LINEBERGER COMPREHENSIVE CANCER CENTER
CLINICAL ONCOLOGY RESEARCH PROGRAM
UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

March 12, 2020

PROTOCOL AMENDMENT # 5

LCCC 1531: Pembrolizumab in Systemic Treatment-Naïve Metastatic Melanoma and Exploration of Use of Baseline ^{11}C-methyl-L-tryptophan (C11-AMT) PET Imaging as a Predictive Imaging Biomarker of Antitumor Response

AMENDMENT INCORPORATES:

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

The motivations for amending the protocol are summarized below:

The main purpose of this amendment is to increase accrual by two additional subjects. Additionally, administrative edits to update items related to serious adverse events and serious suspected adverse reactions reporting and mechanical edits.

Editorial/Administrative Changes

1. Section 7.3.3: Removed GemCRIS as a form to use and added "Multicenter' staff designation where appropriate.
2. Mechanical editing where appropriate.

Scientific Changes

1. Sections 1.1, 4.0, and 8.2: Edits made to the number of subjects to accrue.

The attached version dated March 12, 2020 incorporates the above revisions

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March 12, 2020

Protocol Amendment # 4

LCCC 1531: Pembrolizumab in Systemic Treatment-Naïve Metastatic Melanoma and Exploration of Use of Baseline ^{11}C-methyl-L-tryptophan (C11-AMT) PET Imaging as a Predictive Imaging Biomarker of Antitumor Response

AMENDMENT INCORPORATES:

X Editorial, administrative changes
X Scientific changes (IRB approval)
Methodology changes (IRB approval)
Eligibility Changes (IRB approval)
The motivations for amending the protocol are summarized below:

- Clarify Eligibility Criteria #5
- Modify timeframe for PET/CT imaging

Scientific Changes

2. Section 3.1 clarifies that all archival tissue samples will be considered for tissue analysis.
3. Section 4.1.2 updated with modified timeframe C11-AMT images.

The attached version dated March 31, 2018 incorporates the above revisions

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March 12, 2020

PROTOCOL AMENDMENT # 3

LCCC 1531: Pembrolizumab in Systemic Treatment-Naïve Metastatic Melanoma and Exploration of Use of Baseline ^{11}C-methyl-L-tryptophan (C11-AMT) PET Imaging as a Predictive Imaging Biomarker of Antitumor Response

AMENDMENT INCORPORATES:

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

The motivations for amending the protocol are summarized below:

- The addition of multicenter language to allow the participation of affiliate sites
- Clarify that participants from affiliate sites must come to UNC-CH for their C-11AMT PET scan

Administrative/Editorial

4. Title Page: Updated version date and amendment number; added contact information for UNCCN Project Manager
5. Headers: Updated version date and amendment number
6. Section 4.1.2: Updated to clarify that participants from affiliate sites must come to UNC-CH for their C-11AMT PET scan
7. Section 6.0: Updated to include appropriate multicenter language
8. Section 7.3.3: Updated to include appropriate multicenter language
9. Section 9.3: Updated to include appropriate multicenter language
10. Section 9.4: Updated to include appropriate multicenter language
11. Section 9.5.1: Updated to include appropriate multicenter language
12. Section 9.5.2: Updated to include appropriate multicenter language and single subject exception policy updated per UNC IRB Policy
13. Section 9.5.3: Updated to include appropriate multicenter language
14. Section 9.6: Updated to include appropriate multicenter language
15. Section 9.8: Updated to include appropriate multicenter language

Scientific Changes

16. Section 4.1.2 updated with additional details on methodology used for acquiring 11C-AMT images.

The attached version dated March2, 2018 incorporates the above revisions

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March 12, 2020

PROTOCOL AMENDMENT # 2

LCCC 1531: Pembrolizumab in Systemic Treatment-Naïve Metastatic Melanoma and Exploration of Use of Baseline ^{11}C-methyl-L-tryptophan (C11-AMT) PET Imaging as a Predictive Imaging Biomarker of Antitumor Response

AMENDMENT INCORPORATES:

X Editorial, administrative changes

X Scientific changes (IRB approval)

X Therapy changes (IRB approval)

X Eligibility Changes (IRB approval)

The motivations for amending the protocol are summarized below:

- Clarify that the intended dose of 11C-methyl-L-tryptophan (C11-AMT) is 6.5 mCi +/- 10%, not to exceed 7.15 mCi
- Clarify timelines and sequence of the three baseline tests (e.g. whole body FDG PET and/or CT scan with IV contrast, C11-AMT PET imaging, fresh tumor biopsy collection)
- Update the protocol to include patients with ocular and mucosal melanoma, as well as melanoma of unknown primary origin
- Update the protocol to include patients with intrathoracic-only melanoma and perform an intrathoracic biopsy by interventional pulmonology
- Update the protocol to include patients with a single brain metastasis without the requirement for stereotactic radiosurgery
- Update the protocol to include patients who have received adjuvant ipilimumab or adjuvant high dose interferon alpha 2b at least 30 days prior to C11-AMT imaging
- Allow prior radiographic studies without the need to repeat a second whole body FDG PET scan and/or brain MRI within less than 28 days from the previous one
- Revise information regarding pembrolizumab in the drug information section and add up to date language in the study management and FDA reporting sections of the protocol

Administrative/Editorial

1. Title page: removed names of Co-investigators and reference to UNC cancer network and updated version date.
2. Version date changed to July 14, 2017 in header and amendment # included in the header.
3. Updated TOC.

4. Study Synopsis Section 1.1 revised to reflect updates to the protocol outlined above regarding timings of baseline and research scans in relation to pembrolizumab dosing.
5. Revised Figure 3 in background section 1.4.1.
6. 1.6 Correlative Studies adds language explaining that the mandatory fresh research tumor biopsy will be used to for correlative tumor tissue research in tissue that has been imaged with all 3 imaging modalities (FDG PET, C11 AMT PET, and CT scan with IV contrast). Also explains the possible results and purpose of the tumor biopsy in correlation to C11-AMT PET scan results.
7. Editorial/clarification changes made to Primary (section 2.1) and secondary objectives (section 2.2)
8. Section 4.0 was revised to clarify language on subjects eligible for the trial.
9. Sections 4.1.1 rewritten to clarify requirements for pretreatment baseline scans.
10. Section 4.1.2 revised and rewritten to clarify requirements for C11-AMT PET scan.
11. The text provided underneath the schema in 4.2 was rewritten to clearly explain the schema and study procedures and treatments depicted in the schema.
12. Section 4.2.1 notes that pembrolizumab will be dosed after screening and pretreatment assessments are completed.
13. Cross references updated in sections 4.3.1.2 and 4.3.1.3 and 4.8
14. Section 4.4. cross reference to exclusion criteria added.
15. Section 4.4.1 Concomitant medications received within 28 days of pembrolizumab through 30 days after the last dose of pembrolizumab should be included in the eCRF.
16. Section 5.1.1 Description of IP revised to account for how pembrolizumab is supplied. Pharmacy manual is referenced for additional information.
17. Section 5.1.5 Edited return and retention language
18. Section 6.2.1 Screening period assessments updated based on changes to Time and Events table and language added to clarify coagulation testing requirements for patients on coumadin
19. Section 6.2.2 Added to account for pretreatment assessments added to the Time and Events table (ie, C11-AMT PET scan and research biopsy)
20. Section 6.3.1 Outlines requirements for C1D1 of pembrolizumab visit and includes removal of Thyroid panel which is collected during screening
21. New section 6.3.2 Added to account for tests/assessments required on D1 of cycles 2-4.
22. Section 6.3.3 End of treatment visit revised with minor edits included
23. Section 6.4 Long term follow up: removed reference to ECI guidance document which is no longer required by MERCK and noted that follow up may entail phone contact or chart review.
24. Section 6.5.2 Added clarifications and requirements for Tumor biopsy
25. Section 6.7 minor editorial change to clarify follow up for PFS and OS

26. Section 6.7.1 minor editorial changes made to CT scan (with IV contrast added) and baseline evaluations for efficacy within 4 weeks of pembrolizumab treatment.
27. Sections 7.3.2, and 7.3.3 revised to include up to date language for reporting of AEs
28. Section 8.0 statistical sections revised to clarify study endpoints, plus minor and major editorial changes included in the protocol
29. Sections 9.2, 9.3, 9.4, 9.5.1, 9.5.3 and 9.8 revised to include appropriate up to date study management language

Eligibility

30. Revised inclusion criterion #3 to broaden eligibility for the study (ie, allows for enrollment of cutaneous, mucosal, ocular, and unknown primary melanoma) and clarify that subjects should have unresectable stage III or distant melanoma. Patients with resectable bulky stage IIIB or stage IIIC melanoma may be entered at the discretion of the Principal Investigator, and provides guideance for investigator discretion.
31. Revised inclusion criterion #5 as biopsy is mandatory but not in the case of a subject with lung metastases. In this case the biopsy is optional due to high risk of pneumothorax.
32. Revised inclusion criterion #8 to clarify prior treatments that are allowed/disallowed for enrollment.
33. Revised inclusion criterion #9 to note that screening labs must be obtained within 14 days of C11-AMT PET scan.
34. Revised inclusion criterion #10 to specify requirement to obtain urine or serum pregnancy test in WOCBP within 14 days of C11-AMT PET scan.
35. Added inclusion criterion #12 to allow for prior therapy with adjuvant high dose interferon which should have been completed at least 30 days prior to patient receiving C11-AMT PET scan in this trial.
36. Inclusion criterion #13 added to specify entry requirements for prior adjuvant therapy with ipilumumab.
37. Exclusion criterion #3 revised to note that immunosuppressive therapy should be completed within 7 days prior to C11-AMT PET scan.
38. Exclusion criterion #6 has been separated into 3 separate criteria to better delineate the separated criteria.
39. Exclusion criterion #7 now specifies completion of prior adjuvant cancer treatments at least 30 days prior to C11-AMT PET scan.
40. Exclusion criterion #9 now allows patients with papillary thyroid cancer concurrent with metastatic melanoma.
41. Exclusion criterion #10 was revised to provide clarifying language on conditions that disallow/allow inclusion of patients with brain metastases.
42. Exclusion criterion #19 excludes patients who have received live vaccine with 14 days of C11-AMT PET scan.

Scientific

43. Adverse events recording globally updated to CTCAE v4.03
44. Section 1.2 added additional rationale and background information in support of this proposed trial.
45. Section 1.3.2 added additional background information on pembrolizumab monotherapy in ocular and metastatic mucosal melanoma, deleted old information on Keynote studies 001 and 002 and noted current flat dosing requirement for pembrolizumab.
46. Section 1.5 text pertaining to Keynote studies 002 and 006 deleted because no longer relevant and clarified parameters that will be correlated with antitumor response to pembrolizumab at 12 weeks (or earlier if patient experiences disease progression earlier).
47. Section 1.6 Correlative studies updated to note that patients with metastatic lung disease may be enrolled in the trial. These patients will undergo optional (not mandatory) biopsy if they agree to it due to the high risk for pneumothorax (up to 20%).
48. Primary Endpoint revised to note that patients must be PD-1 inhibitor-naive and correlative endpoint (previously 2.3.2) moved to the secondary endpoints section.
49. Revised Secondary Endpoints in section 2.4 with respect to scans needed in the study. Removed whole body C11-AMT PET wording (2.4.2 and 2.4.3).
50. Section 4.1.2.3 Text updated to match IND indicated dose of C11 – AMT will be 6.5 mCi +/- 10%, not to exceed 7.15 mCi. Text updated to specify that the radionuclide will not be administered via a central line. Text added to note that short half-life of C11-AMT may not allow for whole body scan and that at least 1 or 2 bed positions will be performed depending on the location of the tumor. The three parameters that must be met for bed positions for C11-AMT PET scanning are also clarified.
51. Section 4.1.3 revised to specify requirements for biopsy collection. The biopsies will be collected after C11-AMT PET imaging.
52. Section 4.2 schema revised to more clearly delineate pretreatment scans allowed in relation to the research scan (C11-AMT PET) and fresh biopsy. These procedures are followed by pembrolizumab dosing and FDG-PET with co-registered CTIVC scan at week 12.
53. Section 6.1 revised the Time and Event table to account for Screening and pretreatment periods (new column) that includes research scan and tumor biopsy collection prior to C1D1 of study treatment with pembrolizumab. This also includes minor edits to assessments listed in study procedures column
    a. Added row to Time and Events table to account for eligibility checklist approval
    b. Revised superscripts in table to account for changes to footnotes
    c. Footnote #1 revised and rewritten to account for length of screening and pretreatment periods and timing of screening procedures in relation to C11-AMT-PET, fresh biopsy and first dose of pembrolizumab, etc.

d. Footnote #2 Further clarification provided on timings for scans and that scans should be completed during the screening and pretreatment periods. The requirements for serum/urine pregnancy testing in WOCBP before the research scan and the first dose of pembrolizumab were clarified.
e. Footnote #3 revised to account for study visit/study treatment allowable visit windows and explain lab testing requirements before starting pembrolizumab therapy on C1D1.
f. Footnote #4 removed language referencing health insurance plan and reference to ECI Guidance document and clarified long term follow up requirements.
g. Footnote #10 specifies requirements for urine/serum pregnancy test in WOCBP prior to C11-AMT PET scan and pembrolizumab dosing on D1C1.
h. Footnote #12 specifies requirements for baseline CT scans with IV contrast coregistered with FDG-PET or C11-AMT PET
i. Footnote #13 specifies field requirements for C11-AMT PET scanning and timing of scan in relation to tumor biopsy
j. Footnote #14 specifies requirements for research biopsy ie, mandatory for all patients except for those with lung disease. In this case, biopsy is optional for patients with lung disease.

Therapy Changes

54. Sections 4.3.1.3 and 5.1.6 Management guidelines and AE information added for SJS, TEN and immune-mediated myocarditis.
55. Section 4.4.2 Prohibited meds do not include episodic use (≤7 days) of systemic corticosteroids for general conditions.
56. Section 4.5 clarified that patients responding to pembrolizumab after 4 cycles of study treatment, may continue pembrolizumab per standard of care (not as part of protocol treatment).
57. Section 4.6 revised to clarify conditions for end of treatment visit.
58. Section 4.7 clarified duration of follow up after patients discontinue study treatment
59. Section 4.9 revised to include updated study withdrawal language
60. Section 5.1.7 revised to clarify contraception requirements in relation to the C11-AMT scan (begin at time of consent) and continue through last dose of pembrolizumab (120 days after last dose of pembrolizumab).

The attached version dated July 14, 2017 incorporates the above revisions

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March 12, 2020

PROTOCOL AMENDMENT # 1

LCCC 1531: Pembrolizumab in Systemic Treatment-Naïve Metastatic Melanoma and Exploration of Use of Baseline ^{11}C-methyl-L-tryptophan (C11-AMT) PET Imaging as a Predictive Imaging Biomarker of Antitumor Response

AMENDMENT INCORPORATES:

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

AMENDMENT RATIONALE AND SUMMARY

The amendment includes the following changes:

- Global - updated version date
- Title Page – deleted Merck reference number
- Title Page – inserted IND number for this study
- Section 3.2 – Subject Exclusion Criteria - clarified unclear wording in exclusion criterion 8 to note that patients with symptomatic brain metastases are excluded regardless of tumor lesion size
- Section 4.2 – Schema – removed statement about study completion for clarification purposes
- Section 4.9 – Removal of Patients from Protocol Therapy - corrected reference to section 4.6
- Section 6.1 – Time and Events Table - added bicarbonate to footnote 6 in the Time and Events Table to include bicarbonate in the serum chemistry panel
- Section 6.1 – Time and Events Table - added footnote 9 to ensure that a serum or urine pregnancy test is collected prior to the AMT-PET scan.
- Section 6.2 – Pre-Study Assessments – clarified that a serum or urine pregnancy test must be taken before the AMT PET scan and added bicarbonate to the serum chemistry panel.
- Section 6.3.1 – Study D1 of Cycles 1-4 – added bicarbonate to the serum chemistry panel
- Section 6.3.2 – End of Treatment (or D1 of Cycle 5) – added bicarbonate to the serum chemistry panel
- Section 6.4 – Long term follow up (90 days after week 12 dose of pembrolizumab) – removed tumor imaging requirement as this is not part of the study
- Section 9.4 – Data Management and Monitoring/Auditing – corrected name of audit committee and added statement regarding monitoring

The attached version dated January 12, 2017 incorporates the above revisions

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March 12, 2020

LCCC 1531: Pembrolizumab in Systemic Treatment-Naïve Metastatic Melanoma and Exploration of Use of Baseline ^{11}C-methyl-L-tryptophan (C11-AMT) PET Imaging as a Predictive Imaging Biomarker of Antitumor Response

[redacted]

[redacted]

[redacted]

Sponsor: Lineberger Comprehensive Cancer Center

Funding Source: Merck, Inc.

[redacted]

Protocol Date/Version #: March 12, 2020, v. Amendment 5

March 12, 2020

LCCC 1531: Pembrolizumab in Systemic Treatment-Naïve Metastatic Melanoma and Exploration of Use of Baseline ^{11}C-methyl-L-tryptophan (AMT) PET Imaging as a Predictive Imaging Biomarker of Antitumor Response

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: _____

Version Date/Version #: March 12, 2020, v. Amendment 5

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1.0 BACKGROUND AND RATIONALE

1.1 Study Synopsis

Therapies that inhibit the PD-1/PD-L1 pathway are being used abundantly in the frontline setting for patients with distant metastatic cutaneous melanoma based on data showing higher and potentially durable antitumor activity compared to CTLA-4 inhibitors, in addition to a better manageable safety profile. In contrast to treatments that target the MAPK pathway, for which identification of BRAFV600 mutations are considered robust biomarkers that predict antitumor response (i.e., they are not significantly influenced by stage, tissue biopsy site, and prior treatments) there are no reliable predictors of response to the PD-1/PD-L1 pathway inhibitors; the existing tumor tissue-based biomarkers, such as the immunohistochemical (IHC) expression of PD-L1, suffer from variability in expression and are influenced by site, stage of disease, and use of prior systemic or local treatments (e.g., radiation). It has been previously shown that preexisting melanoma-infiltrating lymphocytes as well as intra-tumoral PD-L1 expression in cutaneous melanoma predicts response to pembrolizumab. In addition, cutaneous melanomas that contain tumor-infiltrating lymphocytes also exhibit other components that regulate immune response, such as a high number of T regulatory cells and high expression of indoleamine 2,3-dioxygenase (IDO). Given that IDO enzymatic activity has been successfully imaged in humans using ^{11}C-methyl-L-tryptophan (C11-AMT) PET Imaging**,** we hypothesize that C11-AMT-avid melanomas will have a higher incidence of clinical benefit to pembrolizumab whereas C11-AMT-low/absent melanoma will not respond to pembrolizumab.

In accordance with our above stated hypothesis, we plan to conduct a phase II trial of pembrolizumab administered at the FDA-approved 200mg flat dose intravenously every 3 weeks in 27 patients (23 evaluable) with systemic treatment-naïve metastatic melanoma. In addition to standard staging with whole body CT scans with IV contrast at baseline and whole body F^{18}-labelled fluorodeoxyglucose (FDG)-PET, we will also perform co-registered PET/non-contrast CT imaging using C11-AMT PET tracer and will obtain tumor tissue biopsies prior to initiation of pembrolizumab treatment. Antitumor response at 12 weeks (or earlier, if patient progresses), which will be measured by RECIST 1.1 criteria, will be correlated with various C11-AMT PET and/or FDG PET parameters (e.g. standard uptake value, K-value, total tumor metabolic volume, measurement of intra-tumoral and inter-lesional heterogeneity). We postulate that there is an intensity threshold for C11-AMT PET parameters that will correlate with response to pembrolizumab; very low C11-AMT PET activity is expected to be associated with lack of response to pembrolizumab, intermediate C11-AMT PET activity is expected to be associated with partial or complete response, whereas extremely high C11-AMT PET activity may be associated with disease stability because high IDO expression and tryptophan depletion is an important immunosuppressive mechanism. In the latter case, combination treatment with pembrolizumab and IDO inhibitors may be required to achieve an antitumor response.

1.2 Disease Background

Over the last 4 years, 9 drugs were FDA-approved for unresectable metastatic melanoma. Of these 9 drugs, 4 are immunotherapies (peg-interferon α-2b, ipilimumab, pembrolizumab, and nivolumab)[1] four are small molecule inhibitors (vemurafenib, dabrafenib, cobimetinib, and trametinib) [2], and one is intratumorally administered oncolytic virotherapy (talimogene laherparepvec, TVEC). While there are predictive biomarkers of response to small molecule inhibitors (e.g. BRAFV600 mutation status), there are no reliable predictive biomarkers of response to any of the 4 immunotherapies. IHC expression of PD-L1 in tumor tissues may be an acceptable predictive biomarker of response to PD-1/PD-L1 immune checkpoint pathway inhibitors [3-5]. Nevertheless, patients may respond to PD-1/PD-L1 pathway inhibitors even if PD-L1 expression by IHC is absent [6]. In addition, there is no consensus agreement about the "optimal" technical details for this test (e.g., which is the best antibody? what are the "right", high/low cutoffs?). Finally, even if implemented, a tumor tissue-based test performed on archived tumor blocks may neither represent the current stage of disease nor take into account heterogeneity in the expression of PD-L1 across different tissues [7]. In support of the latter, at UNC-CH we have performed IHC analysis of PD-L1 expression in formalin-fixed paraffin-embedded melanoma tumor tissues collected during various stages of melanoma progression in 6 patients who died from brain metastases (12-3, 13-3, 14-1, 14-4, 14-5, 14-6). **Figure 1** shows unpublished results from tumor tissues collected at the time of the original diagnosis of cutaneous melanoma, if available (14-1, 14-4, 14-6), or from metastatic tumor, either before, during, or after systemic treatment, or at death (research warm autopsies). Considerable variability in PD-L1 expression can be seen in relation to stage (primary versus metastases), metastatic organs involved at any given time, or in response to systemic treatments. Subject 14-6, in particular, had variable expression of PD-L1 both during and after pembrolizumab administration. Therefore, a non-invasive *in vivo* biomarker predictive of immune response is appealing, because it can take into account the disease as a whole and is contemporary in relation to the intended systemic treatment(s). Unfortunately, to date existing imaging biomarkers rely on non-specific criteria for assessment of immune response [8], such as [^{18}F]-labeled fluoro-2-deoxy-2-D-glucose ([^{18}F]FDG) or [^{18}F]-labeled 3'-fluoro-3'-deoxy-thymidine ([^{18}F]FLT) [9, 10].

In the case of targeting immune checkpoint pathway inhibitors in melanoma, we know that a subset of cutaneous melanomas bear the T-cell "inflamed" signature [11]. In fact, large translational research efforts in cutaneous melanoma, such as the Cancer Genome Atlas Project (TCGA) have identified that up to 50% of tumors bear a "high immune gene" signature [12]. Preclinical studies regarding "inflamed" melanomas have shown that such tumors are formed as a result of early infiltration by $CD8^+$ T cells followed by melanoma cell-mediated upregulation of IDO, the rate-limiting enzyme of the kynurenine pathway [13], upregulation of PD-L1, and recruitment of $FoxP3^+$ regulatory T cells via a negative feedback compensatory mechanism [14]. Indeed, IDO is highly expressed in cutaneous

melanoma tumor tissues, is positively correlated with an increased number of regulatory T cells and a decreased number of effector CD8+ T cells, and is an adverse prognostic factor [15-17]. Larger (n=330) cohorts of cutaneous melanoma tumor tissues that were subjected to RNAseq analysis as part of TCGA have shown that IDO mRNA expression is significantly higher in "inflamed" melanomas [12]. Using the cBioPortal for Cancer Genomics Tool to analyze a more recent updated tumor tissue cutaneous melanoma cohort (n=470) within TCGA, we have confirmed that a significant ($p < 0.001$) positive correlation in mRNA expression exists between IDO1/IDO2, CD8A/CD8B genes (log odds ratio >2.08 for all paired comparisons between these 4 genes; www.cbioportal.org). In addition, upregulation of IDO constitutes one of the resistance mechanisms used by melanoma cells to counter various immune checkpoint pathway inhibitors, including targets against PD-1, PD-L1, CTLA-4, and GITR [14, 18]. Therefore, **seemingly disparate pathways of immunosuppression, such as upregulation of co-inhibitory immune checkpoint proteins and IDO as well as increased numbers of regulatory T cells, appear to be linked with each other and influence response to targeted therapies**. This implies that if it is feasible to image *any* of the components of 'inflamed' cutaneous melanomas (e.g. PD-1, PD-L1, CD8, IDO, FoxP3), we may be able to non-invasively characterize which melanomas may potentially respond to PD-1/PD-L1 pathway inhibitors.

			CD3	PDL1
12-3 BRAF^{E}	PrM	—		
	Pre-Tx	Skin	3	0
	On BRAFi	Skin	3	1+
		Skin-2	3	1+
	Autopsy	Skin-1	3	1+
		Skin-2	3	1+
		Brain-1	3	0
		Brain-2	3	0
		Brain-3	3	1+
		Brain-4	3	1+
		Lymph node	3	1+
		Soft tissue	3	1+
		Trachea	3	1+
		Lung-1	3	1+
		Lung-2	2	1+
		Liver	3	1+
13-3 BRAF^{K}	PrM	—		
	Pre-Tx	—		
	On BRAFi	—		
	Autopsy	Lung	2	2+
		Liver	2	1+
		Lymph node	3	1+
		Spleen-1	2	1+
		Spleen-2	2	1+
		Brain-1	1	1+
		Brain-2	1	1+
		Brain-3	2	1+
		Brain-4	2	2+
14-1 BRAF^{E}	PrM	Skin	3	0
	Pre-Tx	Soft tissue	3	0
		Lymph node	0	0
		Lung	1	1+
	On BRAFi	Brain	3	1+
	Autopsy	Lung-1	3	1+
		Lung-2	3	1+
		Lung-3	3	1+
		Lymph node	3	0
		Soft tissue-1	3	1+
		Soft tissue-2	3	1+

			CD3	PD-L1
14-4 NRAS^{Q61}	PrM		1	2+
	Pre-Tx	Brain-1	1	2+
		Brain-2	1	2+
	On MEKi	—		
	Autopsy	Pericardium	0	1+
		Skeletal Muscle	0	1+
		Diaphragm	0	1+
		Brain-1	0	0
		Brain-2	1	0
14-5 BRAF^{E}	PrM	—		
	Pre-Tx	Brain	0	1+
	On BRAFi	—		
	Autopsy	Pancreas	1	2+
		Soft tissue	0	1+
		Brain-1	0	2+
		Brain-2	0	3+
		Liver	0	1+
		Lung	0	2+
14-6 BRAF^{E}	PrM-1		1	1+
	PrM-2		1	1+
	Pre-Tx	Lymph node	1	1+
		Skin-1	1	1+
		Skin-2	1	1+
	On TMZ after Ipi	Trachea	1	3+
	On Pembro (early)	Trachea	2	2+
	On Pembro (late)	Trachea	1	1+
	On Pembro (late)	Small bowel	1	0
	Autopsy	Lymph node	0	1+
		Skeletal muscle	0	0
		Thyroid	0	1+
		Lung-1	0	1+
		Brain	0	0
		Liver	1	1+
		Heart	1	2+
		Lung-2	1	2+

Figure 1. Expression of PDL1 and CD3 by immunohistochemistry in formalin-fixed, paraffin-embedded (FFPE) tumor blocks obtained from patients who succumb to metastatic melanoma to the brain. Representative tissue sections have been stained with antibodies against CD3 and PDL1, and counterstained with hematoxylin. Scoring is based on a semiquantitative 0 (no stained cells), 1+ (stained cells ≤1%), 2+ (1% <stained cells <10%), 3+ (≥10%) scale and was performed by Dr. Daniel Zedek (dermatopathology). Abbreviations: BRAF^{E}, BRAFV600E; BRAF^{K}, BRAFV600K; NRAS^{Q61}, NRASQ61R; CD3, T cell marker; Tx, treatment; PrM, primary melanoma; BRAFi, BRAF inhibitors; MEKi, MEK inhibitors; TMZ, temozolomide; Ipi, ipilimumab; Pembro, pembrolizumab.

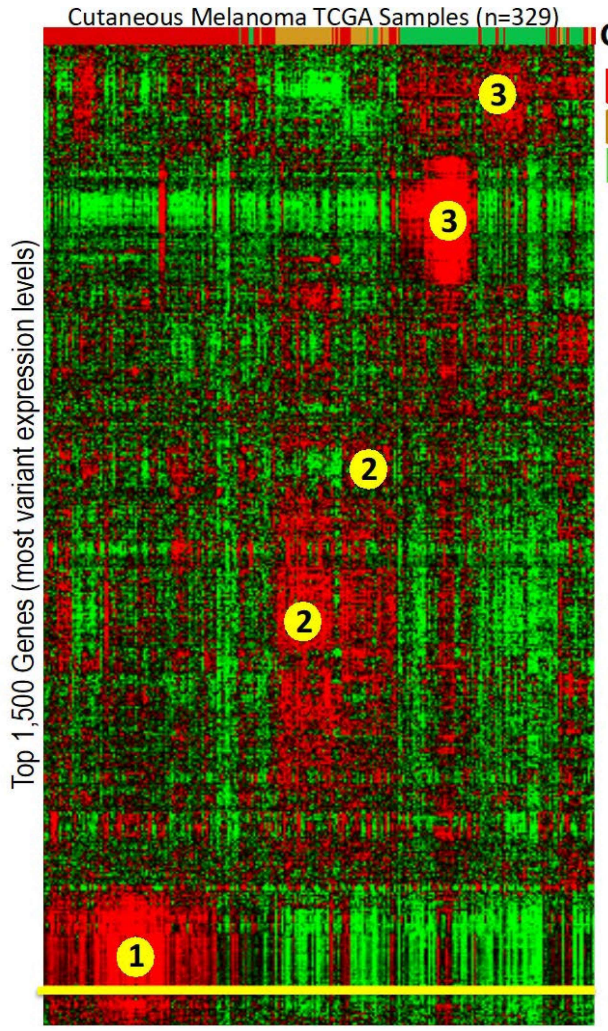

Figure 2. Unsupervised clustering of 329 cutaneous melanoma samples as part of the Cancer Genome Atlas Project. Top: The three cluster group designations [immune-high (red, 1); MITF-low (brown,2), and keratin high (green, 3)]. **Bottom:** heat maps of 1,500 genes with most variant mRNA expression levels (red: upregulated; green: downregulated). Particular sample clusters are shown with numbers. Yellow line corresponds to IDO1 expression across the 329 samples.

Alpha-[^{11}C]methyl-L-tryptophan (C11-AMT) is an amino acid radiotracer that is capable of tracking intra-tumoral tryptophan transport and metabolism via the immunosuppressive kynurenine pathway [19]. Gliomas, both contrast-enhancing and non-enhancing, have increased C11-AMT uptake [20]. In fact, increased C11-AMT trapping in the glioma tissue is associated with upregulation of IDO and correlates with tumor cell infiltration in image-guided stereotactically-acquired tissue samples [21]. More recently, increased C11-AMT uptake by PET imaging was found to be an adverse prognostic factor in high-grade glioma [22]. Similar correlation between IHC expression of components of the IDO pathway [L-type amino acid transporter 1 (LAT1), IDO, and tryptophan hydroxylase (TPH1)] by cancer cells in breast cancer tumor specimens and PET C11-AMT imaging in women with stage II-IV breast cancer has been reported [23]. Unfortunately, none of these molecular imaging studies in gliomas and breast cancer reported any correlations between immune infiltrates and PET C11-AMT imaging. However,

C11-AMT PET imaging may accurately reflect activity of the IDO pathway, which is an inherent component of "inflamed" melanomas.

The ability to *in vivo* image a pathway that, if upregulated within a tumor, is associated with host immune response by restricting the influx of effector $CD8^+$ T cells into the tumor, is important for several reasons. First of all, *in vivo* imaging of pathways may allow us to refine the subset of melanoma cases most likely to respond to PD-1 pathway inhibitors; this is important given the increasing cost of such therapies [24]. Secondly, because response to PD-1 blockade is dependent on the presence of high numbers of pre-existing $CD8^+$ T cells, in addition to expression of PD-1 and PD-L1 by the tumor [25], we would predict that **higher expression and enzymatic activity of IDO would negatively correlate with presence of effector T cells within the tumor** [16]; and thirdly, *in vivo* imaging may **support future studies combining PD-1/PD-L1 pathway inhibitors with either IDO inhibitors** (e.g. INCB024360) **or targets of regulatory T cells (denileukin diftitox).** In support of the latter, a phase Ib study of pembrolizumab in combination with indoximod, an IDO pathway inhibitor, was associated with an overall response rate of 52% and a complete response of 8% in 60 patients with metastatic melanoma[26]. The overall higher response rate of the combination compared to single agent alone (32%), suggests that combined PD-1 and IDO blockade may provide an extra clinical benefit in a patient subgroup yet to be defined. We postulate that this patient subgroup may be those with extremely high baseline C11-AMT PET activity.

1.3 Pembrolizumab (MK-3475)

Pembrolizumab (MK-3475, Keytruda™) is a potent and highly selective intravenous humanized mAb of the immunoglobulin (Ig) G4/kappa isotype 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. Pembrolizumab was approved by the U.S FDA as a front line therapy in metastatic melanoma, metastatic non-small cell lung (NSCLC) cancers that highly express PD-L1, and more recently in recurrent or metastatic squamous cell carcinoma of the head and neck (November 2016).

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades [27]. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies [28-32]. In particular, the presence of $CD8^+$ T-cells and the ratio of $CD8^+$ effector T-cells / $FoxP3^+$ regulatory T-cells correlates with improved prognosis and long-term survival in several solid tumors.

The PD-1/PD-L1 pathway is a major co-inhibitory immune checkpoint pathway that suppresses effector T cell immune response in various cancers. PD-1 is one of the several co-inhibitory immune checkpoint proteins that is normally upregulated

on the cell surface of activated T-cells under physiologic conditions in order to downregulate unwanted or excessive immune responses against exogenous stimuli (viruses, bacteria, cancer), including autoimmune reactions. PD-1 is encoded by the gene PDCD1) and is an Ig superfamily member related to CD28 and CTLA-4. The structure of its murine counterpart reveals that it is a type I transmembrane glycoprotein that contains an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for binding of signaling molecules. The cytoplasmic tail of PD-1 contains two tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules, such as CD3ζ, PKCθ and ZAP70, all of which are involved in the T-cell signaling cascade [33-36]. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4, as both molecules regulate an overlapping set of signaling proteins [13; 14]. PD-1 is expressed on activated lymphocytes including peripheral $CD4^+$ and $CD8^+$ T-cells, B-cells, T regulatory cells and natural killer cells [37, 38].

The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors [39-42]. Both ligands are type I transmembrane receptors containing both IgV- and Ig (constant) C-like domains in the extracellular region and short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectable on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 dampens unwarranted T-cell function in peripheral tissues [43]. Although healthy organs express little (if any) PD-L1, several cancers, including melanoma can overexpress PD-L1 by means of amplification of the gene locus that contains the PD-L1 gene [12]. It is important to emphasize that this mechanism of upregulation is independent from the reactive expression of PD-L1 in response to host immune response, which may have important therapeutic implications [44].

1.3.1 Pre-clinical Studies of Pembrolizumab

Pembrolizumab (MK-3475) strongly enhances T-lymphocyte immune responses in cultured blood cells from healthy human donors, cancer patients, and primates. In *in vitro* assays using T-cells isolated from human donor blood cells, pembrolizumab induces production of interleukin-2 (IL-2), tumor necrosis factor alpha (TNFα), interferon gamma (IFNγ), and activates T cells in subnanomolar EC50 concentrations (i.e. concentrations where 50% of the maximum effect is achieved; ~0.1 - 0.3 nM). It is important to emphasize

that pembrolizumab potentiates existing immune responses only in the presence of antigen and does not nonspecifically activate T-cells.[45]

Using a murine antibody against PD-1 (mDX400), PD-1 blockade has been shown to significantly inhibit tumor growth in a variety of syngeneic murine tumor models. In these experiments in mice, mDX400 treatment is synergistic with chemotherapeutic agents, such as gemcitabine and 5-fluorouracil (5-FU), and combination therapy results in increased complete tumor regression rates *in vivo* [45].

The safety of pembrolizumab was characterized in the one-month repeat-dose toxicity study in cynomolgus monkeys when administered as intravenous (IV) doses of 6, 40 or 200 mg/kg once a week (a total of five doses) and in the 6-month repeat-dose toxicity study in the same species when administered as IV doses of 6, 40 or 200 mg/kg every other week (a total of 12 doses). Pembrolizumab was well tolerated with a systemic exposure [area under the curve (AUC)] of up to ~170,000 µg/day/mL over the course of the one-month study, and with an AUC of up to approximately 67,500 µg/day/mL over the course of the 6-month study. No findings of toxicological significance were observed in either study and the No Observed Adverse Event Level (NOAEL) was ≥200 mg/kg. In addition, no findings of toxicological relevance were observed in the *in vitro* tissue cross-reactivity study using human and cynomolgus monkey tissues.[45]

1.3.2 Summary from Relevant Clinical Studies of Pembrolizumab

In the largest ever conducted disease-specific phase I study of an experimental drug (n=655, KEYNOTE-001) pembrolizumab was administered in various schedules in patients with advanced metastatic melanoma [46, 47]. This phase I study was essentially comprised of 4 different cohorts. The first (n=135) was a study in which patients were treated with 3 different dose schedules; 2mg/kg q3wks, 10mg/kg q3wks, and 10mg/kg q2wks, irrespective of prior treatment with ipilimumab. The second (n=173) was a randomized study of patients who have previously received and have become resistant to ipilimumab (IPI-T), defined as administration of at least 2 ipilimumab doses and equivocal progressive disease within 6 months of first ipilimumab dose; patients were randomized to 2mg/kg q3wks versus 10mg/kg q3wks. The third (n=103) was a randomized study of patients who have never received ipilimumab treatment (IPI naïve; IPI-N). The fourth (n=244) was a randomized study of patients who have been IPI-N and IPI-T to either 10mg/kg q3wks vs. 10mg/kg q2wks. At a cutoff date of April 18, 2015, the median duration of follow-up was 15 months (range 8-29) and 244 patients (37%) were still receiving pembrolizumab. IPI-T (n=342) as opposed to IPI-N (n=313) patients had received a smaller number of pembrolizumab infusions (8 versus 11 doses). No treatment-related deaths were observed in this trial and no differences in grade 3-4 treatment-related events were observed between IPI-T versus IPI-N. The most frequent immune-mediated adverse events

were hypothyroidism (any grade, 7.5%; grade 3-4, 0.2%), hyperthyroidism (2.3%, 0.3%), pneumonitis (2.7%, 0.3%), colitis (1.7%, 1.1%), hepatitis (0.6%, 0.3%), nephritis (0.5%, 0.3%), and uveitis (0.9%, 0%). As of October 18, 2014 and with a median follow-up of 21 months, 581 patients had measurable disease per central RECIST v 1.1 at baseline. In this combined cohort of IPI-T and IPI-N overall response rates were assessed at 33% whereas complete response was 8%. Subgroup analysis revealed that M1b disease, tumor burden less than the median seen in this study, no prior treatment with ipilimumab and normal serum LDH were associated with significantly higher incidence of response rate than 33% whereas prior treatment with BRAF inhibitors, tumor burden higher than the median seen in this study, M1c disease, and elevated serum LDH were associated with significantly lower incidence of response rate than 33%. In this subgroup analysis there were no significant differences in overall response rate between the 2mg/kg q3wk (n=143), 10mg/kg q3wk (n=272), and 10mg/kg q2wks (n=166). The median progression-free survival (PFS) was 4.4 months (PFS rate at 12 months=0.35), whereas the median overall survival (OS) was 22.8 months (OS rate at 12 and 24 months, 0.66 and 0.49)[48].

When pembrolizumab was administered as first-line therapy in patients with advanced cutaneous melanoma, the incidence of complete response and overall response was 13.5% and 45.1%, respectively, whereas the corresponding median PFS and OS were 13.8 months and 31.1 months, respectively. In this study tumor blocks from 67% of the first 411 patients who were enrolled into this study were evaluable for IHC detection of PD-L1 in the tumor. Allred proportion scores (APS) were randomly set for expression of PD-L1; APS=0, corresponds to 0% staining, APS=2 corresponds to 1-10% staining, APS=3 corresponds to 10-33% staining, APS=4 corresponds to 34%-66% staining, and APS=5 corresponds to 66-100% staining. Patients with APS=0 or 1 (n=28, n=24, respectively) had ORR <10% each, patients with APS=2 (n=72) had ORR ~ 20%, patients with APS=3 (n=54) had ORR 40%, patients with APS=4 (n=32) had ORR ~70%, and patients with APS=5 (n=32) had ORR ~ 50% [47]. Pembrolizumab monotherapy has lower clinical benefit when administered in patients with metastatic ocular or metastatic mucosal melanoma[49, 50]. The lower responses in these two non-cutaneous melanoma subtypes is attributed to the lower frequency of “inflamed” types. Pembrolizumab was safe and also showed promising antitumor activity in 18 patients with asymptomatic up to 20-mm active melanoma brain metastases (22% incidence of intracranial antitumor response)[51].

KEYNOTE-006 was a randomized study in which patients with advanced melanoma and no prior treatment with CTLA-4, PD-1, and PD-L1 inhibitors were randomized to pembrolizumab 10mg/kg administered every 2wks (n=279) or every 3 wks (n=277) versus standard of care ipilimumab (3mg/kg, n=278). In the intention-to-treat population the estimated 6-month PFS rates for patients receiving pembrolizumab every 2 or 3 weeks were 47.3% and 46.4%, respectively, whereas the corresponding 6-month PFS rate for ipilimumab-treated patients was 26.5%. Median estimates of PFS were 5.5 months, 4.1 months, and

2.8 months, respectively. At the time of data cutoff for the second interim analysis, which was driven by a minimum follow-up duration of 12 months for all patients, one-year estimates for survival were 74.1%, 68.4%, and 58.2%, respectively. The results from this study suggest that pembrolizumab administered at 10mg/kg irrespective of schedules tested was superior to ipilimumab in the front-line setting (i.e. no prior treatment with co-inhibitory immune checkpoint inhibitors) [52].

KEYNOTE-002 is a randomized study in which patients with advanced melanoma who have previously received and have become resistant to ipilimumab and MAPK pathway inhibitors (if BRAFV600-mutant) were randomized to receive pembrolizumab 2mg/kg every 3wks (n=180), pembrolizumab 10mg/kg every 3 wks (n=181), and investigator's choice chemotherapy (n=171; paclitaxel plus carboplatin, paclitaxel, carboplatin, dacarbazine, or oral temozolomide). At a median follow-up duration of 10 months pembrolizumab administered at either 2mg/kg or 10mg/kg showed significant improvement in PFS compared to investigator's choice chemotherapy (hazard ratio or 0.57 and 0.50, respectively). Response rates in patients who received pembrolizumab at 2mg/kg, 10mg/kg versus investigator's choice chemotherapy were 21%, 25%, and 4%, respectively, whereas the median PFS was 5.4 months, 5.8 months, and 3.6 months, respectively. Incidence of grade 3-4 treatment-related adverse events was higher in those given chemotherapy (26%) than in those given pembrolizumab at 2mg/kg (11%) and pembrolizumab at 10mg/kg (14%). The most common grade 3-4 treatment-related adverse events in the pembrolizumab 2 mg/kg treatment group were fatigue, generalized edema, and myalgia whereas in the pembrolizumab 10mg/kg treatment group common grade 3-4 events were hypopituitarism, colitis, diarrhea, decreased appetite, hyponatremia, and pneumonitis. In the prespecified subgroup analysis of PFS the only subgroup that did not appear to significantly benefit from pembrolizumab 2mg/kg was the BRAFV600 mutant groups [53].

In summary, pembrolizumab administered at 10mg/kg provides better clinical benefit than the standard of care ipilimumab in the front-line setting (i.e., no prior co-inhibitory immune checkpoint inhibitors). Although the FDA-approved 2mg/kg every 3 weeks dose was never directly compared head-to-head against ipilimumab, data from KEYNOTE-001 and KEYNOTE-002 show that there is no significant difference between pembrolizumab administered at 2mg/kg and 10mg/kg every 3 wks. Currently pembrolizumab is administered at 200mg IV, flat/fixed dose every 3 weeks.

1.4 alpha-[^{11}C]-methyl-L-Tryptophan (C11-AMT)

1.4.1 Tryptophan Metabolism

Tryptophan is the largest natural amino acid with polyaromatic (indole; pyrrole plus benzene) side chain. From a chemical viewpoint, it possesses unique

reactivity owing to the electron-richness of the indole system. Enzymes have evolved multiple strategies to break or modify the indole ring. Tryptophan is an essential amino acid that serves as a precursor for several substances, such as alanine, acetoacetyl CoA, niacin and NAD^+, serotonin, melatonin, and hydroxyl indole acetic acid [54]. **Figure 3** shows the 3 major metabolic pathways of tryptophan. For many years, the indoleamine pathway was thought to be the principal pathway of tryptophan metabolism that results in the synthesis of the neurotransmitter, serotonin, and the hormone, melatonin, in the pineal gland and retina. Interest in this pathway was based on the fact that disorders in metabolism of serotonin and melatonin have been associated with affective disorders. In reality, however, only approximately 1% of tryptophan is metabolized through this pathway.

The kynurenine pathway is admittedly the main pathway for tryptophan metabolism and provides precursors that supplement dietary niacin for the biosynthesis of NAD^+ and $NADP^+$, as well as energy production and biosynthesis of other amino acids [55]. The first enzymatic step is the conversion of tryptophan to N-formylkynurenine, which is the rate-limiting step that is regulated by two analogous (i.e. functionally similar but genetically distinct) enzymes: tryptophan dioxygenase (TDO), which is expressed at high levels in the liver and is responsible for regulating systemic tryptophan levels, and indoleamine-2,3-dioxygenase (IDO), an enzyme that is not highly expressed by most tissues, except intestine, although it can be induced in peripheral tissues by pro-inflammatory stimuli (IFNγ, TNFα). For many years, intermediate metabolites of the kynurenine pathway were thought to have little biological activity until research showed that several of these metabolites may have neurotransmitter-like and immunomodulatory properties. For example, quinolinic is an agonist at glutamate receptors that are sensitive to NMDA, kynurenic acid is antagonist at these and other ionotropic glutamate receptors.

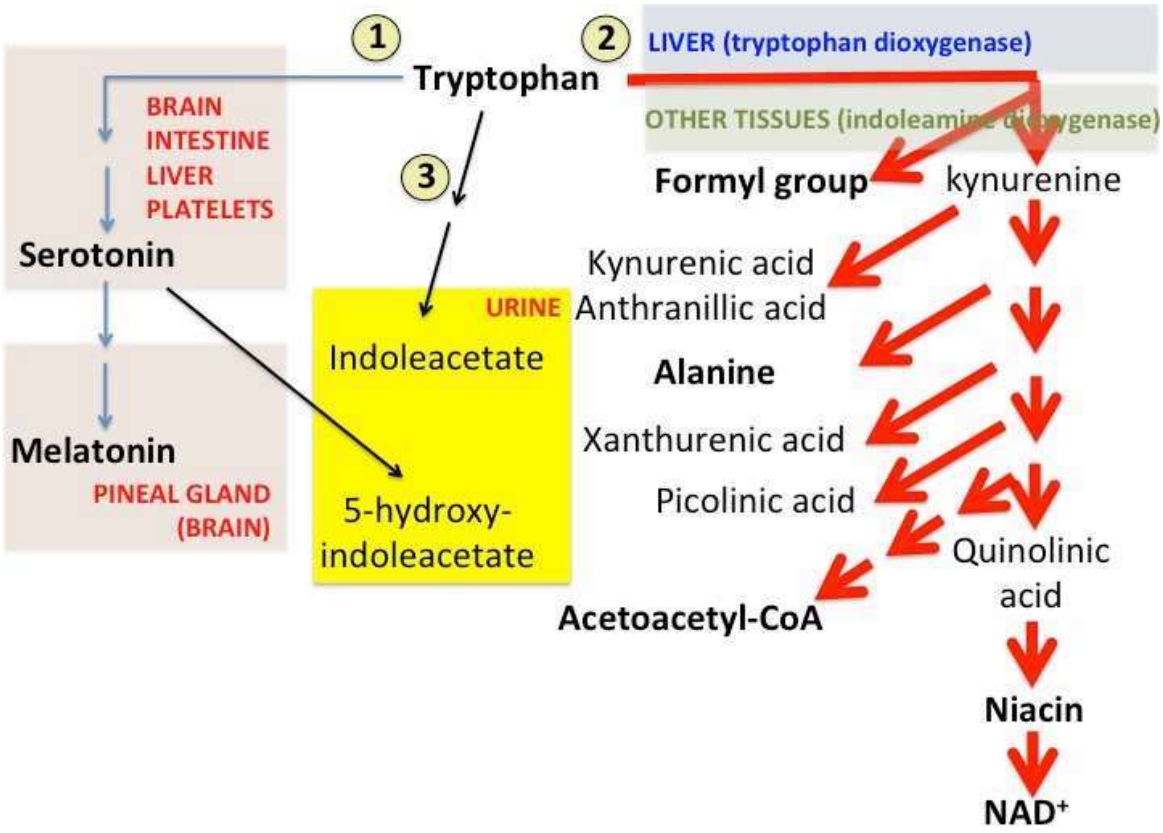

Figure 3. Tryptophan Metabolism. 1. Indoleamine pathway. 2. Kynurenine pathway. 3. The urine excretion (deamination/decarboxylation) pathway.

1.4.2. Immunomodulatory actions mediated by tryptophan metabolism in cancers

Cancer cells or myeloid cells within the tumor microenvironment express high levels of IDO1 and/or TDO. Two mechanisms mediate immunoregulation by tryptophan [56]; under the first, termed "death-by-starvation" paradigm, effector T cells are particularly susceptible to low tryptophan concentrations in the extracellular space, resulting in anergy, cell cycle arrest, and apoptosis. Under the second mechanism, tryptophan metabolites, such as kynurenine, kynurenic acid, 3-hydroxy-kynurenine, and 3-hydroxy-anthranillic acid suppress T-cell function and are capable of inducing T-cell apoptosis via binding to the aryl hydrocarbon receptor (AHR). AHR is a cytoplasmic transcription factor that is activated by xenobiotics, and is therefore important for detection and detoxification of polyaromatic hydrocarbons, as well as endogenous metabolites, such as kynurenine. AHR activation in T cells results in reprogramming the differentiation of naïve $CD4^+$ T-helper cells favoring a regulatory T cell phenotype while suppressing the differentiation into interleukin-17-producing Th cells. In addition, activation of the AHR in dendritic cells promotes a tolerogenic phenotype.

1.4.3 History, Biochemistry, and Applications of C11-AMT PET Imaging in Benign Neurologic Conditions

Alpha-methyl-tryptophan (αMT) is a tryptophan analogue that can undergo early enzymatic reactions as part of the indoleamine and kynurenine pathway and ultimately be converted to alpha-methyl-serotonin (αMS) or alpha-methyl-kynurenine (αMK), respectively. However, neither αMS nor αMK are substrates for downstream enzymes and, as such, they remain in the corresponding tissues for sufficient time that is required to monitor the *in vivo* rate of tryptophan metabolism via the early steps of each of the two corresponding pathways. Based on the favorable properties of αMT, synthesis of carbon-11 at the α-methyl position was feasible during the '80s. C11-AMT PET imaging along with various independent tumor tissues studies in preclinical models showed that C11-AMT PET maps serotoninergic neurons in the brain, an extremely useful property to study in the clinical practice, because alterations in serotonin production have been implicated in various psychopathologic conditions, such as depression, suicide, aggression, and anxiety [57].

Historically, studying the rates of serotonin synthesis in non-malignant brain conditions has been the driving force to apply C11-AMT PET imaging since the late '90s and '00s [58-61]. In fact, in one of these presumably high serotonergic neurologic disorders there was an attempt to correlate C11-AMT PET imaging signal with actual measurements for tryptophan metabolites in the corresponding diseased tissue. More specifically, biochemical studies were conducted in epileptogenic dysplastic tissue obtained from children with tuberous sclerosis complex who first underwent C11-AMT PET imaging prior to surgery to remove epileptic foci. Surprisingly, the increase in C11-AMT uptake in cortical tubers did not represent increased serotonin synthesis, but rather high levels of quinolinic acid [62].

1.4.4 Molecular Imaging of Tryptophan Metabolism Using C11-AMT in Cancers

Publications about the role of the kynurenine pathway in cancers in the '90s and '00s [63] led to clinical investigations about the diagnostic utility of C11-AMT in tumors. C11-AMT imaging in various primary brain tumors revealed overall high standard uptake values, and better discrimination of tumor anatomy compared to FDG PET. In addition, tumors with gadolinium enhancement showed higher SUV for C11-AMT than those with no enhancement and were inferior for recurrence coverage [64]. In addition, the metabolic rate for C11-AMT imaging, a better measure of tryptophan metabolism, was significantly anti-correlated with tumor grade [20]. In fact, AMT metabolic rates were significantly lower in tumor samples with higher and widespread expression for IDO [13]. In addition, C11-AMT PET imaging was significantly better to discriminate between recurrent gliomas from radiation injury [65]. C11-AMT has finally been evaluated in two extra-cranial cancers, non-small cell lung cancer and breast cancer [23, 66]. In

contrast with C11-AMT PET imaging in brain diseases (both malignant and non-malignant), both lung and breast cancer tumors show:

1. higher transport and metabolic rates
2. higher heterogeneity in C11-AMT kinetics. For example, lesions may show earlier peak (5-20min), followed by within-minutes decline versus slower decline in activity. Alternatively, different tracer kinetics for each tumor within a patient with multiple lesions.
3. time-activity curves showed differences in efflux of the tracer or its metabolites (early versus delayed).
4. higher unidirectional uptake of C11-AMT in extra-cerebral tumors (lung>breast) compared to intra-cranial tumors.

In the breast cohort limited tumor tissue analysis confirmed high IHC expression of IDO and TPO as well as high IHC expression of the light chain amino acid transporter, LAT1. No correlation between PET parameters and several parameters of immune response were described. Results suggest that the underlying heterogeneity in C11-AMT PET imaging in lung and breast cancer tumors may explain differential response to immunotherapies.

1.5 Rationale

BRAF V600 codon mutations have been a crucial biomarker in selecting out patients that will most likely respond to MAPK pathway inhibitors. Their success is not only linked to the specific mechanism of action of MAPK pathway inhibitors but also due to the relative ease in their detection using various first- and next-generation sequencing approaches that require only minute amounts of tumor DNA, the latter usually available using minimally invasive biopsies (e.g. fine needle aspiration, or core biopsies) [67]. In addition, oncogenic BRAF (as well as NRAS) mutations are usually present throughout the natural history of cutaneous melanoma and display little intra-patient tumor heterogeneity, because they *de facto* play an important role in melanoma development and progression [68].

Developing biomarkers for co-inhibitory immune checkpoint inhibitors is essential given the high costs associated with their administration [69]. However, such biomarker development, is much more challenging, even if we know that pre-existing tumor-infiltrating $CD8^+$ cells in combination with evidence of the upregulation of tumor tissue biomarkers associated with immune exhaustion (e.g. PD-L1) are fundamental for clinical benefit for various reasons. First, tumor tissue studies by us and others have shown significantly higher heterogeneity in the density of tumor-infiltrating immune cells in relation to stage, organ site, and prior systemic or local treatments [7]. Second, in contrast to oncogenic mutations that are uniformly expressed by nearly all cancer cells within a given tumor biopsy, tumor-associated lymphocytic infiltrates can show a considerable degree of spatial heterogeneity even within the same tumor biopsy, which can be easily underestimated if tumor biopsies are small.

For the above stated reasons, we propose to assess the utility of C11-AMT PET imaging in predicting response to pembrolizumab, since previous clinical studies

with C11-AMT PET have tightly linked C11-AMT PET with enzymatic activity of IDO, an enzyme that is abundant, at least in "inflamed" cutaneous melanoma. We postulate that the ability to image one of the important components of "inflamed" melanomas is far more specific than the existing non-specific methods of assessment of immune response that rely on metabolism ([^{18}F]FDG-PET) or proliferation ([^{18}F]FLT-PET).

In our study we will administer pembrolizumab at the FDA-approved dose and indication (front line setting), i.e. before any type of FDA-approved immunotherapy [high dose bolus IL-2, PD1/PD-L1 pathway inhibitors, CTLA4 inhibitors or other co-stimulatory or co-inhibitory antibody therapies (GITR, LAG3, CD137, etc.)] or targeted therapies (e.g. MAPK pathway inhibitors).

The primary endpoint of our study will be the baseline assessment of the following PET parameters during C11-AMT PET of melanoma: (a) tracer concentration in tumor tissue to the dose injected and the patient mass [SUV=tissue concentration in range of interest (kBq/cm^3)/injected dose per weight (MBq/kg)]. These parameters will be correlated with antitumor response to pembrolizumab at 12 weeks (or earlier if patient progresses).

1.6 Correlative Studies

Under this, patients who participate in this trial will be asked to undergo mandatory fresh biopsies at baseline, namely before initiation of pembrolizumab and after C11-AMT PET imaging. Patients with metastatic lung disease (M1b), the metastatic melanoma subgroup with the highest clinical benefit from pembrolizumab[48], may be enrolled in the trial but will only undergo optional biopsies given the high risk (up to 20%) of pneumothorax. "Mandatory" means that it would occur irrespective of prior available tumor tissue. "Research" means that no diagnostic report will be generated; also, it has to occur in previously imaged tumor with all three modalities (FDG PET, C11 AMT PET, and CT scan with IV contrast). This will provide a unique opportunity to correlate the PET parameters of the particular tumor tissue that will be biopsied after C11-AMT PET imaging; this is very important due to the heterogeneity in the parameters that has been described among different tumors within the same patient in the lung cancer cohort [66]. Given the specific focus of this trial, we will give the highest priority to first generate representative tissue sections from these tumors to analyze the following by immunohistochemistry and/or 2-color immunofluorescence:

a. Expression of the two analogous rate-limiting enzymes of the kynurenine pathway, IDO and TDO in melanoma (S100+) and stromal [i.e. hematopoietic cells (CD33+)]. We anticipate that expression of any these two enzymes, or both, will be correlated with SUVmean and the metabolic rate constant, k_3.
b. Expression of the rate-limiting enzyme of the indoleamine pathway, tryptophan hydroxylase. We anticipate that tryptophan hydroxylase will not be highly expressed by melanoma (S100+) as opposed to stromal (hematopoietic) cells (CD33+).

c. Expression of components of system L, the amino acid transporter systems of L-tryptophan, LAT1 and CD98. We anticipate that LAT1 and/or CD98 to positively correlate with K_1 constant.
d. Expression status of effector T cells (CD8), IDO, and FoxP3+ mononuclear cells using IHC or 3-color immunofluorescence.

We postulate that high SUVmean values for C11-AMT PET will be correlated with high protein expression levels of IDO within the tumor. In addition, expression levels of IDO will be significantly correlated with immune score, PD-L1, and $FoxP3^+$ mononuclear cells. However, biopsy collection and assessment of the proteins listed will helps us interpret counterintuitive results from C11-AMT PET imaging in relation with antitumor response (or lack of) to pembrolizumab. If, let's say, we notice high C11-AMT PET imaging but no antitumor response, is it because: a) tumors express too much of IDO? b) C11-AMT is transported very efficiently into the cell (L amino acid transporter systems CD98 and LAT1) but it is trapped inside the cell without being metabolized because cells do not express either IDO or TPO? c) do tumors express too little of IDO and paradoxically express too much of TPO which is NOT regulated by IFN gamma, e) do tumors express high IDO via mechanisms other than IFN gamma; in other words there is constitutive expression of IDO but there are no tumor infiltrating lymphocytes? f) Are tumors truly 'inflammed" but their adaptive immune resistance is not simply dependent on the PD-1/PD-L1 pathway but to other pathways (CTLA4 inhibition, IDO inhibition? LAG3 inhibition?). If tumors express, let's say low signal of C11-AMT but are FDG-PET avid and respond to pembrolizumab, how do the tumor characteristics explain the PET results? Is it because: A) Neither IDO nor TPO are expressed into the tumor, B) Tumors do not express the essential amino acid transporters and therefore no matter whether tumor express IDO or TPO, as long as C11-AMT does not reach the cell cytoplasm it will never be retained and metabolized, C) do tumors contain sufficient number tumor-infiltrating immune cells but these are not lymphocytes?

If the results from C11-AMT imaging were straightforward (i.e. low C11-AMT equals no response to pembrolizumab, high C11-AMT equals response to pembrolizumab) there would be no need to do research biopsies, but this examination has never been attempted previously. Biopsy collection serves the fundamental translational purpose of explaining at the tissue level what is or is not observed on the C11-AMT PET Scan.

Subjects may also be asked to participate in the Merck-sponsored UNC Immunotherapy Patient Centered Translational research biorepository (IMPACT), LCCC 1528 (PI Jon Serody, MD, UNC). This will involve optional co-enrollment in the IMPACT biorepository, which will be a separate consent but linked to IRB-approved Merck immunotherapy protocols.

2.0 STUDY OBJECTIVES

2.1 Primary Objective

Explore association between intensity of C11-AMT PET at baseline, as measured by mean standardized uptake value (SUVmax at each lesion), total tumor metabolic volume, measurement of intra-tumoral and inter-lesional heterogeneity), with objective response rate (ORR) at 12 weeks as defined via RECIST1.1 to pembrolizumab in patients with PD-1 inhibitor-naïve unresectable stage III or distant metastatic (AJCC stage IV) melanoma.

2.2 Secondary Objectives

2.2.1 Estimate ORR (CR + PR) by RECIST 1.1 at 12 weeks to pembrolizumab in patients with PD-1 inhibitor-naïve unresectable stage III or distant metastatic melanoma (AJCC stage III/IV).

2.2.2 Estimate progression-free survival (PFS) in patients with unresectable stage III or distant metastatic melanoma treated with pembrolizumab as front-line therapy.

2.2.3 Explore associations in SUVmax and other PET parameters (e.g. total tumor metabolic volume, measurement of intra-tumoral and inter-lesional heterogeneity) between C11-AMT PET and FDG-PET at baseline.

2.2.4 Explore associations between SUVmax, and other PET parameters (e.g. total tumor metabolic volume, measurement of intra-tumoral and inter-lesional heterogeneity) identified at baseline C11-AMT PET imaging with expression of components of the IDO pathway detected by immunohistochemistry (IHC) or immunofluorescence (LAT1, IDO, TPH1), lymphocyte subtypes (CD4, CD8, FoxP3, MDSC), PD-1/PD-L1, and other immune checkpoint pathways (LAG3, GITR, TIM3) in freshly acquired tumor specimens prior to treatment with pembrolizumab.

2.2.5 Assess metabolic changes at week 12 (or earlier, if patient progresses) following treatment with pembrolizumab using baseline and week 12 ^{18}F-FDG PET.

2.3 Primary Endpoints

The primary endpoints are assessment of SUVmax, and other PET parameters (e.g. total tumor metabolic volume, measurement of intra-tumoral and inter-lesion heterogeneity), from C11-AMT PET at baseline and correlation with ORR (CR + PR) at 12 weeks as defined via RECIST1.1 to pembrolizumab in patients with PD-1 inhibitor-naïve metastatic melanoma.

2.4 Secondary Endpoints

2.4.1 Assessment of PFS, defined as D1 of treatment until progression or death from any cause in patients with metastatic melanoma treated with pembrolizumab as front-line therapy.

2.4.2 Correlate tumor lesions imaged at baseline by C11-AMT PET with whole body CT scan with IV contrast (CTIVC).

2.4.3 Correlate tumor lesions imaged at baseline by C11-AMT PET with whole body ^{18}F-FDG PET at baseline and at 12 weeks (or earlier, if patient progresses).

2.4.4 Perform immune score, IHC, and/or immunofluorescence to detect underlying host-immune response, expression of components of the IDO pathway (LAT1, IDO, TPH1), lymphocyte subtypes (CD4, CD8, FoxP3, MDSC), PD-1/PD-L1, and other immune checkpoint pathways (LAG3, GITR, TIM3) in freshly collected and archived tumor specimens from patients who will receive pembrolizumab. Correlate immune score and other IHC with SUVmax of the previously imaged lesion by C11-AMT PET.

3.0 PATIENT ELIGIBILITY

3.1 Inclusion Criteria

To be eligible for participation in this trial, the subject must:

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

2. Subject must be ≥ 18 years of age on the day of signing informed consent.

3. Have histologic or cytologic biopsy-proven diagnosis of unresectable stage III or distant metastatic melanoma, irrespective of histologic type (i.e. cutaneous, unknown primary, mucosal, or ocular). Patients with resectable bulky stage IIIB or stage IIIC melanoma (for example at least 2.5-cm in shortest diameter for lymph nodes infiltrated by tumor and at least 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. Have measurable disease based on RECIST v1.1. for solid tumors.

5. Be willing to undergo fresh tumor tissue biopsy of an accessible tumor lesion prior to pembrolizumab. A mandatory fresh biopsy will be collected following C11-AMT PET imaging. Subjects for whom fresh samples cannot be provided (e.g. inaccessible or subject safety concern) or do not agree to this fresh tumor research biopsy of accessible tumor will be deemed ineligible for study participation.

Exception to the mandatory tumor tissue collection are patients with metastatic lung lesions as the only site of metastatic disease. Fresh biopsy collection from these subjects will be optional, due to high risk of pneumothorax.

6. Be willing to allow for investigators to collect archival tumor tissues from surgical procedures that may have been performed before or after enrollment into this trial for research purposes (in-house cases and/or outside cases). These samples will be obtained by study staff as long as subject continues on follow-up. Blocks of tissue will be requested, and if blocks are not able to be obtained, 5micron slides (10-15) will be sufficient.

7. Be willing to be injected with ^{11}C-methyl-L-tryptophan (C11-AMT).

8. Have a performance status of 0 - 2 on the ECOG Performance Scale.

9. Has not received prior therapy with CTLA-4, PD-1/PD-L1 inhibitors, other co-stimulatory or co-inhibitory immune checkpoint antibody therapies (e.g. LAG3, TIM3, CD137, KIR3DL, CD70, and CD27) for distant metastatic melanoma. Patients who have received MAPK inhibitors are allowed on condition that they have recovered from adverse events to at most Grade 1 by CTCAE v4.03 and at least 15 days have elapsed between last dose of MAPK inhibitors and C11-AMT imaging. Patients who have previously received CTLA-4 inhibitors in the adjuvant setting are allowed to participate as long as they discontinued CTLA-4 treatment at least 30 days ago and meet criteria outlined in inclusion #14. Patients who have previously received adjuvant PD-1 inhibitors are excluded.

10. Demonstrate adequate organ function as defined in the table below; all screening labs to be obtained within 14 days prior to C11-AMT PET scan.

System	Laboratory Value
Hematological	
Hemoglobin (Hgb)	≥ 9 g/dL or ≥ 5.6 mmol/L without transfusion or EPO dependency (within 7 days of assessment of Hgb)
Absolute Neutrophil Count (ANC)	≥ 1,500/mm^3
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 x ULN **OR** ≥ 60 mL/min using the Cockcroft-Gault formula for subject with creatinine levels > 1.5 X institutional upper limits of normal (ULN)
Hepatic	
Serum Total Bilirubin	≤ 1.5 X ULN
Aspartate aminotransferase (AST)	≤ 2.5 X ULN OR < 5 X ULN for subjects with liver metastases

Alanine aminotransferase (ALT)	≤ 2.5 X ULN OR < 5 X ULN for subjects with liver metastases
Albumin	≥ 2.5 mg/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT)	≤1.5 X ULN, unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN, unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants

11. Female subjects of childbearing potential should have a negative urine or serum pregnancy within 14 days prior to C11-AMT PET scan. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

12. Female subjects of childbearing potential must be willing to use adequate methods of contraception as outlined in Section 5.1.7 – Contraception for the course of the study through 120 days after the last dose of study medication. Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.

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

    Male subjects should agree to use an adequate method of contraception as outlined in Section 5.1.7- Contraception starting with the first dose of study therapy through 120 days after the last dose of study therapy.

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

13. Patients who have received prior adjuvant high dose interferon are allowed to participate as long as the last injection was given at least 30 days prior to the C11-AMT PET scan and they have fully recovered from side effects (i.e., Grade ≤1 or permanent side effects that require hormone replacement therapy).

14. Patients on adjuvant ipilimumab are allowed to participate at least 30 days from drug discontinuation as long as they have at most Grade 1 adverse events (or grade 2 if they have to received hormone replacement therapy for their otherwise grade 1 ipilimumab-induced autoimmune endocrinopathies).

3.2 Subject Exclusion Criteria

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

1. Is currently participating and receiving study therapy for his/her advanced melanoma or has participated in a study of an investigational agent and received study therapy in the advanced melanoma setting.

2. Has received prior treatment with PD-1/PD-L1 pathway inhibitors in the adjuvant setting.

3. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to C11-AMT PET scan.

4. Has a known history of active tuberculosis (Bacillus Tuberculosis)

5. Hypersensitivity to pembrolizumab or any of its excipients described in section 5.1.1.

6. Has had prior monoclonal antibody (mAb) targeting immune checkpoint proteins, for distant metastatic melanoma and have progressed or have developed intolerable side effect.

7. Adjuvant anticancer treatments are allowed if at least 30 days has elapsed between the infusion/injection and C11-AMT PET scan as part of this study.

8. Prior radiation therapy for metastatic melanoma is allowed as long as the patient bears measurable actively growing disease outside the previously irradiated field. **NOTE:** If subject received major surgery, they must have recovered adequately from the toxicity (i.e., all symptoms ≤ grade 1) and/or complications from the intervention prior to starting therapy.

9. 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 patient not under active systemic treatment other than hormonal therapy and with documented undetectable PSA (<0.2ng/mL),
   - CLL/SLL provided patient has isolated lymphocytosis (Rai stage 0), and does not require systemic treatment [for "B" symptoms, Richter's transformation, lymphocyte doubling time (<6 months), lymphadenopathy or hepatosplenomegaly],
   - Lymphoma or any type or hairy-cell leukemia provided patient is not on active systemic treatment and is in complete remission, as evidenced by PET/CT scans and bone marrow biopsies for at least 3 months,
   - Papillary thyroid cancer. Since this malignancy infrequently metastasizes

distantly, patients with concurrent metastatic melanoma can be enrolled. Patients can be enrolled regardless of if they meet any of the following: A) have completed a thyroidectomy within the last 2 years, B) have or have not received adjuvant radioactive iodine therapy, or C) were only recently diagnosed with asymptomatic papillary thyroid cancer and their surgery is pending.

- History of malignancy provided patient has completed therapy and is free of disease for ≥ 2 years. If patient had other malignancy within the last 2 years from which he may have been completely cured by surgery alone, he may considered to be enrolled on condition that the risk of development of distant metastatic disease based on AJCC staging system is less than 30%.

10. Has known active parenchymal central nervous system (CNS) metastases that are symptomatic, and/or more than one lesions, and/or their largest diameter is > 5-mm and/or require antiepileptic drugs or corticosteroids. Patients with carcinomatous meningitis are also excluded. Exceptions are: subjects with previously treated brain metastases provided they are stable (without evidence of progression by imaging) for at least 2 weeks prior to C11-AMT and any neurologic symptoms have returned to baseline, have no evidence of new or enlarging brain metastases, and are not using ongoing steroids for at least 7 days prior to C11-AMT. Patients with active (i.e. not treated with stereotactive radiosurgery), single, asymptomatic, up to 5-mm in largest diameter brain metastases (measured either by brain MRI with IV contrast or head CT with IV contrast measured within 2 weeks prior to C11-AMT) are allowed.

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

12. Has known history of (non-infectious) pneumonitis that required steroids, or any evidence of current pneumonitis.

13. Has an active infection requiring systemic therapy.

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

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

16. 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.

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

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

19. Has received a live vaccine within 14 days of C11-AMT PET scan.

    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.

4.0 TREATMENT PLAN

This is a phase 2, single-arm, open-label study of pembrolizumab that will enroll up to 27 subjects who have not received prior PD-1 inhibitor therapy for their recent diagnosis of unresectable stage III or distant metastatic melanoma. Section 4.1 describes the tests and sequence of procedures that will be performed at baseline prior to initiation of pembrolizumab treatment.

4.1 Baseline (pretreatment) imaging requirements

4.1.1 ^{18}F-fluorodeoxyglucose (FDG) PET/CT scanning

Whole body FDG PET/CT scan with IV contrast is the preferred method of evaluation at baseline following study entry and will be performed at least 24 hours before C11-AMT PET scanning at the molecular CT (mCT) scanner at UNC-Chapel Hill Hospitals. Although, the FDG PET/CT scan with IV contrast is preferred the following baseline measurements may be used if they have occurred within the below specified windows:

a. **whole body FDG PET/CT scan without IV contrast**, will be accepted for study purposes (i.e. correlation between baseline FDG PET scan and baseline C11-AMT scan) if it has occurred within 28 days before the C11-AMT PET scan. In this case, the patient will only be required to have a baseline CT scan of the chest, abdomen, and pelvis (also neck, if applicable) with IV contrast within 28 days of starting pembrolizumab. The CT scan with IV contrast will be the primary method of assessment of treatment response by RECIST v1.1 criteria. PET scan should encompass the whole body and/or lesions of interest as defined in Sections 6.7.2 Baseline Documentation of Target and Non-Target Lesions at discretion of the treating investigator.

b. **CT scan with IV contrast** will be accepted for study purposes (i.e. baseline tumor assessment) if it has occurred within 28 days of starting pembrolizumab. In this case, the patient will only be required to have a baseline PET scan without CT coregistration 28

days prior to C11-AMT. This is to correlate baseline FDG PET with baseline C11-AMT PET parameters.

4.1.2 C11-AMT PET scan co-registered with CT scan without IV contrast

C11-AMT PET will be performed before starting pembrolizumab treatment and at least 24 hours after FDG PET/CT scan. This test will be performed at the PET/CT scanner available at the Biomedical Research Imaging Center (BRIC). Please note that due to the short half-life of the C11-AMT tracer (20 minutes) and the need to perform dynamic imaging, C11-AMT PET scan may not be whole body and can be limited to only 1 or 2 bed positions, at the discretion of the radiologist depending on where the metastatic lesions of interest are anatomically located and should be similar to the area scanned with FDG PET to ensure ability to correlate baseline FDG PET and baseline C11-AMT parameters, and encompasses target lesions as described in section 6.7.2. Subjects will remain in the scanner continuously from the time of injection to target time for imaging 5 – 50 minutes following the injection. PET data will be acquired during the initial phase following the injection to determine whether or not 30 min images reflect steady-state distribution. Images acquired 20-40 min (+/- 5 min) following the injection will be used for data analysis purposes. For subjects who cannot be imaged with a single bed position, two bed positions will be utilized and images acquired using alternating bed positions. CT scan without IV contrast will be co-registered with C11-AMT PET to assist in defining regions of interest (ROI) for downstream AMT PET analysis. **If for whatever reason C11-AMT was not produced appropriately or the PET scan failed, patients may undergo a repeat C11-AMT PET scan**. Participants from affiliate sites must have their C11-AMT PET scan performed at UNC-CH. The following steps describe synthesis, quality control testing, administration, and analysis of AMT PET scan.

4.1.2.1 Synthesis of α-[11C]-methyl-L-tryptophan

The protocol for C11-AMT production is based on a simplified 2-step hydrolysis procedure that has been previously extensively applied for routine clinical use [70]. The product will be manufactured Dr. Zibo Li's group at the Translational Imaging Core, BRIC, where a cyclotron and radiochemistry modules are in place to ensure semi-automated radioisotope production under Good Medical Practice (GMP) guidelines. Given the short half-life of C11-AMT, it will be produced on the same day immediately before the AMT PET imaging. This method eliminates the need for high performance liquid chromatography (HPLC) isolation and purification of the final product, thus saving considerable time and eliminating potential pyrogenicity and production contamination problems associated with the multiple reuse of a semi-prep HPLC column. Five elements are important in this process: First, lithium diisopropylamide (LDA), which is required for the first step, will be freshly made within a week from the actual AMT production; alternatively, it can be purchased. Second, the AMT precursor, (dimethyl 2(S), 3a(R), 8a(S)-(+)-hexahydro-8(phenylsulfonyl)pyrrolo[2, 3-b]indole-1,2-dicarboxylate) will be purchased from a commercial vendor (Sigma-Aldrich) and stored a 4°C. An aliquot will be thawed to room temperature for 30-60 min on the day of C11-AMT synthesis. Third, production of $^{11}CH_3I$. [^{11}C]Carbon dioxide will be produced by the $14N(p,\alpha)^{11}C$ reaction from the GE PETtrace 880 Cyclotron (Biomedical

Research Imaging Center, UNC-CH). [^{11}C] methyl iodide will be prepared using the GE FX-FN module, by converting [^{11}C] CO_2 to [^{11}C] CH_4, followed by iodination. Fourth, production of C11-AMT will be performed from its precursor and other molecules (LDA, $^{11}CH_3I$, THF, TFA, KOH), as shown in Figure 4. Fifth, C11-AMT will be purified using commercially available C-18 Sep-Pak cartridges.

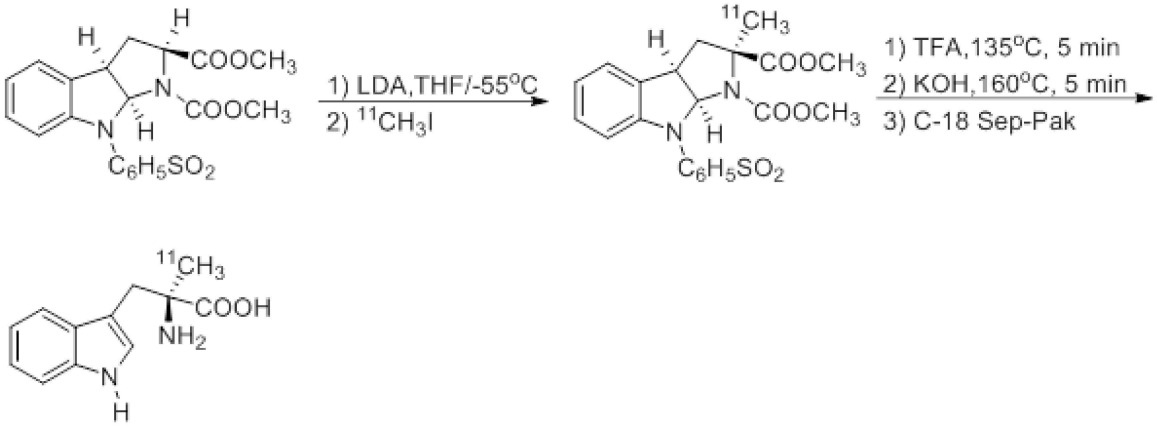

Figure 4. Procedure for Synthesis of α-[^{11}C]-methyl-L-tryptophan.
Abbreviations: LDA: lithium diisopropylamide; THF, tetrahydrofuran; TFA, trifluoroacetic acid; C-18 Sep-Pak, solid phase extraction chromatography cartridges. Adapted from[70]

4.1.2.2 Quality control analysis of α-[11C]-methyl-L-tryptophan prior to administration

Immediately following preparation the C11-AMT product solution will undergo a series of quality tests to ensure the product is suitable for human administration. Release of the product for human use will be the responsibility of Dr. Eric Smith's group, Radiopharmacist at the BRIC. More specifically, radiochemical purity will be performed by radio-HPLC to ensure greater than 90% of the radioactivity is in the proper chemical form (C11-AMT). Visual inspection to evaluate for particulates will be performed on each batch. The pH of the solution will be measured and adjusted using 8.4% sodium bicarbonate and 23.4% sodium chloride, if necessary. Osmolality will be evaluated via an osmometer. Residual solvents will be assessed via gas chromatography to ensure chemicals used to clean the reaction vessel have been properly removed. The product will also undergo sterility and pyrogenicity testing. A filter integrity test will provide an immediate indication of the sterility of the batch, and an endotoxin test will confirm the lack of pyrogens. Samples from the batch will also be inoculated and monitored for full sterility analysis. Table 1 below summarizes the required tests and the specifications for release.

QUALITY CONTROL TEST	DESCRIPTION	REQUIREMENTS FOR PASS	REQUIRES TEST PASS PRIOR TO PRODUCT RELEASE
Chemical Purity	Visual inspection for color and particulates	Clear and Colorless	Yes
Filter Integrity	Test bubble point	Meet pressure specified by manufacturer	Yes
pH	pH as per USP test method 791	5.0 – 7.5	Yes
Chemical & Radiochemical Purity	HPLC consistent with guidelines of General Chapter <621>, Chromatography subsection HPLC	Radiochemical Purity > 95% Other UV (list nm) peaks ≤ 35 µg in final product	Yes
Radiochemical Purity	TLC	Rf > 0.5 and Purity ≥ 95%	Yes
Residual Solvent Levels	Gas Chromatography	Acetone < 5,000 ppm Acetonitrile < 400 ppm	Yes
Radionuclidic Purity	Half-life Determination	18.4 – 22.4 min	Yes
Bacterial Endotoxin Levels	Limulus Amoebocyte Lysate (LAL) by PTS	< 175 EU per dose	Yes
Sterility	USP <71> sterility test	No growth observed in 14 days	No

Table 1. Summary of quality control tests required prior to release of radiopharmaceutical product for human studies. Abbreviations: USP, United States Pharmacopeia; HPLC, high pressure liquid chromatography; TLC, thin layer chromatography; Rf, relative front (measure of solubility); ppm, parts per million; EU, endotoxin units.

4.1.2.3 Administration, scanning and analysis of α-[11C]-methyl-L-tryptophan

Patients will fast for at least 6 hours before the C11-AMT PET study to ensure stable plasma levels of tryptophan and large neutral amino acids. Initially, a venous line will be established for the administration of C11-AMT, irrespective of whether the patient already has a central line (e.g. Mediport, Port-a-cath, PICC line). In other words, these central lines will NOT be used for infusion of the radionuclide agent. Intravenous administration of ^{11}C-AMT [6.5mCi, maximum7.15mCi, which is within the 10% variation from the standard dose)] will be administered under the supervision of Dr. Terence Wong or another qualified nuclear medicine physician from the Nuclear Medicine Department. C11-AMT PETv imaging will commence 5-50 minutes following injection. A low-dose scout CT scan (120 kVp, 10 mA) will be initially acquired for patient positioning and determining dose modulation parameters. CT scan (120 kVp, automated dose

modulation, dose-equivalent of 100 mAs) will be subsequently acquired for attenuation correction and anatomic localization. This scan takes less than 20 seconds and will be performed with the patient suspending respiration in the end-tidal volume phase. This will provide the most accurate alignment of the PET and CT images. The automated CT tube current modulation allows the scanner to adjust the tube current (and therefore CT dose) during the scan and enables the desired image quality to be maintained using the lowest dose possible. The acquisition time for each bed position on the PET emission scans will be adjusted during the scan to optimize the count statistics (i.e. progressively longer acquisition time for later bed positions). PET images will be acquired using 3D acquisition with time-of-flight. Given the short half-life of C11-AMT it may not be possible to perform whole body C11-AMT imaging. Depending on the anatomic location of the tumors, at least 1 or 2 bed positions will be performed instead. These bed positions may not be contiguous but rather include the tumors of interest based on the following three parameters: (a) tumors must have been previously imaged by both CT scan with IV contrast as well as FDG-PET, to ensure correlation between FDG-PET parameters, precise measurements by CT scan with IV contrast, and C11-AMT PET parameters, (b) tumors that are included in assessing antitumor response to pembrolizumab by RECIST criteria are included, (c) the index tumor lesion that has been previously selected for biopsy or surgical excision is included.

Melanoma tumors will be visually identified conjointly by the CT scan and C11-AMT PET images. To objectively determine the tumor region of interest (ROI), we will initially determine the voxel with the highest ^{11}C-AMT tracer concentration as well as background regions in close proximity to the location of the tumor. Using commercial software (MimVista), the PET data will be evaluated; proposed study parameters include: standardized uptake value (SUV_{max}) at each lesion, total tumor metabolic volume (TMV) to reflect tumor burden over all lesions, and measurements of intra-tumoral and inter-lesional heterogeneity.

Following completion of C11-AMT PET imaging, biopsy of the tumor tissue that was ideally PET imaged by both FDG and C11-AMT will be performed either by excisional or CT-guided biopsy.

The selection of the lesions targeted for biopsy will be determined in part by the location and ability to access safely as well the imaging properties (^{11}C-AMT uptake). The lesion selected for biopsy will be documented and annotated on cross sectional images to enable the most accurate correlation between imaging and histopathology.

4.1.3 Fresh Biopsy Collection

Biopsy of tumors of interest will be performed following C11-AMT PET imaging and before pembrolizumab administration. Depending on tumor location, biopsies can be excisional (the preferred method), punch (acceptable), or core biopsies using an 18g needle. When possible, the biopsy site will be documented with imaging, to enable

precise correlation of local C11-AMT PET parameters with histopathology. Excised tumors will be fixed in formalin and paraffin-embedded for the histopathologic studies outlined above (Section 1.5).

4.2 Schema

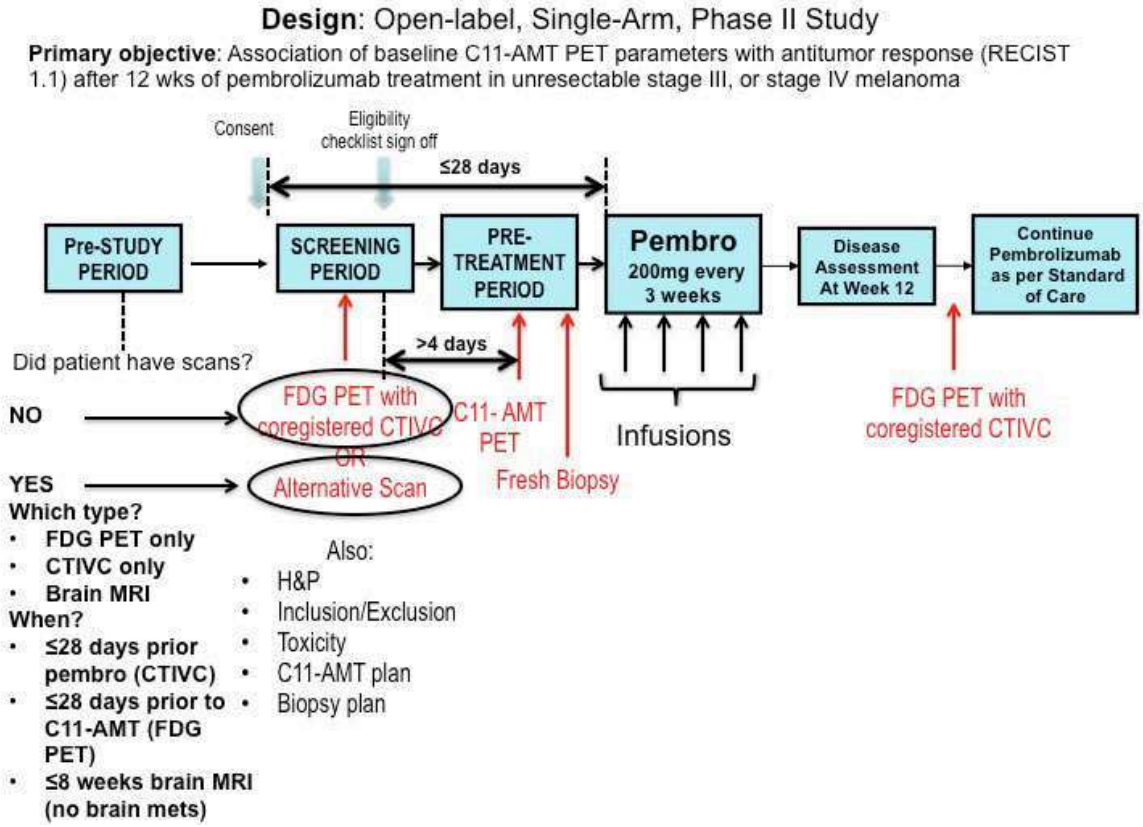

Figure 4. Treatment schema. Scans during the pre-study period may be accepted for baseline standard of care imaging if they have occurred within a particular timeframe in relation with C11-AMT or pembrolizumab in administration. An alternative scan to the FDG-PET coregistered with CT with IV contrast can be considered if patients had previously whole-body PET scan with non-contrast CT (alternative scan would then be CT of the chest, abdomen, and pelvis; neck, if applicable) or CT scan with IV contrast (alternative scan would be whole body PET scan). Nevertheless, FDG PET with coregistered CT scan with IV contrast (CTIVC) is the preferred method of baseline assessment of disease prior to pembrolizumab initiation. The evaluation period begins with signed informed consent and ends on the day of pembrolizumab infusion; it should not exceed 28 days. The evaluation period consists of two periods: screening period and pre-treatment period. During the screening period FDG PET with CTIVC, history and physical exam (H&P), review of inclusion/exclusion criteria, blood work, and planning for subsequent baseline studies is completed. As soon as screening is complete, the eligibility checklist is approved and signed by the treating physician. Under no circumstances is the time between checklist sign off and C11-AMT PET imaging less than or equal to 4 days. Pre-treatment period includes C11-AMT PET imaging (at least 1

bed position), fresh biopsy collection, and repeat of blood/urine tests. Up to four infusions of pembrolizumab are administered before the restaging FDG PET whole body with CTIVC scan is performed.

FDG PET coregistered with CT scan with IV contrast (CTIVC) is the preferred method of evaluation at baseline within the defined windows and at 12 weeks (or earlier, if the patient progresses). However alternative scan tests will be acceptable for the study as long as they fulfill certain criteria:

A. If the whole body PET scan has been performed previously, time elapsed between pre-study PET scan and baseline C11-AMT PET scan should be within 28 days. In this scenario, a baseline CT scan with IV contrast of the chest, abdomen, and pelvis (add neck, if applicable) will be performed within 28 days prior to starting pembrolizumab.

B. If whole body CT scan with IV contrast has been previously performed, time elapsed between the pre-study "diagnostic" CT scan with IV contrast and treatment initiation with pembrolizumab should be at most 28 days. In this case, a whole body PET scan must be performed within 28 days prior to C11-AMT PET imaging.

C. If both whole body PET scan and a CT scan with IV contrast (chest, abdomen, pelvis and neck if applicable) have been performed separately prior to study entry, this is acceptable as long as the whole body PET scan is within 28 days of C11-AMT PET imaging and the CT with IV contrast is within 28 days of starting pembrolizumab.

Following pembrolizumab infusions administered every 3 weeks x 4 cycles, antitumor response will be assessed at 12 weeks by performing a whole body FDG PET scan co-registered with CTIVC. As an alternative, CTIVC of the chest, abdomen, and pelvis (add neck, if applicable) is acceptable and can be used for this assessment of antitumor response by RECIST v1.1 criteria. It is quite possible that patients may progress earlier than 12 weeks; in this case, an evaluable patient will be a patient who has received at least two pembrolizumab infusions and subsequently had restaging scans to document radiographic progression and measurement by RECIST v1.1 criteria. Antitumor response will then be correlated with various C11-AMT PET parameters at baseline (e.g. SUVmax, total tumor metabolic volume, measurement of intra-tumoral and inter-lesion heterogeneity), which is the primary endpoint of the study. Toxicity will be assessed during treatment via NCI CTCAE v4.03. Following week 12, subjects will have completed study treatment requirements, and after discussion with their doctor they will be allowed to continue pembrolizumab per standard of care until disease progression or other reasons (e.g., unacceptable toxicity). Long-term follow up and treatment of all patients who continue to have clinical benefit from pembrolizumab (complete response, partial response, stable disease) will be maintained as per standard of care.

4.2.1 Treatment Dosage and Administration

After screening and pretreatment, treatment will consist of the following:

Pembrolizumab 200mg IV flat dose will be administered over 30 minutes on Day 1; Pembrolizumab dosing will be repeated every 3 weeks until progression or subject withdrawal for other reasons.

Agent	Dose/Frequency	Route	Schedule
Pembrolizumab	200mg every 3 weeks	IV over 30 minutes	Starting on D1 of study; Every 3 weeks (21 days) until disease progression or withdrawal for other reasons

On-study treatment is 12 weeks of pembrolizumab.

4.3 Toxicities and Dosing Delays/Dose Modifications

4.3.1 Pembrolizumab Administration

Pembrolizumab 200 mg will be administered as a 30-minute IV infusion starting on Day 1 (D1) of the study (hematology labs must meet inclusion criteria to initiate therapy with pembrolizumab). Every effort should be made to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min). The Pharmacy Manual contains specific instructions for the preparation of the pembrolizumab infusion fluid and administration of infusion solution. This manual is provided as a document separate from the protocol.

4.3.1.1 Management of Infusion Reactions for Pembrolizumab

Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Refer to the table below for infusion reaction treatment guidelines:

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab (MK-3475) with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

4.3.1.2 Other Dose Modifications for Pembrolizumab

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per Table below. Also see section 4.3.1.3 for supportive care guidelines, including use of corticosteroids.

Dosing interruptions are permitted in the case of medical/surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated

medical events, patient vacation, and/or holidays). Subjects should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the patient's study record.

If a dose of pembrolizumab is delayed, then the subsequent dose should be administered when symptoms have resolved to a level permissive for re-initiation of therapy.

Dose Modification Guidelines for Pembrolizumab

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

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

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

[2b] If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose; Refer to 4.3.1.1 – Infusion Treatment Guidelines for further management details.

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

4.3.1.3 Rescue Medications and Supportive Care for Pembrolizumab

Supportive Care Guidelines

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

Note: if after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance (as outlined below). Refer to Section 4.3.1.2 for dose modifications.

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

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

- **Diarrhea/Colitis**:

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

  - All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
  - For **Grade 2 diarrhea/colitis** administer oral corticosteroids.
  - For **Grade 3 or 4 diarrhea/colitis**, treat with intravenous steroids followed by high dose oral steroids.

- When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

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

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

- **Hypophysitis**:

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

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

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

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

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

- **Steven's Johnson Syndrome (SJS) and Toxic Epidermal Necrolysis (TEN)**
  - For signs and symptoms of SJS or TEN, withhold pembrolizumab and refer the patient for specialized care for assessment and treatment
  - If SJS or TEN is confirmed, permanently discontinue pembrolizumab

- **Immune-mediated myocarditis management**
  - For suspected immune-mediated myocarditis, ensure adequate evaluation to exclude other etiologies and administer corticosteroids as appropriate

- **Infusion Reaction:**

  See section 4.3.1.1

4.4 Concomitant Medications/Treatments

Medications or vaccinations specifically prohibited in the exclusion criteria provided in section 3.2 are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the Merck Clinical team. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician.

4.4.1 Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care.

All concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF.

All concomitant medications received within 28 days before the first dose of drug treatment and 30 days after the last dose of pembrolizumab should be recorded. Concomitant medications administered within 30 days after the last dose of pembrolizumab treatment should be recorded for SAEs and events of clinical interest (ECIs) as defined in Section 7.3.3.

4.4.2 Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase

- Anti-cancer systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the Principal Investigator. **Note**: This does not include episodic use (up to 7 consecutive days) of systemic corticosteroids for other general conditions (e.g. pre-medication for radiographic imaging due to IV contrast allergy, COPD exacerbation, poison ivy, etc.)

- Radiation therapy
  - Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed at the investigator's discretion.

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

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

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

4.5 Duration of Therapy

Treatment may continue until one of the following occurs:

- Disease progression
  Note: For unconfirmed radiographic disease progression, please see Section 6.7
- Inter-current illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Pregnancy
- Patient decides to withdraw from study treatment, **OR**
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator

Pembrolizumab is an FDA-approved therapy for metastatic melanoma. In addition, the primary endpoint of the study is correlation between baseline AMT PET parameters and antitumor response by RECIST criteria at 12 weeks. Secondary endpoint is correlation of AMT PET parameters with PFS. It is anticipated that a considerable number of patients will have durable responses to therapy. To balance between study endpoints, sufficient follow up time, and resources patients who have had clinical benefit (complete response, partial response, stable disease) and can receive pembrolizumab per standard of care (not as part of protocol treatment).

4.6 End of Treatment

End of treatment visits can occur under the following circumstances:

-Patients permanently stop study treatment for disease progression or investigator/patient preference. Such visits should be performed 30 days (±7 days) after the last dose of pembrolizumab.

-Patients have on ongoing ≥grade 2 or serious AE (SAE). They should continue to be followed until the event is resolved or deemed irreversible by the investigator.

4.7 Duration of Follow Up

Subjects will be followed for up to 3 years after removal from study treatment or until death, whichever occurs first. Patients removed from study for unacceptable adverse events (AEs) will be followed until resolution or stabilization of the event(s).

The long-term follow-up visit is defined as 90 days (±15 days) after the last dose of treatment (see Section 6.1). All adverse events should be followed until this initial long-term follow-up visit (90 days after last pembrolizumab dose). Subsequent follow-up visits, defined as every 90 days thereafter (±15 days) for up to 3 years or until death (whichever is first) may be conducted via telephone or

chart review. These visits will be limited to history of any subsequent cancer treatments, an assessment of any SAE's considered to be possibly or probably related to study treatment until resolution, and survival status.

4.8 Removal of Patients from Protocol Therapy

Patients will be removed from protocol therapy and the PI notified when any of the criteria listed in section 4.5 apply. The reason for discontinuation of protocol therapy will be documented on the eCRF.

In case a patient decides to prematurely discontinue protocol therapy ("refuses treatment"), the patient 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.

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

4.9 Study Withdrawal

If a patient 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 as completely as possible the reason for the study withdrawal.

- The patient 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 patient's study withdrawal should be obtained with an explanation of why the patient is withdrawing from the study.
- If the patient 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 patient from the study is an adverse event, the principal specific event will be recorded on the eCRF.

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

5.0 DRUG INFORMATION

5.1 Pembrolizumab

5.1.1 Description

Investigational Clinical Supplies will be provided by Merck as summarized in the table below.

Product Name & Potency	Dosage Form
100 mg/4 mL (25 mg/mL)	Solution

Pembrolizumab (MK-3475) 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 (MK-3475). The product is preservative-free, latex free solution which is essentially free of extraneous particulates.

See the pharmacy manual, provided as a separate document from the protocol.

5.1.2 Supplier/How Supplied

Pembrolizumab will be provided at no cost to the study patient by Merck, the manufacturer of the drug, for the 12week duration of the study. Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

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

5.1.3 Handling and Dispensing

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label. *See the pharmacy manual, provided as a separate document from the protocol*.

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.

5.1.4 Storage and Stability

As per the pharmacy manual, provided as a document separate from the protocol.

5.1.5 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 investigational product will be discarded at the site per institutional policy (e.g., 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.

5.1.6 Adverse Events Associated with Pembrolizumab

The most common adverse reactions (reported in ≥20% of patients in clinical trials of pembrolizumab) included fatigue, cough, nausea, pruritus, rash, decreased appetite, constipation, arthralgia, and diarrhea. The following warnings are associated with the use of pembrolizumab:

Immune-Mediated Pneumonitis
Pneumonitis occurred in ~3% of melanoma patients treated in clinical trials of pembrolizumab. The median time to development of pneumonitis was 5 months with a median duration of 4.9 months. The one patient with Grade 3 pneumonitis required initial treatment with high-dose systemic corticosteroids (greater than or equal to 40 mg prednisone or equivalent per day) followed by a corticosteroid taper. Pneumonitis completely resolved in seven of the nine patients with Grade 2-3 pneumonitis.

Immune-Mediated Colitis
Colitis (including microscopic colitis) occurred 1% of melanoma patients treated in clinical trials of pembrolizumab. The median time to onset of was 6.5 months with a median duration of 2.6 months. All three patients with Grade 2 or 3 colitis were treated with high-dose corticosteroids (greater than or equal to 40 mg prednisone or equivalent per day).

Immune-Mediated Hepatitis
Hepatitis (including autoimmune hepatitis) occurred in 0.5% of melanoma patients treated in clinical trials of pembrolizumab. The time to onset was 22 days for the case of Grade 4 hepatitis, which lasted 1.1 months. The patient with Grade 4 hepatitis permanently discontinued pembrolizumab and was treated with high-dose (greater than or equal to 40 mg prednisone or equivalent per day) systemic

corticosteroids followed by a corticosteroid taper. Both patients with hepatitis experienced complete resolution of the event.

Immune-Mediated Hypophysitis

Hypophysitis occurred in 0.5% of melanoma patients treated in clinical trials of pembrolizumab. The time to onset was 1.7 months for the patient with Grade 4 hypophysitis and 1.3 months for the patient with Grade 2 hypophysitis. Both patients were treated with high-dose (greater than or equal to 40 mg prednisone or equivalent per day) corticosteroids followed by a corticosteroid taper and remained on a physiologic replacement dose.

Renal Failure and Immune-Mediated Nephritis

Nephritis occurred in 3 (0.7%) patients of melanoma patients treated in clinical trials of pembrolizumab, consisting of one case of Grade 2 autoimmune nephritis (0.2%) and two cases of interstitial nephritis with renal failure (0.5%), one Grade 3 and one Grade 4. The time to onset of autoimmune nephritis was 11.6 months after the first dose of pembrolizumab (5 months after the last dose) and lasted 3.2 months; this patient did not have a biopsy. Acute interstitial nephritis was confirmed by renal biopsy in two patients with Grades 3-4 renal failure. All three patients fully recovered their renal function with treatment with high-dose corticosteroids (greater than or equal to 40 mg prednisone or equivalent per day) followed by a corticosteroid taper.

Immune-Mediated Hyperthyroidism

Hyperthyroidism occurred in 5 (1.2%) of 411 melanoma patients treated in clinical trials of pembrolizumab. The median time to onset was 1.5 months and the median duration was 2.8 months (range 0.9 to 6.1). One of two patients with Grade 2 and the one patient with Grade 3 hyperthyroidism required initial treatment with high-dose corticosteroids (greater than or equal to 40 mg prednisone or equivalent per day) followed by a corticosteroid taper. One patient (0.2%) required permanent discontinuation of pembrolizumab due to hyperthyroidism. All five patients with hyperthyroidism experienced complete resolution of the event.

Immune-Mediated Hypothyroidism

Hypothyroidism occurred in 34 (8.3%) of 411 melanoma patients treated in clinical trials of pembrolizumab. The median time to onset of hypothyroidism was 3.5 months. All but two of the patients with hypothyroidism were treated with long-term thyroid hormone replacement therapy. The other two patients only required short-term thyroid hormone replacement therapy. No patient received corticosteroids or discontinued pembrolizumab for management of hypothyroidism. Thyroid disorders can occur at any time during treatment.

Other Immune-Mediated Adverse Reactions

Other clinically important immune-mediated adverse reactions can occur. The following clinically significant, immune-mediated adverse reactions occurred in

less than 1% of patients treated with pembrolizumab, including exfoliative dermatitis, uveitis, arthritis, myositis, pancreatitis, hemolytic anemia, partial seizures arising in a patient with inflammatory foci in brain parenchyma, and adrenal insufficiency.

Across clinical studies with pembrolizumab in approximately 2,000 patients, the following additional clinically significant, immune-mediated adverse reactions were reported in less than 1% of patients: myasthenic syndrome, optic neuritis, and rhabdomyolysis.

Embryofetal Toxicity
Based on its mechanism of action, pembrolizumab may cause fetal harm when administered to a pregnant woman. Animal models link the PD-1/PD-L1 signaling pathway with maintenance of pregnancy through induction of maternal immune tolerance to fetal tissue.

Steven's Johnson Syndrome (SJS) and Toxic Epidermal Necrolysis (TEN)
The risk of SJS and TEN is reported at approximately 0.4 – 7 cases per million patient years in the general adult population. Independent risk factors include certain medications such as anticonvulsants, sulfonamides, aminopenicillins, allopurinol, and NSAIDs. Non-medication triggers include infection, contrast media, and vaccinations. Malignancy is associated with an increased mortality rate in patients with SJS and TEN.

Myocarditis
A total of 6 cases of myocarditis have been reported in patients treated with pembrolizumab in clinical trials in an expanded access program. There was one fatal case reported in a clinical trial. Immune-mediated myocarditis should be suspected if other causes of myocarditis such as infection or prior radiation therapy have been excluded. Risk factors include certain medications and treatment modalities such as radiation, anthracycline, alkylating agents and most recently checkpoint inhibitors.

5.1.7 Contraception

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

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 120 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. In order to participate in the study subjects of childbearing potential must adhere to the contraception requirement (described above) before C11-AMT PET imaging is performed starting at the time of consent and continued throughout the study period up to 120 days after the last dose of trial therapy (i.e., pembrolizumab). 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.

5.1.8 Use in Pregnancy

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

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

5.1.9 Use in Nursing Women

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

5.1.10 Overdose

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

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

If a dose of Merck's product meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect."
All reports of overdose with and without an adverse event must be reported within 24 hours to the Sponsor and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220) (see section 7.3.3.1)

5.1.11 Risks Associated with C11-AMT PET

As with any radiotracer, subjects will be exposed to additional radiation above the natural background/cosmic radiation exposure. In particular, the radiation dose following the C11-AMT will be approximately 0.325 rem, which is equivalent to extra 1.1 years of the radiation exposure that everyone receives from annual natural background radiation. In addition, there is at least a theoretical risk of infection after injection of C11-AMT due to bacterial contamination of the product during the process of manufacturing C11-AMT. This is minimized by filtering the product and performing sterility testing prior to injection. Finally, the product may contain impurities other than C11-AMT; these are either byproducts of the chemical reactions used to generate C11-AMT or solvents used during the process to purify C11-AMT. This will be controlled by testing the final product using gas chromatography to ensure chemicals used to clean the reaction vessel have been properly removed. It is possible that up to 5% of ethanol may be present in the final product. Given that the total amount to be injected will be only a few mL, subjects may rarely experience symptoms similar to ingestion of less than a glass of wine.

6.0 EVALUATIONS AND ASSESSMENTS

6.1 Time and Events Table (After screening perform C11-AMT scan and then a research biopsy prior to Cycle 1/Day 1 of pembrolizumab)

Procedure	Screening Period 1[1,2]	Pretreatment Period[1]	D1 Cycle 1[3]	D1 Cycle 2[3]	D1 Cycle 3[3]	D1 Cycle 4[3]	End of StudyTreatment[4]	Long-term Follow-up[4]
Informed Consent	X							
Medical History	X[5]							X
Physical Exam	X[5]		X[5]	X[5]	X[5]	X[5]	X[5]	
ECOG Performance Status	X		X	X	X	X	X	
CBC w/differential	X		X	X	X	X	X	
Hematology[6]	X		X	X	X	X	X	
INR/PT and PTT	X							
Serum chemistries[7]	X		X	X	X	X	X	
Liver function tests (LFTs)[8]	X		X	X	X	X	X	
Thyroid panel[9]	X				X		X	
Urine or serum pregnancy	X[2,10]		X[10]					
Pembrolizumab IV			X	X	X	X		
Brain MRI[11]	X[2]							
FDG PET[11]	X[2]						X	
CT scan neck chest, abdomen/pelvis[11]	X[2,12]						X	
AMT PET[13]		X						
Toxicity evaluations[14]	X		X	X	X	X	X	X
Fresh tumor biopsy		X						

Concomitant Medications	X		X	X	X	X	X	
Eligibility checklist	X							
Survival documentation								X

Key to Time and Events Table

[1]The time interval between informed consent and initiation of pembrolizumab consists of a screening period and a pretreatment period and overall it should not exceed 28 days. The screening period starts from informed consent and ends immediately before C11-AMT imaging and biopsy which will be performed at UNC Main Campus. This period of C11AMT scanning and research biopsy are inclusively called the "pre-treatment period." During the pre-treatment period, the procedure for fresh biopsy collection will also be performed at UNC. Patients must be deemed eligible prior to entering the pre-treatment period. During the screening period patients will be assessed for eligibility based on history, physical examination, inclusion/exclusion criteria, and blood work. Baseline standard of care radiographic imaging will be performed taking into account timing and type of radiographic imaging prior to study enrollment. Based on the pattern and anatomic location of tumors decisions regarding the bed positions that will be held during the C11-AMT PET imaging session as well as the optimal method and location for fresh biopsy collection are made. Finally, the eligibility will be reviewed by the UNC study coordinator and signed off by the Principal Investigator and Multicenter Project Manager. Submission of source documents will be required at least 4 days before C11-AMT PET imaging is performed. The pre-treatment period starts from the day of C11-AMT PET imaging and ends with initiation of pembrolizumab. It involves assessing the quality of C11-AMT PET imaging with the option to repeat once more if necessary. During the pre-treatment period the procedure for fresh biopsy collection is performed.

[2]Unless otherwise noted, screening evaluations and eligibility packet submissions will be completed at a minimum of 4 days prior to C11-AMT PET imaging. Tumor imaging for clinical purposes (i.e. whole body FDG PET coregistered with whole body CT scan with IV contrast) and assessment of clinical response will be performed at least 5 days before C11-AMT PET scan. The serum β-hCG pregnancy test should be done within 14 days of C11 AMT scan and within 72 hours of initiating pembrolizumab treatment in women of childbearing potential. Brain MRI should be obtained within 8 weeks of C11-AMT PET imaging.

[3]A window of ±2 days will apply to all study visits including treatment visits. CBC w/diff, chemistry, and LFTs on day 1, cycle 1 of pembrolizumab will be repeated only if >7 days have elapsed between screening laboratory tests and day 1.

[4]Study-related treatment with pembrolizumab is 12 weeks (or earlier if patient progresses). The end of study treatment visit should occur under the following circumstances: (1) Patients permanently stop pembrolizumab for disease progression or investigator/patient preference. Such visits should be performed 30 days (±7 days) after the last dose of pembrolizumab. (2) Patients who have an ongoing ≥grade 2 or serious AE (SAE) should be followed until the event is resolved or deemed irreversible by the investigator. (3) Patients who are still experiencing clinical benefit (partial response, complete response, disease stability) after 12 weeks of pembrolizumab treatment will discuss with their clinician to continue pembrolizumab as per standard of care (i.e., off study-related treatment but follow up will continue as specified in Section 6.4), especially if they are tolerating treatment well (i.e., are experiencing no ongoing SAEs), wish to continue receiving pembrolizumab.

The first long-term follow-up visit is defined as 90 days (±15 days) after the last dose of on-study treatment. Serious adverse events (SAEs; or follow-up to any SAEs) that occur within 90 days of the end of pembrolizumab (or prior to start of new anti-cancer therapy, whichever is earlier) or any grade of Events of Clinical Interest (see section 7.3 that occur within 90 days of the end of pembrolizumab or 30 days after the initiation of a new anti-cancer therapy (whichever is earlier) must be recorded. Subsequent follow-up visits, defined as every 3 months (90 days) thereafter (±15 days) for up to 3 years or until death (whichever is first) may be conducted via telephone or

chart review. These follow up visits will be limited to history of any subsequent cancer treatments, an assessment of any SAE's considered to be possibly or probably related to pembrolizumab treatment until resolution, date of disease progression on pembrolizumab, and survival status.

[5]Complete history at baseline, physical exam to include height (baseline only), weight and vital signs (BP, respiratory rate, and temperature).

[6]Hgb, ANC, and platelets

[7]Serum chemistries and electrolytes to include sodium, potassium, bicarbonate, chloride, LDH, BUN, serum creatinine, glucose, calcium, magnesium, total protein, albumin.

[8]LFTs include total bilirubin, AST and ALT.

[9]Thyroid panel includes TSH, T3 and free T4. This test should be performed at screening, D1 of cycle 3, and at the end of treatment.

[10]A urine or serum pregnancy test (β-hCG) is required for all women of childbearing potential at screening within 14 days of the C11-AMT scan and 72 hours before the first dose of pembrolizumab.

[11]Optimal radiographic tumor imaging for baseline clinical purposes includes whole body CT scan (chest, abdomen, and pelvis; add neck, if clinically necessary) with IV contrast coregistered with whole body ^{18}F-fluorodeoxyglucose (FDG) PET. Brain MRI (or head CT with IV contrast, if MRI is not applicable) is not required at screening if it has been performed within 8 weeks from C11-AMT imaging and was negative for parenchymal brain metastases or leptomeningeal disease. Tumor imaging requirements at screening may be adjusted to take into account the type of tumor imaging done before study enrollment (i.e. whole-body CT scan –chest, abdomen, and pelvis; add neck, if applicable- with IV contrast alone or whole body FDG PET coregistered with CT scan without IV contrast) in relation with timing of C11-AMT PET imaging or pembrolizumab initiation. See section 4.1 for details. Scans for disease assessment will be done at baseline and week 12 (or earlier if patient progresses). If patients continue to have clinical benefit (complete response, partial response, disease stability) after 12 months from pembrolizumab treatment they can continue to be imaged per standard of care.

[12]CT scan of the chest abdomen and pelvis (add neck, if applicable) will be performed twice at baseline: (A) coregistered with FDG PET; in this case IV contrast will be administered, (B) coregistered with C11-AMT PET; in this case no IV contrast will be administered with CT and radiology report will be issued for clinical assessment of tumor burden.

[13]Limited (i.e. not whole body; at least 1 bed position) scan will be performed as described in sections 4.1.1 and 4.1.2 and before fresh tumor biopsy.

[14]Observer-rated toxicity will be assessed using the NCI-CTCAE v4.03

[15]Fresh biopsy is mandatory for all patients (except for patients with metastatic lung disease in which case it is optional) and will be performed during the pre-treatment period at least 24 hours following C11-AMT PET imaging and before pembrolizumab. Further details to be provided in a study manual.

6.2 Pre-Study Assessments

6.2.1 Screening Period

Clinical evaluation: complete history; physical exam to include height (baseline only) and weight; ECOG performance status (see Appendix A ECOG Performance Status)

Laboratory studies:

- **Pregnancy Test**: A urine or serum pregnancy test (β-hCG) is required for all women of childbearing potential at screening within 14 days prior to C11-AMT PET imaging
- **Hematology**: Hgb, absolute lymphocyte count, and platelet count
- **CBC w/differential**
- **Serum Chemistries**: These include the following parameters: sodium, potassium, bicarbonate, chloride, LDH, BUN, creatinine, glucose, calcium, magnesium, albumin, and total protein (or calculate creatinine clearance via Cockcroft-Gault (see Appendix B Cockcroft-Gault Formula and as noted in inclusion criterion #9)
- **LFTs:** These include total bilirubin, AST (SGOT), ALT (SGPT)
- **INR and PT or PTT** (Note: Patient entering the study while receiving coumadin for anti-coagulation therapy or those who will initiate coumadin for anti-coagulation therapy should have their coagulation test performed per standard of care)
- **Thyroid Panel:** TSH, free T4 (prior to cycles 1 and 3)

Concomitant Medications**:** Review (see section 4.4)

Toxicity Assessment**:** Perform per NCI CTCAE v4.03

Tumor imaging for clinical purposes**:** Baseline tumor imaging for clinical purposes should include ^{18}F-fluorodeoxy glucose (FDG PET) whole body co-registered with computed tomography (CT) of the neck, chest, abdomen and pelvis) with IV contrast and brain MRI.

6.2.2 Pre-treatment Period

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- **Tumor imaging for research purposes**: Baseline tumor imaging should include C11-AMT PET co-registered with computed tomography (CT) of the neck, chest, abdomen and pelvis) with no IV contrast.
- **Fresh tumor biopsy:** Collect a fresh biopsy to support correlative studies. Ideal fresh tumor biopsy type is excisional followed by (in order of preference) punch, followed by core needle biopsy using a 18g needle.

6.3 Treatment Assessments

6.3.1 Study D1 of Cycle 1 (Perform all assessments prior to study drug administration)

Clinical evaluation: physical exam; ECOG performance status (see Appendix A ECOG Performance Status)
Laboratory studies (*Repeat if > 7 days have elapsed since screening labs obtained) :

- ***Hematology**: Hgb, ANC, and platelet count
- ***CBC w/differential**
- ***Serum Chemistries**: These include the following parameters: sodium, potassium, bicarbonate, chloride, LDH, BUN, creatinine, glucose, calcium, magnesium, albumin, and total protein (or calculate creatinine clearance via Cockcroft-Gault (see Appendix B Cockcroft-Gault Formula)
- **Pregnancy test in WOCBP** (w/in 72 h of pembrolizumab dosing)
- ***LFTs:** These include total bilirubin, AST (SGOT), ALT (SGPT)

Pembrolizumab administration: 200mg, IV flat dose over 30 min
Concomitant Medications**:** Review (see section 4.4)
Toxicity Assessment**:** Perform per NCI CTCAEv4.03

6.3.2 Study D1 of Cycles 2-4 (Perform all assessments prior to study drug administration)

Clinical evaluation: physical exam; ECOG performance status (see Appendix A ECOG Performance Status)
Laboratory studies:

- **Hematology**: Hgb, ANC, and platelet count
- **CBC w/differential**
- **Serum Chemistries**: These include the following parameters: sodium, potassium, bicarbonate, chloride, LDH, BUN, creatinine, glucose, calcium, magnesium, albumin, and total protein (or calculate creatinine clearance via Cockcroft-Gault (see Appendix B Cockcroft-Gault Formula)
- **LFTs:** These include total bilirubin, AST (SGOT), ALT (SGPT)
- **Thyroid Panel:** TSH, T3, free T4 (prior to cycle 3)

Pembrolizumab administration: 200mg, IV flat dose over 30 min
Concomitant Medications**:** Review (see section 4.4)
Toxicity Assessment**:** Perform per NCI CTCAEv4.03

6.3.3 End of treatment (or D1 of cycle 5*)

***Note**: If subject is benefitting he/she may continue pembrolizumab per SOC after discussion with subject's oncologist (i.e., off study-related treatment but follow up will continue per standard of care if subject continues pembrolizumab and as specified in Section 6.4)

Clinical evaluation: physical exam; ECOG performance status (see Appendix A ECOG Performance Status)
Laboratory studies:

- **Hematology**: Hgb, ANC, and platelet count
- **CBC w/differential**
- **Serum Chemistries**: These include the following parameters: sodium, potassium, bicarbonate, chloride, LDH, BUN, creatinine, glucose, calcium, magnesium, albumin, and total protein (or calculate creatinine clearance via Cockcroft-Gault (see Appendix B Cockcroft-Gault Formula)
- **LFTs:** These include total bilirubin, AST (SGOT), ALT (SGPT)
- **INR and PT or PTT** (Note: Patient entering the study while receiving anti-coagulation therapy or those who have the initiation of an anti-coagulation therapy should have their coagulation test performed on a weekly basis)
- **Thyroid Panel:** TSH, T3, free T4

Concomitant Medications**:** Review (see section 4.4)
Toxicity Assessment**:** Perform per NCI CTCAEv4.03
Tumor imaging for clinical purposes on D1 of Cycle 5**:** Tumor imaging to assess metabolic response following 12 weeks on pembrolizumab should include ^{18}F-fluorodeoxy glucose (FDG PET) whole body coregistered with computed tomography (CT) of the neck, chest, abdomen and pelvis) with IV contrast.

6.4 Long-term follow up (90 days after week 12 dose of pembroluzimab)

Clinical evaluation: focused history on symptoms/toxicity.
Toxicity: Per NCI CTCAEv4.03; Serious adverse events (SAEs; or follow-up to any SAEs) that occur within 90 days of the end of pembrolizumab (or prior to start of new anti-cancer therapy, whichever is earlier) or any grade of Events of Clinical Interest (see section 7.3) that occur within 90 days of the end of pembrolizumab or 30 days after the initiation of a new anti-cancer therapy (whichever is earlier) must be recorded.

Survival analysis:

Note: Subsequent follow-up visits to occur every 90 days (± 15 days) for up to 3 years or until death (whichever is first) may be conducted via telephone or chart review, and will be limited to history, date of progression on or following pembrolizumab therapy, if any subsequent cancer treatments were given, an assessment of any SAE's considered to be possibly or probably related to study treatment until resolution, and survival status.

6.5 Correlative Studies Procedures

These are described in more detail in the study procedures laboratory manual.

6.5.1 Tumor Biopsy

All patients must provide a fresh tumor biopsy prior to initiation of therapy. Exceptions to the mandatory biopsy requirement include patients with lung parenchymal disease; in that case, optional biopsy will be offered with the caveat that the risk of pneumothorax can be up to 20%. See accompanying laboratory manual for additional details, including details on shipping and storage.

6.5.2 Handling of Biospecimens Collected for Correlative Research

Biospecimens collected for this study will be stored in the Lineberger Comprehensive Cancer Center (LCCC) Tissue Procurement Facility (TPF), or if needed, in a secure off-site storage facility. All biospecimen samples will be obtained in accordance with procedures outlined in the LCCC1528 Study Laboratory Manual and stored in containers with controlled access. Each sample will be assigned a unique code number and no identifiable personal health information (PHI) will be on the specimen label. Information about the patient'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 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 patient's participation in the clinical study may also be included. This information may be important for understanding how the patient'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 patient to use their leftover tissue for other research purposes (e.g., TPF consent form was signed by the patient). 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. In this case, if consent was obtained, leftover tissue will be available for analysis, as described in LCCC 1528).

Compliance Statement

Biospecimen collection for this study will be conducted in full accordance to all applicable University of North Carolina (UNC) 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 and assent (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.

6.6 Assessment of Safety

Any patient who receives treatment on this protocol will be evaluable for toxicity. Each patient will be assessed periodically for the development of any toxicity according to the Time and Events table (section 6.0). Toxicity will be assessed according to the NCI CTCAE v4.03. Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

For subjects receiving treatment with pembrolizumab, all adverse events (AEs) of unknown etiology associated with pembrolizumab exposure should be evaluated to determine if it is an Event of Clinical Importance (ECI) with a potential immunologic etiology (termed immune-related adverse events, or irAEs); see the separate ECI guidance document (provided as a document separate from this protocol) regarding the identification, evaluation and management of potential irAEs.

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

Please refer to section 7.0 for detailed information regarding the assessment of toxicity and recording of AEs.

6.7 Assessment of Efficacy

Subjects who have completed C11-AMT PET, FDG PET and CT scan of the neck, chest, abdomen, and pelvis and have received at least 2 doses of pembrolizumab will be evaluable for assessment of response and progression. Subjects whose cancer growth is documented by physical examination without imaging confirmation will count as progression. Patients who end study therapy for any reason (e.g., toxicity of treatment, decide to withdraw) will still be followed for PFS and OS.

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

See the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee, version 1.1 [71] for additional details on RECIST1.1.

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:

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

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

All other lesions, including small lesions (longest diameter <10mm or pathological lymph nodes with ≥10 to <15mm 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 within 4 weeks from pembrolizumab treatment. The same method of assessment and the same technique should be used to characterize each identified and reported 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 ≥10mm diameter as assessed using calipers (e.g. skin nodules). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesions is recommended.

6.7.2 Baseline Documentation of Target and Non-Target Lesions

All measurable lesions up to a maximum of 5 lesions total (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".

6.7.3 Evaluation of Target Lesions using RECIST 1.1 Criteria

NOTE: See Response Evaluation Criteria in Solid Tumors (RECIST) Committee, version 1.1[71] for special notes on the assessment of target lesions.

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 <10mm.

Partial response (PR)−At least a 30% decrease in the sum of the 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 5mm. 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.

6.7.4 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 (<10mm short axis).

Non-complete response (non-CR)/non-progression (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.

6.7.5 Evaluation of Best Overall Response using RECIST 1.1 Criteria

NOTE: Please see [71] for complete details.

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 patient 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

6.7.6 Immune-related Response Criteria

For all patients who experience disease progression on study by RECIST 1.1 criteria, they are allowed to be treated beyond initial progressive disease per immune-related response criteria (irRC).

Definitions of measurable and non-measurable disease

Measurable disease: Neoplastic masses that can be precisely measured in 2 in-plane perpendicular diameters. Both its longest diameter and its longest perpendicular must be greater than or equal to 10 mm or 2 times the axial slice thickness if greater than 5 mm. Lymph nodes must have a short-axis line-length of ≥ 15 mm. Malignant lymph nodes must be measurable in 2 perpendicular diameters. Both its longest diameter and it longest perpendicular must be greater than or equal to 15 mm. The quantitative endpoint will be defined as the product of the longest diameter with its longest perpendicular.

Non-measurable disease: Non-measurable lesions are those that are not suitable for quantitative assessment over time. These include:
1) Neoplastic masses that are too small to measure, because their longest uninterrupted diameter or longest perpendicular are less than 10 mm.
2) Neoplastic masses whose boundaries cannot be distinguished. This includes masses which cannot be demarcated from surrounding tissue

7.0 ADVERSE EVENTS

7.1 Definitions

7.1.1 Adverse Event (AE)

An adverse event (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 patient or clinical investigation subject administered a pharmaceutical product 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.

Adverse events may occur during the course of the use of Merck product in clinical trials, or as prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

Progression of the cancer under study is not considered an adverse event.

All adverse events that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.
From the time of treatment through 30 days following cessation of treatment, all adverse events must be reported by the investigator. Such events will be recorded at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in section 7.2.3.1. The investigator will make every attempt to follow all subjects with non-serious adverse events for outcome.

Adverse events will not be collected for subjects during the pre-screening period (for determination of archival tissue status) as long as that subject has not undergone any protocol-specified procedure or intervention. If the subject requires a blood draw, fresh tumor biopsy etc., the subject is first required to provide consent to the main study and AEs will be captured according to guidelines for standard AE reporting.

7.1.2 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 adverse event 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

7.1.3 Unexpected AE or SAR

An AE or SAR is considered unexpected if the specificity or severity of it is not consistent with the applicable product information (e.g., Investigator's Brochure (IB) 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 IB 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.

7.1.4 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 serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in 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 serious adverse events.

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

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

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

7.2 Documentation of non-serious AEs or SARs

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

Collected information should be recorded in the Case Report Forms (CRF) for that patient. 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.

7.3 SAEs or Serious SARs

7.3.1 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 90-day follow-up period after treatment is discontinued (or to the initiation of new anti-cancer treatment, whichever is earliest).

7.3.2 Documentation and Notification

These events (SAEs or Serious SARs) must be recorded in the SAE console within Oncore™ for that patient within 24 hours of learning of its occurrence.

7.3.3 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 (see section 9.5.3) within 7 days of the Investigator becoming aware of the problem.

Affiliate sites (Required wording if multicenter):

- 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 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

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them) that occurs during the trial.

Pregnancies and lactations that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if they cause the subject to be excluded from the trial or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

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).

Pregnancies and lactations that occur from the time of treatment allocation/randomization through 120 days following cessation of Sponsor's product, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, must be reported by the investigator. All reported pregnancies must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

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

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 Multicenter 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.

FDA Expedited Reporting requirements:
A sponsor must report any suspected adverse reaction that is both serious and unexpected to the FDA. The sponsor must report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event, such as:

- A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome);
- One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g. tendon rupture);
- An aggregate analysis of specific events observed in a clinical trial (such as known consequences of underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

The sponsor must submit each IND safety report on FDA Form 3500A. Each notification to FDA must bear prominent identification of its contents, i.e., "IND Safety Report," and must be transmitted to the review division that has the responsibility for review of the IND. In each IND safety report, the sponsor must identify all IND safety reports previously submitted to FDA concerning a similar suspected adverse reaction and must analyze the significance of the suspected adverse reaction in light of previous, similar reports or any other relevant information.

Timing

FDA must be notified of potential serious risks within 15 calendar days after the sponsor determines the event requires reporting. FDA must be notified of unexpected fatal or life-threatening suspected adverse reactions as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information. For Multicenter trials, Lineberger is the sponsor, therefore, the Multicenter Project Manager must be notified of the SAE within 24 hours of the event. If the results of a sponsor's investigation show that an adverse event not initially determined to be reportable is reportable, the sponsor must report such suspected adverse reaction in an IND safety report as soon as possible, but in no case later than 15 calendar days after the determination is made.

Follow-up

The sponsor must promptly investigate all safety information it receives. Relevant follow-up information to an IND safety report must be submitted as soon as the information is available and must be identified as such, i.e., "Follow-up IND Safety Report." Additionally, upon request from FDA, the sponsor must submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

Notification of Investigators

The sponsor must notify all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting.

Process

If the sponsor deems that an event is both a serious SAR AND unexpected, it must also (in addition to Oncore) be recorded on the MedWatch Form 3500A as per 21 CFR 312.32. Unexpected adverse events 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.

The MedWatch form and supporting documents defining the event and causality should be faxed to the Multicenter Project Manager at 919-966-4300 (or emailed, with address provided at the Start up Meeting (SIM)) along with supporting documentation defining the event and causality. The Multicenter Project Manager will then send the report to the Funding Source and notify the Multicenter and CPO Regulatory Associate of the event.
The MedWatch 3500a form can be accessed at:
http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm.

(Please be sure and access form 3500a, and not form 3500).

Once the UNC Principal Investigator determines an event is a serious SAR AND unexpected, the MedWatch 3500A form will be submitted to the FDA. If the event is serious, unexpected and considered to be possibly-, probably- or definitely-related to the study treatment, the Multicenter Project Manager will inform the Regulatory Associate at UNC and IND Specialist. The MedWatch form will be submitted according to LCCC SOP for safety reporting for a multi-site study.

All IND safety reports must be submitted on Form 3500A and be accompanied by Form 1571. The FDA must be notified or any unexpected or life-threatening suspected adverse reactions as soon as possible, but no later than 7 calendar days of learning of the event.

The Multicenter Project Manager will also be responsible for informing each Affiliate site of all serious and unexpected SARs reported to the FDA via fax as soon as possible.

Additional Reporting Requirements
The following additional items must be reported via IND safety report:

- *Findings from other studies.* The sponsor must report any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies, whether or not conducted under an IND, and whether or not conducted by the sponsor, that suggest a significant risk to humans exposed to the drug.
- *Findings from animal or in vitro testing.* The sponsor must report any findings from animal or *in vitro* testing, whether or not conducted by the sponsor, that suggest a significant risk in humans exposed to the drug, such as reports of mutagenicity, teratogenicity, or carcinogenicity, or reports of significant organ toxicity t or near the expected human exposure.
- *Increased rate of occurrence of serious suspected adverse reactions.*

Additional Guidance
Please refer to 21CFR312.32 and "Guidance for Industry and Investigators: Safety Reporting Requirements for INDs and BA/BE Studies" for additional information

and reporting requirements. All IND Safety Reports will be submitted in accordance with these regulations/guidances.

7.3.3.1 Merck Reporting Requirements:

For the time period beginning when the consent form is signed until treatment allocation/randomization, any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any subject must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study whether or not related to the Merck product, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to Merck product that is brought to the attention of the investigator at any time following consent through the end of the specified safety follow-up period specified in the paragraph above, or at any time outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor and to Merck Global Safety.

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

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

All 15-Day Reports and Annual Progress Reports must be submitted as required to FDA. Investigators will cross-reference these reports to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally investigators will submit a copy of these reports to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215 993-1220) at the time of submission to FDA.

Events of Clinical Interest

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

For the time period beginning when the consent form is signed until treatment allocation, any ECI, or follow up to an ECI, that occurs to any subject must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to Merck product, must be reported within 24 hours to the Sponsor and within 24 hours to Merck Global Safety.

Events of clinical interest for this trial include:
1. An overdose of Merck product, as defined in section 5.1.10
2. An elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.

* *Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology. Asymptomatic Gilbert's disease will be an allowed exception.

Pregnancy and Lactation
See above in this section for additional information. Such events must be reported within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220).

Protocol-Specific Exceptions to Serious Adverse Event Reporting
Efficacy endpoints as outlined in this section will not be reported to Merck as unless there is evidence suggesting a causal relationship between the drug and the event. Any such event will be submitted to the Sponsor within 24 hours and to Merck Global Safety within 2 working days either by electronic or paper media. Specifically, the suspected/actual events covered in this exception include any event that is disease progression of the cancer under study.

The Sponsor will monitor efficacy endpoint events and safety data to ensure the safety of the subjects in the trial. Any suspected endpoint which upon review is not progression of the cancer under study will be forwarded to Merck Global Safety as a SAE within 2 working days of determination that the event is not progression of the cancer under study

Hospitalization related to convenience (e.g. transportation issues etc.) will not be considered a SAE.

7.4 Data and Safety Monitoring Plan

The Principal Investigator will provide continuous monitoring of patient safety in this trial with periodic reporting to the Data and Safety Monitoring Committee (DSMC).

Meetings/teleconferences will be held at a frequency dependent on study accrual, and in consultation with the study Biostatistician. These meetings will include the investigators as well as protocol nurses, clinical research associates, regulatory associates, data managers, biostatisticians, 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 patients 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.

8.0 STATISTICAL CONSIDERATIONS

8.1 Study Design/Study Endpoints

This study is designed to explore associations between various parameters from FDG PET and C11-AMT PET imaging at baseline (SUVmax, $k_{1\text{-}4}$ rate constants as assessed using the 3-compartmental model, total tumor metabolic volume, measurement of intra-tumoral and inter-lesion heterogeneity) and antitumor response to pembrolizumab at week 12 (or earlier, if patient progresses) that will be assessed using CT scan of the chest, abdomen and pelvis (add neck, if applicable) with IV contrast and defined via RECIST v1.1. Evaluable patients will be defined as those subjects who have successfully completed baseline FDG PET, C11-AMT PET and CT scan of the chest, abdomen, and pelvis (add neck, if

applicable) with IV contrast, have received at least 2 pembrolizumab infusions every 3 weeks (if progressed earlier than 12 weeks), and have had restaging CT scans with IV contrast to document response, disease stability, of tumor progression.

For quantitation of expression of various proteins or immune cell subsets in representative tissue sections from archived tumor blocks by immunohistochemistry or immunofluorescence, we will use the H-score, as previously described.

8.2 Sample Size and Accrual

A total of 27 patients will undergo C11-AMT PET imaging prior to treatment with pembrolizumab as part of the trial. Of these subjects, we anticipate <5% dropout rate, due to intolerance (pembrolizumab has a safe toxicity profile) or rapid tumor progression. Therefore, we anticipate that the total number of evaluable subjects will be 23.

Based on early clinical trials of patients who received pembrolizumab in the front line setting, the overall response rate (the sum of complete or partial response) is 45% [47, 52]. Therefore, we anticipate that 10/23 patients will respond to treatment. To test the null hypothesis that C11-AMT PET SUVmax is not correlated with response to pembrolizumab in the front line setting, we will compare the means of C11-AMT PET between responders and non-responders. With 10 responders and 13 non-responders we will have 80% power to reject the null hypothesis if the effect size is 1.1. One-sided 0.05 level test will be used.

8.3 Data Analysis Plans

Apart from comparing the means of baseline FDG PET and C11-AMT PET SUVmax in responders and non-responders we will fit logistic a regression model with responder status as outcome and AMT PET SUVmax as a covariate. We will also model time-to-progression as a function of C11-AMT PET using Cox model. These analysis will be repeated with k3 as an outcome.

8.3.1. C11-AMT uptake

Lesions of interest will be identified on clinical non-contrast CT scan and evaluated for C11-AMT uptake. The following C11-AMT PET parameters will be assessed: SUVmean SUVmean tumor-to-background ratio, SUVmax tumor-to-background ratio, total metabolic volume. In addition, intra-tumoral histogram analysis, and interlesion uptake ratios may be applied to investigate intra- and interlesional tumor heterogeneity. Image-guided biopsies will enable local C11-AMT uptake at the biopsy site to be correlated with immunohistochemical findings.

8.3.3. Immunohistochemical analysis of tumor tissues for components of the tryptophan metabolism and classification of tumors as inflamed or not inflamed

Biopsies from tumor tissues that were previously analyzed with FDG PET and C11-AMT PET imaging will provide insights about underlying immune response (inflamed versus inflamed melanomas) and the relative abundance of various

enzymatic components of tryptophan uptake and metabolism. 2-color immunofluorescence analysis will enable us to attribute the signal of various enzymatic components to melanoma (S100+) as opposed to other stromal cells (hematopoietic cells, CD33+). Based on previous reports we already anticipate that presence of CD8+ cells in the vicinity of PD-L1-expressing cells will correlate with response to pembrolizumab. What we do not know is whether TPH, IDO and TDO are also expressed in melanoma tissues along with L system transporters (LAT1, CD98). Perhaps TPH may be also expressed by melanoma cells and/or component of the tumor microenvironment, in which case we should anticipate AMT to be also metabolized via the IDO pathway. It is also quite possible that differences in SUVmax of C11-AMT PET activity may reflect differences in protein abundance of these enzymes/transporters as well as the different cell types that express these enzymes (cancer versus stromal cells). Furthermore, various rate constants may significantly correlate with the expression of any of these enzymes/transporters. For example IDO/TDO may correlate with k_3, and LAT1/CD98 expression may correlate with k_1 or k_4.

Pearson's correlation coefficient will be computed between C11-AMT PET values and expression of components of the IDO pathway (IDO, LAT1, TPH), expression of various immune checkpoint proteins, and density of various immune cell subsets (CD8+, FoxP3+, CD11b+/CD33+). We will also assess the association between expression of components of the IDO pathway (IDO, LAT1, TPH) and k_3, and LAT1/CD98 expression may correlate with C11-AMT uptake.

9.0 STUDY MANAGEMENT

9.1 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 patient 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 patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB-approved consent form.

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

9.2 Required Documentation

Before the study can be initiated at any site, the following documentation must be provided to the Clinical Protocol Office (CPO) at the University of North Carolina.

- 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.
- Form FDA 1572 appropriately filled out and signed with appropriate documentation
- Financial disclosures
- CAP and CLIA Laboratory certification numbers and institution lab normal values
- Executed clinical research contract

9.3 Registration Procedures

[redacted]

For Affiliate patients, at the time of consent, please complete the New Subject Registration Patient Form. Scan and email the un-redacted signed consent and registration form to the Multicenter Project Manager, UNC Study Coordinator and UNC PI on the day of consent. A sequence number will be confirmed on the New Subject Patient Registration Form to the Affiliate Site, UNC Study Coordinator and UNC PI by the Multicenter Project Manager. Please allow 4 days between submission of source documents and potential C11AMT scan date (first event within the pre-treatment period) for review of source and eligibility confirmation.

[redacted]

9.4 The UNC Study Coordinator will review source documents for preparation of the Pre-Treatment Period that will occur at the UNC-Main Campus. Once Eligibility is verified by the UNC PI and Multicenter Project Manager, the patient will be registered as eligible to enter the pre-treatment period. 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 affiliate study teams at participating institutions. The investigators at each site will allow monitors to review all source documents supporting data entered into OnCore®. [redacted]

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.

9.5 Adherence to the Protocol

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

9.5.1 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.

<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>
<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:</u>

- 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).

9.5.2 Single Patient/Subject Exceptions

For Institutions Relying on UNC's IRB:
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.

9.5.3 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:

For Institutions Relying on UNC's IRB:
Protocol Deviations: UNC 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.

For Institutions Relying on Their Own IRB:
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 Regulatory Associate/Multicenter Project Manager within 5 days. UNC-CH will determine if the violation affects the safety of the patient and integrity of the data. Once your institution's IRB response is received, please forward to the Multicenter Regulatory Associate.

Unanticipated Problems:

UNC
Any events that meet the criteria for "Unanticipated Problems" as defined by UNC's IRB must be reported 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 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.

9.6 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator at UNC. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the patient, 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'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 multi-center studies, any affiliate site must submit their informed consent

revisions to the Multicenter Regulatory Associate prior to submission to their IRB.

9.7 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 patient 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 study 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.

9.8 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 at each institution or site is responsible for personally overseeing the treatment of all study patients. 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 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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11.0 APPENDICES

11.1 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.	

11.2 Appendix B Cockcroft-Gault Formula

$$\text{Estimated creatinine clearance (mL/min)} = \frac{(140 - \text{age in years}) \times (\text{weight in kg})}{72 \times (\text{serum creatinine in mg/dL})}$$

For females, use 85% of calculated creatinine clearance value.