sum of the longest dimension (LD) of the target lesions taking as reference the baseline sum LD.

Progressive Disease (PD) − At least a 20% increase in the sum of the LD of the target lesions taking as reference the smallest sum LD recorded since the treatment started, including baseline, if that is the smallest on study. In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5-mm. The appearance of one or more new lesions also constitutes PD.

Stable disease (SD) − Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as references the smallest sum LD since the treatment started.

7.5.4 Evaluation of Non-Target Lesions using RECIST 1.1 Criteria

Complete Response (CR) − Disappearance of all non-target lesions and normalization of tumor marker levels. All LN must be non-pathological in size (<10-mm short axis).

Non-CR)/non-PD − Persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal limits.

Progressive Disease (PD) − Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions.

7.5.5 Evaluation of Best Overall Response using RECIST v1.1 Criteria

The best overall response is the best response recorded from the start of the study treatment until the end of treatment provided the confirmation criteria are met. To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat studies that should be performed > 4 weeks after the criteria for response are first met. If a CR/PR cannot be confirmed the original "response" should be considered stable disease. The best overall response will be defined according to the following table:

Overall Response First Time Point	Overall Response Subsequent Time Point	BEST Overall Response
CR	CR	CR
CR	PR	SD, PD, or PR[1]
CR	SD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	NE[2]	SD provided minimum criteria for SD duration met, otherwise, NE[2]
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
PR	NE[2]	SD provided minimum criteria for SD duration met, otherwise, NE[2]
NE	NE[2]	NE[2]

[1] If a CR is truly met at first time point, then any disease seen at a subsequent time point, even disease meeting PR criteria relative to baseline, makes the disease PD at that point (since disease must have reappeared after CR). Best response would depend on whether minimum duration for SD was met. However, sometimes 'CR' may be claimed when subsequent scans suggest small lesions were likely still present and in fact the subject had PR, not CR at the first time point. Under these circumstances, the original CR should be changed to PR and the best response is PR. [2] NE, inevaluable

7.5.6 Other Efficacy Parameters

In addition to response rate by RECIST version 1.1 criteria, the study will calculate progression-free survival at 6-months and overall survival at 1-year, given that these two have proven to be valid early surrogate endpoints of clinical benefit of all FDA approved treatments for metastatic melanoma over the last 5 years.

8.0 ADVERSE EVENTS

8.1 Definitions

8.1.1 Adverse Event (AE)

AE is any untoward medical occurrence (e.g., an abnormal laboratory finding, symptom, or disease temporally associated with the use of a drug) in a subject who has been administered a pharmaceutical product as part of clinical investigation and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product.

Hospitalization for elective surgery or routine clinical procedures that are not the result of an AE (e.g., surgical insertion of central line) need not be considered AEs and should not be recorded as an AE. Disease progression should not be recorded as an AE, unless it is attributable by the investigator to the study therapy.

From the time of treatment through 30 days following cessation of treatment, all AEs must be reported by the investigator. Such events will be recorded at each examination on the Adverse Event CRFs. The reporting timeframe for AEs meeting any serious criteria is described in section 7.3.3. The investigator will make every attempt to follow all subjects with non-serious AEs for outcome.

8.1.2 Suspected Adverse Reaction (SAR)

A suspected adverse reaction (SAR) is any AE for which there is a *reasonable possibility* that the drug is the cause. *Reasonable possibility* means that there is evidence to suggest a causal relationship between the drug and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any AE caused by a drug.

Causality assessment to a study drug is a medical judgment made in consideration of the following factors: temporal relationship of the AE to study drug exposure, known mechanism of action or side effect profile of study treatment, other recent or concomitant drug exposures, normal clinical course of the disease under investigation, and any other underlying or concurrent medical conditions. Other factors to consider in considering drug as the cause of the AE:

- Single occurrence of an uncommon event known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson syndrome)
- One or more occurrences of an event not commonly associated with drug exposure, but otherwise uncommon in the population (e.g., tendon rupture); often more than once occurrence from one or multiple studies would be needed before the sponsor could determine that there is *reasonable possibility* that the drug caused the event.

- An aggregate analysis of specific events observed in a clinical trial that indicates the events occur more frequently in the drug treatment group than in a concurrent or historical control group

8.1.3 Unexpected AE or SAR

An AE or SAR is considered unexpected if its specificity or severity is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product). Unexpected also refers to AEs or SARs that are mentioned in the Investigator's 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.

8.1.4 Serious AE or SAR

An AE or SAR is considered serious if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death;
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- Requires inpatient hospitalization (>24 hours) or prolongation of existing hospitalization;*
- Results in congenital anomaly/birth defect;
- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a SAE drug experience when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definition. For reporting purposes, also consider the occurrences of pregnancy as an event, which must be reported as an important medical event.

*Hospitalization for anticipated or protocol-specified procedures such as administration of chemotherapy, central line insertion, metastasis interventional therapy, resection of primary tumor, or elective surgery, will not be considered SAE.

Pregnancy that occurs during the study must also be reported as an SAE.

Note: In addition to the above criteria, AE meeting either of the below criteria, although not serious per ICH definition, are reportable to Amgen in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by Amgen or collection purposes.

- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose.

8.2 Documentation of non-serious AEs or SARs

For non-serious AEs or SARs, documentation must begin from day 1 of study treatment and continue until the 30-day follow-up period after treatment is discontinued.

Collected information should be recorded in the CRF for that subject. Please include a description of the event, its severity or toxicity grade, onset and resolved dates (if applicable), and the relationship to the study drug. Documentation should occur at least monthly.

8.3 SAEs or Serious SARs

8.3.1 Timing

After informed consent but prior to initiation of study medications, only SAEs caused by a protocol-mandated intervention will be collected (e.g. SAEs related to invasive procedures such as biopsies, medication washout).

For any other experience or condition that meets the definition of an SAE or a serious SAR, recording of the event must begin from day 1 of study treatment and continue through the 30-day follow-up period after treatment is discontinued.

8.3.2 Documentation and Notification

SAEs or Serious SARs must be recorded in the SAE console within OnCore® for that subject within 24 hours of learning of its occurrence.

8.3.3 Reporting

IRB Reporting Requirements:

UNC**:**

The UNC-IRB will be notified of all SAEs that qualify as an Unanticipated Problem as per the UNC IRB Policies using the IRB's web-based reporting system within 7 days of the Investigator becoming aware of the problem.

Affiliate sites:

For affiliate sites using a local IRB of record, please submit adverse events per local IRB policy.

For affiliate sites relying on the UNC-IRB, an aggregated list of all SAEs will be submitted to the UNC IRB annually at the time of study renewal according to the UNC IRB policies and procedures. In addition, any SAEs that qualify as an Unanticipated Problem will be entered into OnCore® by the affiliate site and reported to the UNC IRB by the Multicenter Regulatory Associate using the IRB's web-based reporting system within 7 days of the Investigator becoming aware of the problem.

Pregnancy

Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female subject occurring while the subject is on study, or within 28 days of the subject's last dose of study should be recorded as SAEs. The subject is to be discontinued immediately from the study.

For Affiliate sites, the pregnancy, suspected pregnancy, or positive pregnancy test must be reported to the Multicenter Project Manager immediately (within 24 hours) via email (preferred) or facsimile to 919-966-4300. The Multicenter Project Manager will then report the event to the Funding Source (see requirements below).

The female subject should be referred to an obstetrician-gynecologist, preferably one experienced in reproductive toxicity for further evaluation and counseling.

The Investigator will follow the female subject until completion of the pregnancy, and must document the outcome of the pregnancy (either normal or abnormal outcome) and report the condition of the fetus or newborn to the UNC Project Manager. If the outcome of the pregnancy was abnormal (e.g., spontaneous or therapeutic abortion), the Investigator should report the abnormal outcome as an AE. If the abnormal outcome meets any of the serious criteria, it must be reported as an SAE.

Amgen Reporting Requirements:

Please refer to the table below regarding policy for reporting SUSARs, and other safety related data to Amgen.

Safety Data	Timeframe for Submission to AMGEN
Suspected Unexpected Serious Adverse Reaction (SUSARs)	Sent to AMGEN at time of regulatory submission to FDA
Pregnancy/Lactation	Within 10 days of Sponsor awareness

To report suspected adverse reactions, contract Amgen at 1-855-465-9442) and FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

Aggregate Reports Required for All Amgen Studies:

Safety Data	Timeframe for submission to Amgen
Annual Safety Report (e.g., EU Clinical Trial Directive [CTD] Annual Safety Report, and US IND Annual Report)	Annually
Other Aggregate Analyses (any report containing safety data generated during the course of a study)	At time of ISS sponsor submission to any body governing research conduct (eg, RA, IRB, etc)
Final (End of Study Report, including:	At time of ISS sponsor submission to any body governing research conduct (eg, RA,

Safety Data	Timeframe for submission to Amgen
• Unblinding data for blinded studies • Reports of unauthorized use of a marketed product	IRB, etc.) but not later than 1 calendar year of study completion

Merck Reporting Requirements:

To report suspected adverse reactions, contact Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., at 1-877- 888-4231 and FDA at 1-800-FDA-1088 or www.fda.gov/medwatch

8.3.3.1 Events of Clinical Interest

Selected SAEs are also known as Events of Clinical Interest (ECI)

ECIs for this trial include:

1. ONJ
2. Life-threatening hypocalcemia
3. Serious infection attributed to denosumab
4. Autoimmune hepatitis or an elevated AST or ALT lab value that is greater than or equal to 3 × the ULN and an elevated total bilirubin lab value that is greater than or equal to 2 × the ULN and, at the same time, an alkaline phosphatase lab value that is <2 × the ULN, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

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

Although not considered an event of clinical interest, subjects receiving denosumab and PD-1 inhibitor in combination may have an increased risk for autoimmune colitis.

If an investigator deems that an event is both a serious SAR and unexpected based on the extensive knowledge of AE attributed to PD-1 inhibitor alone and denosumab alone (e.g. stroke, serious infusion-related reaction, myocardial infarction), it must also (in addition to OnCore®) be recorded on the MedWatch Form 3500A, as per 21 CFR 312.32. Unexpected AE or adverse reaction refers to an event or reaction that is not listed in the investigator's brochure or is not listed at the specificity or severity that has been observed; or if an investigator's brochure is not required or available, is not consistent with the risk information described in the general investigation plan or elsewhere in the current IND application.

Process

If the sponsor deems that an event is both a serious adverse reaction AND unexpected, it must also (in addition to OnCore®) be recorded on the MedWatch Form 3500A as per 21

CFR 312.32. The MedWatch form should be submitted on MedWatch by the study coordinator.

The MedWatch 3500A form can be accessed at: http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm.

8.4 Data and Safety Monitoring Plan

The Principal Investigator will provide continuous monitoring of subject safety in this trial with periodic reporting to the UNC LCCC Data and Safety Monitoring Committee (DSMC). As discussed in detail in the Statistical Considerations Section 8.1, continuous toxicity monitoring will be applied to the incidence of SAE attributed to PD-1 inhibitor alone (colitis, hepatitis, hypophysitis, pneumonitis, T1DM) as well as to denosumab alone (hypocalcemia, hypophosphatemia, osteomyelitis, ONJ). Any trends regarding significantly higher incidence for any of these AEs compared to their previously described incidence with either drug alone will imply significant interaction between denosumab and PD-1 inhibitor and depending on the severity and onset, it may lead to discontinuation of denosumab and/or changes in denosumab scheduling.

Meetings/teleconferences will be held at a frequency dependent on study accrual, and in consultation with the study Biostatistician (Dr. Anastasia Ivanova). These meetings will include the investigators from all three sites (Drs Moschos, Shirai, and Hoimes) as well as protocol nurses, clinical research associates, regulatory associates, data managers, and any other relevant personnel the principal investigators may deem appropriate. At these meetings, the research team will discuss all issues relevant to study progress, including enrollment, safety, regulatory, data collection, etc.

The team will produce summaries or minutes of these meetings. These summaries will be available for inspection when requested by any of the regulatory bodies charged with the safety of human subjects and the integrity of data including, but not limited to, the oversight Office of Human Research Ethics (OHRE) Biomedical IRB, the Oncology Protocol Review Committee (PRC) or the North Carolina TraCS Institute Data and Safety Monitoring Board (DSMB).

The UNC LCCC Data and Safety Monitoring Committee (DSMC) will review the study on a regular (quarterly to annually) basis, with the frequency of review based on risk and complexity as determined by the UNC Protocol Review Committee. The UNC PI will be responsible for submitting the following information for review: 1) safety and accrual data, including the number of subjects treated; 2) significant developments reported in the literature that may affect the safety of participants or the ethics of the study; 3) preliminary response data; and 4) summaries of team meetings that have occurred since the last report. Findings of the DSMC review will be disseminated by memo to the UNC PI, PRC, and the UNC IRB and DSMB.

9.0 STATISTICAL CONSIDERATIONS

This is a phase II study of two FDA-approved drugs, one of which is standard of care for metastatic melanoma (e.g. pembrolizumab or nivolumab) whereas the other is FDA-approved for a specific condition (e.g. bone metastases), irrespective of the cancer type. We believe that a dedicated phase I study is not required and that a phase II study using those two drugs at the FDA-approved doses will be able to systematically investigate both safety and efficacy of this treatment combination.

9.1 Study Design/Study Endpoints

All subjects will receive nivolumab (or pembrolizumab) plus denosumab for up to year. Drugs will be sequentially introduced, such that correlative analysis can be performed to investigate the mechanistic (direct antitumor vs. indirect immunomodulatory effect) and pharmacodynamic (target tissue saturation studies) effect of denosumab alone and in combination with nivolumab (or pembrolizumab) by performing serial peripheral blood collections (up to 5 timepoints) and tumor collection (up to 3 time points). Assessment of response to the treatment combination will be assessed at week 16 and then every 12 weeks of nivolumab (or pembrolizumab) treatment for up to 1-year.

9.1.1 Definitions

Evaluable subjects are those who complete all activities by at least day 22, namely: (a) they have received the two denosumab injections on day 1 and day 8, (b) they have received the first nivolumab (or pembrolizumab) infusion on day 22, and (c) they have completed day 22 mandatory tumor biopsy. The rationale for this is to answer the co-primary translational endpoints regarding denosumab-alone immunomodulatory effects in peripheral blood and tumor tissue with sufficient power versus denosumab and nivolumab (or pembrolizumab) in combination. Accruing the projected number of subjects along with baseline and day 22 peripheral blood and tumor tissue endpoints is considered one of the "translational successes" of this study.

SAEs are grade 3 or higher AE, irrespective of attribution to study drug(s).

PFS is measured from the date of enrollment on study to the date of documented progression. The rate of subjects who are progression-free will be specifically calculated at 6 months from study entry.

OS is measured from the date of enrollment on study to the recorded date of death. The rate of subjects who are alive at 1-year will be specifically calculated for the study.

9.1.2 Correlative Studies

This study is unusual for "traditional" phase II cancer trials in that the primary endpoints are translational correlatives and not a clinical efficacy endpoint. Correlative analysis relies on the collection of peripheral blood and tumor tissue on which power size calculations are based (see Section 8.2, Sample Size and

Accrual). Specific data analysis plans for the correlative studies are presented in Section 8.3.

9.1.3 Toxicity Assessment

Toxicity assessment is an important secondary endpoint, because toxicity from concurrent administration of these two drugs has not been systematically addressed, despite the fact that denosumab and nivolumab (or pembrolizumab) are frequently co-administered in community medical oncology practice, in particular for subjects with bone metastases from metastatic melanoma. Given the presumed mechanism of action of denosumab, namely increased thymic output of autoreactive T cells, we cannot exclude the possibility that the incidence of several immune-mediated SAE attributed to nivolumab (or pembrolizumab) may actually be increased due to concurrent administration of denosumab. We will therefore apply continuous toxicity monitoring focusing on the incidence of SAE attributed to nivolumab (or pembrolizumab) and with toxicity boundary rules that should not by any chance occur more frequently that the well-established immune-mediated effects from the concurrent ipilimumab-nivolumab administration. More specifically, from the large phase III study of pembrolizumab versus ipilimumab in subjects with metastatic melanoma the most significant SAEs have been colitis (2.5%), hepatitis (1.8%), hypophysitis (0.4%), pneumonitis (0.4%), type I diabetes mellitus (0.4%)[10]. In the phase III study of subjects with metastatic melanoma who were randomized to nivolumab alone, versus ipilimumab alone, versus nivolumab plus ipilimumab, the following frequent SAE were observed in the concurrent ipilimumab-nivolumab arm. Diarrhea (9.3%), fatigue (4.2%), rash (4.8%), ALT increase (8.3%), AST increase (6.1%), colitis (7.7%). In addition, a total of 29.4% patients who were treated with ipilimumab-nivolumab were discontinued from the study due to treatment-related AE [9]. If the incidence of any of the above stated SAE that were observed in the ipilimumab-nivolumab trial is reached at any point in our trial, we will consider that the denosumab and PD-1 inhibitor combination is toxic, and we will therefore discontinue denosumab administration; instead patients will only be treated with PD-1 inhibitor alone. Trial can also permanently stop at any point during the study, if there is evidence that the probability for a patient of prematurely stopping denosumab due to treatment-related AEs is 30% or higher.

In the large phase II study of denosumab alone in patients with giant cell tumor of the bone the incidence of SAE attributed to denosumab alone was 0% for grade ≥3 hypocalcemia, 3% for hypophosphatemia, 1% for osteomyelitis, and 1% for osteonecrosis of the jaw [70]. These four AEs will be monitored throughout our study and toxicity stopping rules will apply. Denosumab alone can be discontinued within a particular patient if such SAE attributed to denosumab alone may occur and cannot be treated with calcium supplementation. However, denosumab (and therefore the study overall) can prematurely stop if the incidence of any of the four stated SAE attributed to denosumab exceeds 10%. In addition, these four distinct SAEs attributed to denosumab will be monitored throughout the study.

Denosumab will be discontinued if excessive numbers of SAEs known to be associated with denosumab are seen, that is, if the number of SAEs is equal to or exceeds b_n out of n subjects (see table). This is a Pocock-type stopping boundary that yields the probability of crossing the boundary at most 0.1 when the SAE rate is equal to the acceptable rate of 0.1.

Number of subjects, n	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Boundary, b_n	-	2	2	3	3	3	3	3	4	4	4	4	4	5	5	5	5	5	5	5
Number of subjects, n	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Boundary, b_n	6	6	6	6	6	6	7	7												

Nivolumab (or pembrolizumab) will be discontinued if excessive numbers of SAEs known to be associated with nivolumab (or pembrolizumab) are seen, that is, if the number of SAEs is equal to or exceeds b_n out of n subjects (see table). This is a Pocock-type stopping boundary that yields the probability of crossing the boundary at most 0.2 when the SAE rate is equal to the acceptable rate of 0.1.

Number of subjects, n	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Boundary, b_n	-	-	3	3	4	4	4	5	5	5	6	6	6	7	7	7	7	8	8	8
Number of subjects, n	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Boundary, b_n	9	9	9	9	10	10	10	11												

The trial will be halted if there is evidence that the rate of SAEs that have never been previously described with any drug alone and are attributed to the denosumab and nivolumab (or pembrolizumab) combination (e.g. stroke, hemorrhage, myocardial infarction) is 5% or higher.

9.1.4 Clinical Efficacy Endpoints

Clinical efficacy endpoints constitute a secondary endpoint and involves assessment of antitumor response, 6-month PFS and 1-year OS rate, which have all been important early surrogate endpoints of clinical benefit in previously conducted clinical trials using PD-1/PD-L1 inhibitors in melanoma[9,10,71]. Despite the fact that our study is underpowered to investigate clinical benefit based on these clinical endpoints, we felt that the most credible endpoint, namely 1-year OS, should be adequately computed, which is the reason why the study will last up to a year, unless subjects progress or develop toxicity.

9.2 Sample Size and Accrual

Our goal is to enroll 25 evaluable subjects; therefore, we expect to enroll about 28 subjects. The immune-mediated mechanism of action of denosumab alone (co-primary translational endpoints) versus the combination of denosumab and nivolumab (or pembrolizumab) will be evaluated in blood using multiparameter flow cytometry and ELISA assays and in tumor samples through IHC and IF. Blood samples will be collected at baseline (week 0) and at weeks 3, 16, 28 and 40. Tumor samples will be collected at baseline and weeks 3 and 16. For each subject and for each of these measurements, we will compute the difference from the corresponding baseline, week 0 for denosumab alone and week 3 for the combination of denosumab and nivolumab (or pembrolizumab). The differences will be tested with one-sample *t*-tests at one-sided 0.05 level each. No adjustment for multiple testing will be performed. With the sample size of 25 we will be able to detect a significant change from baseline with power 80% if the true effect size is 0.51. This is in line with previously published numbers [65,72-74].

9.3 Data Analysis Plans

For each assay in the serum or peripheral blood there will be between 3 to 5 time points (baseline, day 22, week 16, week 28, week 40), depending on whether subjects continue to have clinical benefit to the denosumab-anti-PD-1 agent combination beyond the week 16 time-point of first assessment of antitumor and throughout the year-long duration of the study. Descriptive statistics will be applied. We will plot means and standard errors for each time point; logarithmic or other transformation may be applied for data normalization, if necessary. Particular emphasis will be put on assessment of changes attributed to denosumab alone (comparison between the day 22 and baseline) as well as changes attributed to denosumab-anti-PD-1 agent combination (comparison between the week 16 and baseline). Serum measurements of free RANKL and OPG levels are continuous variables (from zero to infinity, in mol/L) whereas peripheral blood immune cell subsets described in Section 1.10.1.2 (items 1, 2, 5, 6, 7, and 8) are percentages of cell subset (from 0 to 100). CD4+ and CD8+ sjTREC (item 3 in Section 1.10.1.2) are continuous variables (from zero to infinity in number of cells per 5 x 10^7 PBMCs). All the above analyses will be performed by Dr. Anastasia Ivanova.

TCR repertoire analysis will be performed by Dr. Benjamin Vincent, whose laboratory has developed and published methods for assessment of clonality and diversity of the immune receptor repertoire in cancer [75].

Tumor tissues will be collected at baseline and day 22. Week 16 tumor tissue collection is optional. For all tumor tissue studies, which are described at length in section 1.10.2, quantitative computer imaging analysis will be applied using commercially available computer imaging software (Definiens Tissue Studio and Visiopharma). Descriptive statistics will be applied. We will plot means and standard errors for each time point; logarithmic or other transformation may be applied for data normalization, if necessary. The differences from corresponding

baseline for each measurement and at for each time point will be tested with one-sample *t*-tests at one-sided 0.05 level each.

AEs of the denosumab-anti-PD-1 agent combination will be tabulated and 95% confidence interval will be computed. The overall RR (CR + PR) at 16 weeks will be estimated as a proportion of subjects with RR and 95% confidence interval will be computed. PFS at 6 months and OS at 1 year will be estimated using Kaplan-Meier method.

10.0 STUDY MANAGEMENT

10.1 Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the subject will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the subject and the investigator is assured that the subject understands the implications of participating in the study, the subject will be asked to give consent to participate in the study by signing an IRB-approved consent form.

Prior to a subject's participation in the trial, the written informed consent form should be signed and personally dated by the subject and by the person who conducted the informed consent discussion.

10.2 Required Documentation

Before the study can be initiated at any site, the following documentation must be provided to the CPO at UNC-CH.

- A copy of the official IRB approval letter for the protocol and informed consent.
- IRB membership list.
- CVs and medical licensure for the principal investigator and any sub-investigators who will be involved in the study.
- Financial Disclosures
- Form FDA 1572 appropriately filled out and signed with appropriate documentation
- CAP and CLIA Laboratory certification numbers and institution lab normal values.
- Executed clinical research contract.

10.3 Registration Procedures

All subjects must be registered with the LCCC CPO Multicenter Office at the University of North Carolina before enrollment to study. To register a subject call the Multicenter office at 919-966-7359 Monday-Friday 8:30 am – 5:00 pm EST. Scan and email the UNC Project Manager (CPOMultiCenter@med.unc.edu; preferred) or fax (919-966-4300) the registration form, signed informed consents, signed eligibility form and all source

documents to confirm eligibility. When sending registration request with eligibility documentation, please allow 24 hours for source to be reviewed.

10.4 Data Management and Monitoring/Auditing

The CPO Multicenter Office of the UNC LCCC will serve as the coordinating center for this trial. Data will be collected through a web based clinical research platform, OnCore®. Other study institutions will be given a password to directly enter their own data onto the web site via electronic case report forms (eCRFs). Multicenter personnel will coordinate and manage data for quality control assurance and integrity.

All data will be collected and entered into OnCore® by the affiliate study teams at participating institutions. The investigators at each site will allow monitors to review all source documents supporting data entered into OnCore®. The Multicenter Data Coordinator can be reached at 919-843-2742 or 1-877-668-0683.

All data will be monitored and source data will be verified on selected subjects. Queries will be issued on an ongoing basis on all subjects. Participating sites should respond to data queries within 14 days of receipt. The LCCC compliance committee or their designee will audit trial sites every twelve months while still enrolling or subjects are still on treatment. Participating sites must send source and regulatory documents to LCCC upon request, for remote monitoring and/or audit review.

10.5 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study subject requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

10.5.1 Emergency Modifications

UNC and affiliate investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior UNC or their respective institution's IRB/IEC approval/favorable opinion.

For any such emergency modification implemented, a UNC-CH IRB modification form must be completed by UNC Research Personnel within five (5) business days of making the change.

<u>For Institutions Relying on UNC's IRB:</u>
For any such emergency modification implemented, a UNC IRB modification form must be completed by UNC Research Personnel within five (5) business days of making the change.

<u>For Institutions Relying on Their Own IRB:</u>
For Affiliate investigators relying on their own institution's IRB, as soon as possible after the modification has been made, the implemented deviation or change and the reasons for it should be submitted to:

- To UNC Principal Investigator for agreement
- The Affiliate institution's IRB for review and approval. (Once IRB's response is received, this should be forwarded to the Multicenter Regulatory Associate).

10.5.2 Single Subject Exceptions

Eligibility single subject exceptions are not permitted for Lineberger Comprehensive Cancer Center Investigator Initiated Trials under any circumstances. Other types of single subject exceptions may be allowed if proper regulatory review has been completed in accordance with Lineberger Comprehensive Cancer Center's Single Subject Exceptions Policy.

10.5.3 Other Protocol Deviations/Violations

According to UNC's IRB, a protocol deviation is any unplanned variance from an IRB approved protocol that:

- Is generally noted or recognized after it occurs
- Has no substantive effect on the risks to research participants
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected
- Did not result from willful or knowing misconduct on the part of the investigator(s).

An unplanned protocol variance is considered a violation if the variance meets any of the following criteria:

- Has harmed or increased the risk of harm to one or more research participants.
- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies.

If a deviation or violation occurs, please follow the guidelines below.

<u>For Institutions Relying on UNC's IRB</u>:
Protocol Deviations: UNC or Affiliate personnel will record the deviation in OnCore®, and report to any sponsor or data and safety monitoring committee in accordance with their policies. Deviations should be summarized and reported to the IRB at the time of continuing review.

Protocol Violations: Violations should be reported by UNC personnel within one (1) week of the investigator becoming aware of the event using the same IRB online mechanism used to report Unanticipated Problems.

<u>For Institutions Relying on Their Own IRB:</u>
In addition to adhering to the policies regarding protocol compliance set forth by your institution's IRB, the following is also required:

Protocol Deviations: In the event a deviation from protocol procedures is identified, record the deviation in OnCore®.

Protocol Violations: Any protocol violation that occurs must be reported to your IRB per institutional policies and reported to the UNC Multicenter Project Manager within 5 days. UNC will determine if the violation affects the safety of the subject and integrity of the data. Once your institution's IRB response is received, please forward to the Multicenter Regulatory Associate.

Unanticipated Problems:
Any events that meet the criteria for "Unanticipated Problems" as defined by UNC's IRB must be reported by the Study Coordinator using the IRB's web-based reporting system.

Affiliate Sites:
Any events that meet the criteria for "Unanticipated Problems (UPs)" as defined by UNC's IRB must also be reported to the UNC Multicenter Project Manager. The Multicenter Regulatory Associate will report the event to the UNC IRB using the IRB's web-based reporting system. Examples of such UPs include a lost or stolen laptop computer that contains sensitive study information.

10.6 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator at UNC-CH. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the subject, a revised consent form might be required.

For Institutions Relying on UNC's IRB:
The written amendment, and if required the amended consent form, must be sent to UNC-CH's IRB for approval prior to implementation.

For Institutions Relying on Their Own IRB:
Investigators must submit the amendment to their institution's IRB for approval. For multicenter studies, any multicenter site must submit their informed consent revisions to the Multicenter Regulatory Associate prior to submission to their IRB.
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10.7 Record Retention

Study documentation includes all eCRFs, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed subject consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the Principal Investigator. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

10.8 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study subjects. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered into the eCRFs. Periodically, monitoring visits will be conducted, and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all eCRFs will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

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12.0 APPENDICES

12.1 Appendix A. ECOG Performance Status

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

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

12.2 Appendix B. Time and Events Table (For Subjects Receiving Pembrolizumab)

(**Note**: Week 1 includes days 1 through 7; week 2 includes days 8 through 14; week 3 includes days 15 through 21, etc.)

Assessments[1]	**Pre-Study[1]**	**Study Treatment (cycle 1 = 21 days)**									**End of Tx**	**Long-term follow up[21]**
		C1D1	**C1D8**	**C2D1**	**C3D1**	**C3D15**	**C4D1**	**C5D1**	**C6D1**	**C7D1 – C17D1**		
	Day-21 to -0	**Wk1 D1±1d**	**Wk2 D8±1d**	**Wk4 D22±2d**	**Wk7 D43±3d**	**Wk9 D56±7d**	**Wk10 D64±7d**	**Wk13 D85±7d**	**Wk16 D106±7d**	***Wks17-49[20] (Wks22/28/34/40/46±2wks)**	**Wk52**	
Informed Consent	×											
History, PE[2]	×	×		×	×		×		×	×	×	×
Serum Pregnancy Test	×[3]											
ECOG Performance Status	×	×[4]		×	×		×		×	×	×	
Concomitant meds review	×	×	Throughout the study								×	
CBC with differential[5]	×	×[4]		×	×		×		×	×	×	
Serum chemistries[6]	×	×[4,6]		×	×		×	×	×	×	×	
Ca+, Mg+, phosphorus[7]			×			×				×[20]		
Endocrine tests[8]	×						×		×	×[20]	×	
Urinalysis	×	×[4]							×	×	×	
Toxicity assessment[9]	×	×	×	×	×	×	×	×	×	×	×	
Brain MRI[10]	×											
Whole body CT[11]	×								×	×[11]	×[11]	
Denosumab[12]		×	×	×		×		×	×	×[20]		
Pembrolizumab[13]				×	×		×	×	×	×[13, 20]	×[14]	
Peripheral blood mononuclear cells[15]		×		×					×	×[15,20]	×	
Archival tissue (baseline) / Tumor Biopsies[16]	×[17]			×[18]					×[19]			
Dental examination	×											

Footnotes to Time and Events Table

1. All screening labs to be obtained within 72 hours prior to registration. All study visits should be performed within ± 1 day for the first two weeks of study therapy. For the Day 21 visit the window is ± 2 days, then ± 3 days for assessments scheduled on Day 28 (week 5) through Day 42 (Week 7) and then ± 7 days for all subsequent visits.

2. Complete history/physical at baseline only, thereafter focused history/physical exam on symptoms/toxicity.

3. Serum pregnancy test should be performed within 72 hours prior to the first dose of denosumab

4. All screening labs to be obtained within 72 hours prior to registration.

5. Will be performed at baseline and every other pembrolizumab infusion starting from day 21, as per standard of care.

6. Include sodium, potassium, chloride, CO_2, BUN, creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase, magnesium, phosphorus, LDH, amylase and lipase. All listed serum chemistries will be performed before each pembrolizumab infusion as per standard of care.

7. On the days of denosumab injections that do not coincide with pembrolizumab infusions, calcium, magnesium, and phosphorus will be performed immediately before denosumab injections.

8. Include cortisol levels, free T4 (fT_4)/TSH, prolactin (women only), and testosterone (males only).

9. Toxicity assessments per NCI-CTCAE version 5.0.

10. Is performed at baseline; on future visits only if clinically indicated, as per standard of care.

11. Includes CT of the neck (if applicable), chest, abdomen, pelvis with IV contrast. Scans are performed at baseline, week 16, and then every 12 weeks thereafter (i.e., weeks 28, 40, and 52). Scans may be performed within ±7 days of the scheduled visit.

12. Denosumab will be administered s.c. as a flat dose, 120 mg every 4 weeks, as per FDA-approved dose, for up to a year until intolerable toxicity or disease progression occurs. An extra 120 mg loading dose of denosumab will be administered on day 8. See Dosing schema in section 4.2.1. Denosumab will not be continued beyond the one-year duration of the trial, irrespective of treatment response.

13. Pembrolizumab will be administered every 3 weeks, 200 mg, for up to a year, as per FDA-approved dose starting on D21 of the study until intolerable toxicity or disease progression occurs. See Dosing schema in section 4.2.1.

14. Following completion of the trial at 1 year and on condition that there is no disease progression, pembrolizumab can be continued at the opinion of the investigator, as per standard of care guidelines.

15. PBMCs will be isolated from peripheral blood (approximately 100 mL at each time point) using the Ficoll isolation technique; an aliquot will be used for multiparameter flow cytometry, another for immune receptor repertoire analysis, and another for sjTCR excision cycle analysis. Samples will be collected at baseline on D1/wk1 and on D21/wk4, D106/wk16, wk28, and wk40.

16. Archival tissue or fresh tumor will be collected at baseline and a mandatory excisional biopsy will be performed on D21 of the study. An optional excisional biopsy should be obtained on D106 (week 16). Tumor tissue will be submitted to the Tissue Pathology Lab (TPL) for IHC and multicolor IF.

17. If archived tumor blocks are not available, subject can still be enrolled on condition that he/she has a tumor biopsy prior to study enrollment.

18. Mandatory (excisional, punch or CT-guided).

19. Optional.

20. Following radiographic disease assessment on week 16, subjects will be seen by the investigator on every other pembrolizumab infusion (i.e., 6 weeks on D1 of weeks 22, 28, 34, 40, 46 and at the end of treatment visit at week 52) for safety assessment and on every fourth pembrolizumab infusion (i.e., 12 weeks) for response assessment for up to a year. See sections identified below for details of scheduled assessments.

21. Subjects who have not progressed on pembrolizumab plus denosumab after a year of treatment will be followed every 3 months for up to 2 years (i.e. a total of 3 years since study initiation) with at least phone calls. The following information will be recorded: (a) details regarding systemic treatment for their melanoma that they are currently on (type, response if any, duration on treatment, treatment-related side effects), (b) whether they have progressed (and when) from current PD-1 inhibitor treatment, (c) whether treatment-related adverse events that subjects may have developed during the year of denosumab-pembrolizumab treatment have resolved, and when, (d) whether they are alive or not; if dead, precise date of death. Subjects who have progressed on pembrolizumab plus denosumab within the year of the study duration will be followed every 3 months for up to 3 years since study initiation with at least phone calls. The following information will be recorded: (a) systemic treatment for their melanoma they are currently on (type, response if any, duration on treatment, treatment-related side effects), (b) whether treatment-related AEs that subjects may have developed during the year of denosumab-pembrolizumab treatment have resolved, and when, (c) whether they are alive or not; if dead, precise date of death