DUHS IRB Application (Version 1.4)

General Information

***Please enter the full title of your protocol:**

Understanding Immunotherapy Resistance Mechanisms in Advanced Melanoma

***Please enter the Short Title you would like to use to reference the study:**

Understanding Immunotherapy Resistance Mechanisms in Advanced Melanoma
* This field allows you to enter an abbreviated version of the Study Title to quickly identify this study.

Add Study Organization(s):

List Study Organizations associated with this protocol:

Primary Dept?	Department Name
◆	**DUHS** - Duke Default Department

Assign key study personnel (KSP) access to the protocol

*** Please add a Principal Investigator for the study:**

(Note: Before this study application can be submitted, the PI MUST have completed CITI training)

Hanks, Brent

3.1 If applicable, please select the Key Study personnel: (Note: Before this study application can be submitted, all Key Personnel MUST have completed CITI training)

A) Additional Investigators, Primary Study Coordinator (CRC), and the Primary Regulatory Coordinator (PRC):

Nixon, Jennifer
Primary Regulatory Coordinator
Wiggs, Carol
Primary Study Coordinator (CRC/CRNC/RPL)

B) All Other Key Personnel

Al-Rohil, Rami, M.D.
Pathologist
Beasley, Georgia
Sub-Investigator
Devito, Nicholas
Collaborator

Eggertson, Shauna
Study Coordinator (CRC/CRNC/RPL)
Goodwin, Jenna
Data Manager
Herrmann, Tara
Sub-Investigator
Honeycutt, Wanda
Study Coordinator (CRC/CRNC/RPL)
Hyslop, Theresa
Statistician
Jung, Sin-Ho
Statistician
Leddy, Margaret
Research/Physician Assistant
Marin, Daniele, M.D.
Collaborator
May, Mitzi
Study Coordinator (CRC/CRNC/RPL)
Mosca, Paul
Sub-Investigator
Oswalt, Cameron
Collaborator
Salama, April
Sub-Investigator
Scheri, Randall
Sub-Investigator
Sturdivant, Michael
Collaborator
Thomas, Joshua
Data Manager
Wiggs, Carol
Study Coordinator (CRC/CRNC/RPL)

***Please add a Study Contact:**

Hanks, Brent
Nixon, Jennifer
Wiggs, Carol

The Study Contact(s) will receive all important system notifications along with the Principal Investigator. (e.g., The study contact(s) are typically the Principal Investigator, Study Coordinator, and Regulatory Coordinator.)

Oncore

Please select the Library for your Protocol:

This field is used in OnCore. Determines the Reference Lists, Forms, Protocol Annotations, Notifications, and Signoffs available for the protocol. Protocols that require reporting to the NCI (National Cancer Institute), must select the Oncology library.

- (•) Oncology
- ( ) Non-Oncology

Protocol Application Type

Select the type of protocol you are creating:

Please see additional criteria and information in the policy titled “Reliance on the IRB of Another Institution, Organization, or an Independent IRB" on the **IRB web site**.

- (●) Regular Study Application - Most common. The IRB will determine if the study is eligible for expedited review or requires full board review upon submission.
- ( ) Application for Exemption from IRB Review - Includes Exempt, Not Human Subject Research, & Not Research.
- ( ) External IRB Application - Any study using an external IRB as the IRB-of-Record.
- ( ) Trainee Research While Away from Duke - Research conducted by medical students overseen by the Office of Curriculum & other student/trainee research away from Duke.
- ( ) Individual Patient Expanded Access, Including Emergency Use - Use of an investigational product under expanded access, including emergency use of an investigational drug or biologic or emergency use of an unapproved device.

Conflict of Interest

Do any of the participating study investigators or other key personnel (or their immediate family/significant other) have a financial or intellectual interest in, or are receiving compensation from, the sponsor or the drugs, devices or technologies used in this research?

( ) Yes (●) No

Are any key personnel an inventor of any of the drugs, devices or technologies used in this research?

( ) Yes (●) No

Do any key personnel have or anticipate (within the year) any financial relationships (e.g., consulting, speaking, advisory boards, patents, equity, options) that could be perceived to overlap or present a conflict of interest with the current research?

( ) Yes (●) No

Do any key personnel have a conflict of interest management plan (issued by the Duke University School of Medicine Research Integrity Office) with this company?

( ) Yes (●) No

Oversight Organization Selection

CRU (Clinical Research Unit) or Oversight Organization Selection:

Please select the CRU.

Oncology

The Clinical Research Unit that takes responsibility for this study.

- More information on CRUs can be found on the Duke Office of Clinical Research (DOCR) website, **http://docr.som.duke.edu**
- Questions concerning CRU selection should be directed to docr.help@dm.duke.edu.
- For questions about the Campus Oversight Organization, please visit **Campus Oversight Organization**.

List all Key Personnel on the study who are outside Duke:

- **Note:** You will also need to attach the documentation of Human Subjects Certification for each individual, if they have completed the certification somewhere other than Duke.
- **If outside key personnel will have access to Duke PHI, a data transfer agreement AND external site IRB approval (or IRB authorization agreement) will be needed.** See HRPP policy **Use of Research Data by Former Duke Students or Former Duke Faculty and Employees**
- In the panel below, "PHI" is Protected Health Information.

Entry 1

Name	
Study Role	
Email Address	
Institution / Organization	
Will he/she have access to Duke P.H.I.?	○ Yes ○ No
Is he/she an unpaid volunteer at Duke on the study?	○ Yes ○ No

Indicate the Protocol source below:

The protocol source is the author of the protocol. If the protocol is a joint authorship between multiple sources, select the primary author.

An IRB fee may be assessed for all research that is supported by for-profit entities and requires full board review. For additional information, see the **IRB fees section of the IRB web site**

- ◉ PI initiated
- ○ Commercial / Industry (for-profit entity) initiated
- ○ Federal Government initiated
- ○ Cooperative Group Initiated
- ○ Foundation (non-profit group) initiated
- ○ Other

Sponsor and Funding Source

Add all funding sources for this study:

View Details	Sponsor Name	Sponsor Type	Contract Type:	Project Number	Award Number
⊟	Duke University	Institutional			

Sponsor Name:	Duke University
Sponsor Type:	Institutional
Sponsor Role:	Funding Protocol Control Data Coordination Monitoring Auditing Coordinating Center;
Grant/Contract Number:	
Project Period:	From: to:
Is Institution the Primary Grant Holder:	No
if No, then who is the Primary Grantee?	
Contract Type:	

Project Number:	
Award Number:	
Grant Title:	
PI Name: (If PI is not the same as identified on the study.)	
Significant Discrepancy:	

⊟	Merck & Co.	Industry			

Sponsor Name:	Merck & Co.
Sponsor Type:	Industry
Sponsor Role:	Funding
Grant/Contract Number:	
Project Period:	From: to:
Is Institution the Primary Grant Holder:	Yes
Contract Type:	
Project Number:	
Award Number:	
Grant Title:	
PI Name: (If PI is not the same as identified on the study.)	
Significant Discrepancy:	

Is this a federally funded study?

○ Yes ◉ No

As part of this study, will any samples or PHI be transferred to/from Duke to/from anyone other than the Sponsor, a Sponsor subcontractor, or a Funding Source?

◉ Yes ○ No

Is the Department of Defense (DOD) a funding source?

○ Yes ◉ No

Have you successfully synced your protocol to OnCore by clicking the 'Sync Data Over API' button at the top of this page?

Please verify that the protocol has been created in OnCore before submitting this application for PI Signoff.

◉ Yes, I synced my protocol to OnCore and verified it was successfully sent by logging into OnCore.
○ I may have forgotten! I'll click it again right now, just to be sure, and verify it was successfully sent by logging into OnCore.
○ This is a DCRI protocol that is not required to be entered into OnCore.

Mobile Devices and Software

Does this study involve the use of a software or a mobile application?

(●) Yes ( ) No

Please describe the following:

- The developer of the mobile app and how the app will be obtained.
- What PHI will be collected via the app.
- Where the data will be stored and who will have access to it.

MaestroCare

List all software, including third party (non-Duke) and mobile apps, that will be utilized for ascertainment, recruitment, or conduct of the research/project: (eg, MaestroCare, DEDUCE):

MaestroCare

Multi-site Research

Is this a multi-site study?

( ) Yes (●) No

Complete for each site if Duke is the Primary grant awardee or coordinating center:

Research Abstract

Please type your Research Abstract here:

The Research Abstract should summarize the main points of your study in one paragraph. The following guidelines may help you:

1. Purpose and objective (1-2 sentences)
2. Study activities and population group (2-4 sentences)
3. Data analysis and risk/safety issues (1-2 sentences)

This study is designed to investigate three related questions pertinent to understanding the mechanisms of resistance to checkpoint inhibitor immunotherapy. First, we are interested in exploring the genetic alterations occurring in persistent melanomas that have responded but have not been eliminated by checkpoint inhibitor immunotherapies. Second, we are interested in examining potential markers that correlate with treatment failure and disease progression during ongoing checkpoint inhibitor immunotherapy. Third, we will investigate baseline markers that correlate with checkpoint inhibitor therapy response..

Research Summary

State your primary study objectives

understanding the mechanisms of resistance to checkpoint inhibitor immunotherapy

State your secondary study objectives

First, we are interested in exploring the genetic alterations occurring in persistent melanomas that have responded but have not been eliminated by checkpoint inhibitor immunotherapies. Second, we are interested in examining potential markers that correlate with treatment failure and disease progression during ongoing checkpoint inhibitor immunotherapy. Third, we will investigate baseline markers that correlate with checkpoint inhibitor therapy response.

Please select your research summary form:

Standard Research Summary Template

This is the regular (generic) research summary template which is required for all regular applications (unless your protocol fits under the other research summary templates in this category). Use of these instructions is helpful for ensuring that the research summary contains all necessary elements.

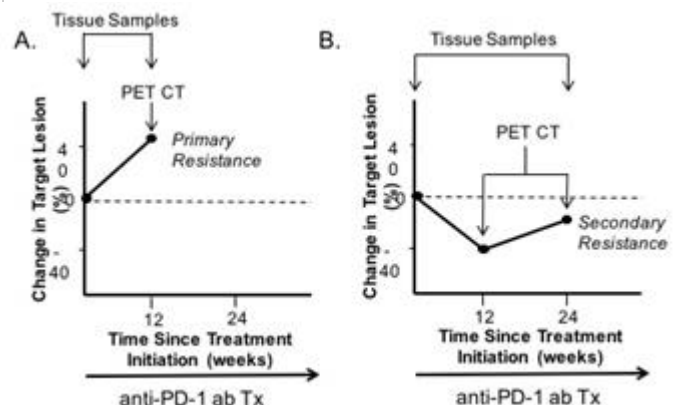
Standard Research Summary

Purpose of the Study

- Objectives & hypotheses to be tested

This study is designed to investigate three related questions pertinent to understanding the mechanisms of resistance to checkpoint inhibitor immunotherapy. First, we are interested in exploring the genetic alterations occurring in persistent melanomas that have responded but have not been eliminated by checkpoint inhibitor immunotherapies. Second, we are interested in examining potential markers that correlate with treatment failure and disease progression during ongoing checkpoint inhibitor immunotherapy. Third, we will investigate baseline markers that correlate with checkpoint inhibitor therapy response.
We are proposing that melanomas which respond and develop eventual disease stability in response to checkpoint inhibitor immunotherapy undergo a genetic program promoting secondary resistance. Understanding these genetic alterations and the factors which contribute to this process would be critical for the identification of novel immunotherapeutic targets which may synergize with the T cell-targeted checkpoint inhibitors. Many patients who exhibit a response to the anti-PD-1 antibodies develop a prolonged course of disease stability which resembles the equilibrium phase of the previously proposed process of cancer immunoediting (8-10) (**Figure 1**). This state of equipoise has been hypothesized to involve genetic alterations that promote immune evasion and, in some cases, lead to the development of tumor escape. Based on our data, we propose that melanomas that develop a period of disease stability in response to anti-PD-1 immunotherapy exhibit genetic alterations that suppress the effectiveness of anti-tumor immunity. Our previous studies indicate that the tumor-derived factors that play a role in the paracrine signaling pathways capable of regulating nearby stromal cell populations are more likely to be critical immune regulators of the tumor microenvironment (5, 6, 11). Therefore, we will focus our studies on those differentially expressed genes which encode soluble proteins using an available prediction algorithm. Those genes identified by this study to be differentially expressed in the melanoma tissues of patients demonstrating a clinical response to immune checkpoint inhibitor therapy will be further evaluated in transgenic autochthonous melanoma models.

Figure 2. Spider Plots of Melanoma Patients Progressing Through Checkpoint Inhibitor Immunotherapy. A. Primary resistance to checkpoint inhibitor immunotherapy. B. Secondary resistance to checkpoint inhibitor immunotherapy.



Previous studies have shown evidence that cancers utilize adaptive resistance mechanisms to evade the anti-tumor immune response. These mechanisms involve the upregulation of negative feedback mechanisms that dampen anti-tumor immunity and include the stimulation of PD-L1 expression as well as the upregulation of the immunoregulatory indoleamine 2,3-dioxygenase (IDO) enzyme (12, 13). The identification of each of these mechanisms has led to the development of effective immunotherapeutic agents that are making

a significant impact on the management of advanced melanoma (2, 14, 15). Based on our preliminary data in a transgenic model of BRAF^{V600E} melanoma, we are proposing the existence of previously unrecognized additional adaptive resistance mechanisms that drive MDSC recruitment and function to suppress the development of an effector T cell response. In order to investigate this hypothesis, we will obtain tissue and blood specimens from advanced melanoma patients at the time of disease progression while undergoing checkpoint inhibitor immunotherapy (**Figure 2**).

Anti-PD-1 antibody immunotherapy is now FDA approved in the adjuvant setting for melanoma patients who previously underwent surgical excision of their disease (16, 17). It is currently unknown how this therapy may modify the behavior of recurrent disease. For example, whether recurrent melanoma in patients who underwent adjuvant anti-PD-1 antibody immunotherapy impacts their sensitivity to future immunotherapy is currently unknown. This protocol will also collect tissue and blood specimens who develop recurrent disease following prior adjuvant anti-PD-1 antibody immunotherapy to investigate the development of potential mechanisms of immunotherapy resistance and to better understand what immunotherapy may be most effective in this setting.

Background & Significance

- Should support the scientific aims of the research

The clinical effectiveness of immunotherapy approaches for the treatment of cancer is now being demonstrated with the recent FDA approvals of the CTLA-4 antagonist, ipilimumab, and the PD-1 antagonists, pembrolizumab and nivolumab, in metastatic melanoma (1, 2). However, the clinical benefit observed for these immunotherapeutic strategies remains restricted to a subset of patients with advanced melanoma and is more limited in patients with other solid tumor malignancies. Emerging data suggest that multiple immune evasion mechanisms utilized by cancers to subvert the host immune response likely play an important role in dampening the clinical effects of these therapies (3, 4). We, as well as others, have shown that many of these mechanisms are dependent upon tumor-induced alterations in local immune cell populations that ultimately lead to the development of an immunotolerant microenvironment (5, 6). However, the identity of many of these tumor-derived factors that suppress the local immune response are unknown and whether or not these processes of immune evasion contribute to resistance to immune checkpoint blockade is unclear.

The achievement of disease stability and the development of clinical progression following a period of disease stability in advanced melanoma patients undergoing treatment with an immune checkpoint inhibitor is becoming a more common clinical scenario. This setting represents a period of immune resistance to these immunotherapeutic agents that remains poorly understood. The cancer immunoediting hypothesis as posited by Schreiber, R. et al. indicates that a period of equilibrium exist between the tumor and the host immune system whereby tumor cells that have undergone various genetic alterations are unable to be eliminated by effector T cell populations due to the development of an array of immune evasion mechanisms (6, 7). To date, very little work has been conducted to characterize this state of host-tumor equilibrium and our understanding of the biochemical pathways that contribute to this state of immune tolerance is incomplete. We hypothesize that this period of disease control in melanoma patients undergoing immune checkpoint inhibitor therapy represents a state of equilibrium between the host immune system and the tumor and that this state is accompanied by alterations in gene expression which promote treatment resistance. We therefore propose that the identification of these gene expression alterations during active checkpoint inhibitor immunotherapy will provide important insight into both secondary and primary resistance mechanisms to these immunotherapeutic agents.

Using an autochthonous melanoma model, we have identified an adaptive resistance mechanism to anti-PD-1 antibody immunotherapy that promotes the recruitment of myeloid-derived suppressor cell (MDSC) populations to the tumor bed. This mechanism involves the upregulation of CXCR2 chemokine ligands by melanomas escaping anti-PD-1 antibody therapy. We have found that CXCL5 chemokine expression levels by these melanomas increase at the time of disease progression and can be monitored in the plasma of these tumor-bearing mice. These data suggest that CXCR2 chemokines as well as myeloid cell markers may be useful for monitoring the response and resistance of advanced melanoma patients to ongoing checkpoint inhibitor immunotherapy. The soluble nature of these CXCR2 chemokines raises the possibility that these ligands can be monitored in the plasma, making this approach particularly translatable to clinical practice.

References:

Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *NEJM.* 2010;363(8):711-23.

Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med.* 2012;366(26):2443-54.

Swann JB, and Smyth M. Immune Surveillance of Tumors. *J Clin Invest.* 2007;117(5):1137-46.

Stewart TJ, and Smyth MJ. Improving cancer immunotherapy by targeting tumor-induced immune suppression. *Cancer metastasis reviews.* 2011;30(1):125-40.

Hanks BA, Holtzhausen A, Jamieson R, Gimpel P, Campbell O, Sun L, Tewari A, George A, Starr M, Nixon A, et al. Type III TGF-β Receptor Downregulation Generates an Immunotolerant Tumor Microenvironment. *J Clin Invest.* 2013;123(9):3925-40.

Holtzhausen A, Zhao F, Evans K, Tsutsui M, Orabona C, Tyler DS, and Hanks BA. Melanoma-derived Wnt5a Promotes Local Dendritic-Cell Expression of IDO and Immunotolerance: Opportunities for Pharmacologic Enhancement of Immunotherapy. *Cancer Immunol Res.* 2015.

Schreiber RD, Old LJ, and Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science.* 2011;331(6024):1565-70.

Dunn GP, Bruce AT, Ikeda H, Old LJ, and Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. *Nat Immunol.* 2002;3(11):991-8.

Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, Hassel JC, Rutkowski P, McNeil C, Kalinka-Warzocha E, et al. Nivolumab in Previously Untreated Melanoma without BRAF Mutation. *N Engl J Med.* 2014.

Topalian SL, Sznol M, McDermott DF, Kluger HM, Carvajal RD, Sharfman WH, Brahmer JR, Lawrence DP, Atkins MB, Powderly JD, et al. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. *J Clin Oncol.* 2014;32(10):1020-30.

Holtzhausen A, Zhao F, Evans K, and Hanks BA. Early carcinogenesis involves the establishment of immune privilege via intrinsic and extrinsic regulation of indoleamine 2,3-dioxygenase-1: translational implications in cancer immunotherapy. *Frontiers Immunology.* 2014;5(1-9.

Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nature reviews Cancer.* 2012;12(4):252-64.

Munn DH, and Bronte V. Immune suppressive mechanisms in the tumor microenvironment. *Current opinion in immunology.* 2015;39(1-6.

Liu X, Shin N, Koblish HK, Yang G, Wang Q, Wang K, Leffet L, Hansbury MJ, Thomas B, Rupar M, et al. Selective inhibition of IDO1 effectively regulates mediators of antitumor immunity. *Blood.* 2010;115(17): 3520-30.

Khleif S, Munn DH, Nyak-Kapoor A, Mautino, Mario R., Kennedy E, Vahanian NN, and Link CJ. First-in-human phase I study of the novel indoleamine-2,3-dioxygenase (IDO) inhibitor NLG-919. *J Clin Oncol.* 2014;32(5s):suppl; abstr TPS3121.

r J, Mandala M, Del Vecchio M, Gogas HJ, Arance AM, Cowey CL, Dalle S, Schenker M, Chiarion-Sileni V, Marquez-Rodas I, et al. Adjuvant Nivolumab versus Ipilimumab in Resected Stage III or IV Melanoma. *N Engl J Med.* 2017.

Eggermont AMM, Blank CU, Mandala M, Long GV, Atkinson V, Dalle S, Haydon A, Lichinitser M, Khattak A, Carlino MS, et al. Adjuvant Pembrolizumab versus Placebo in Resected Stage III Melanoma. *N Engl J Med.* 2018;378(19):1789-801.

Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, et al. The sequence of the human genome. *Science.* 2001;291(5507):1304-51.

Hamid O, Schmidt H, Nissan A, Ridolfi L, Aamdal S, Hansson J, Guida M, Hyams DM, Gomez H, Bastholt L, et al. A prospective phase II trial exploring the association between tumor microenvironment biomarkers and clinical activity of ipilimumab in advanced melanoma. *Journal of translational medicine.* 2011;9(204.

Anders S, and Wolfgang H. *European Molecular Biology Laboratory (EMBL)* Heidelberg, Germany; 2012.

Robinson MD, McCarthy DJ, and Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics.* 2010;26(1):139-40.

20. Ching T, Huang S, and Garmire LX. Power analysis and sample size estimation for RNA-Seq differential expression. *Rna.* 2014;20(11):1684-96.

Design & Procedures

- Describe the study, providing detail regarding the study intervention (drug, device, physical procedures, manipulation of the subject or the subject's environment, etc.). Discuss justifications for placebo control, discontinuation or delay of standard therapies, and washout periods if applicable. Identify procedures, tests and interventions performed exclusively for research purposes or more frequently than standard of care. Include alternative therapies, concurrent therapies discontinued per protocol, risk benefit ratio, and use of tissue /specimens. Discuss monitoring during washout periods if applicable. Include brief description of follow-up, if any.

Arm 1 - Stage IV/Unresectable Stage III Melanoma Patients.

Prior to Treatment.

Within twenty-eight (28) days prior to initiating an anti-CTLA-4 antibody, an anti-PD-1 antibody, an anti-PD-L1 antibody, or combination anti-CTLA-4/anti-PD-1 antibody therapy, patients will be consented for participation and will undergo both a tumor tissue biopsy and a blood draw (Table 1). Imaging by CT or PET CT will be used as a baseline tumor assessment and will be performed ≤ 42 days prior to the treatment start date . Those patients with only cutaneous disease will be assessed by caliper/ruler measurement, their disease will be photographed, and the size documented. Please see Inclusion Criteria below describing measurable disease requirements for study participation.

A tissue biopsy will be performed on an accessible lesion. This will involve a 5-6 mm punch biopsy on superficial lesions or an ultrasound-guided core needle biopsy on lesions that are deemed to be low risk and clinically appropriate if more accessible disease cannot be identified. Each patient's case will be reviewed between the study PI, a study co-investigator, and an independent interventional radiologist to determine if a lesion is appropriate for biopsy. Lesions that are felt to be at lower risk for complications and will be considered for biopsy include soft-tissue lesions and superficial hepatic lesions that are not near critical anatomic structures. Patients with only pulmonary nodules and/or deep hepatic lesions felt to be higher risk will not be considered for tissue biopsy on this study. An excisional biopsy rather than a punch biopsy will also be considered for smaller superficial lesions on a case by case basis. A blood sample (70 cc) will also be collec ted from the patient as described below. Note that only blood specimens will be

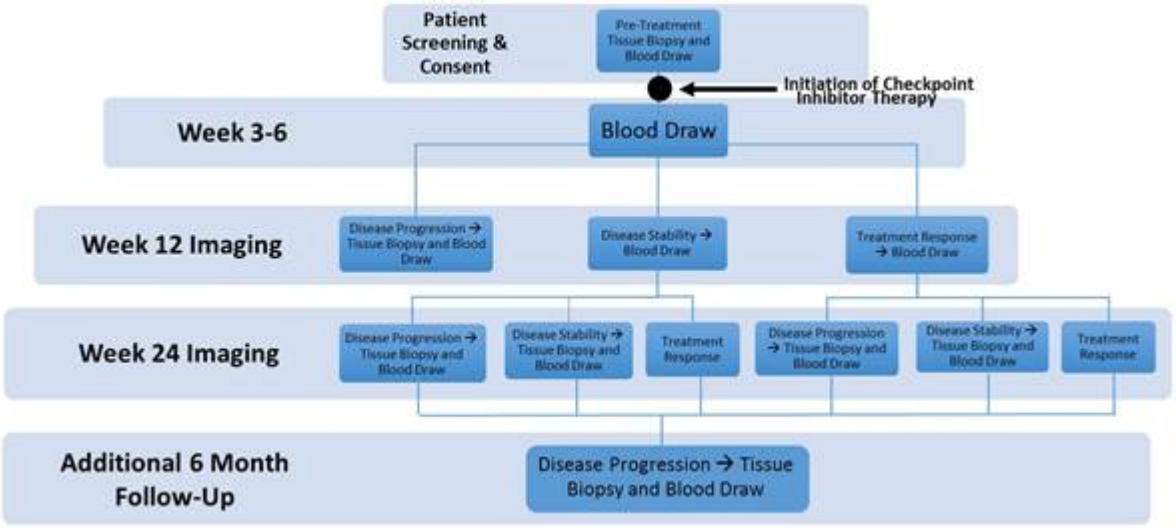


Figure 3. Overview of Tissue Acquisition Protocol. Disease progression will be defined based on traditional RECIST1.1 criteria as a tumor burden increase ≥ 20%. Treatment response will be based on modified criteria and defined as a tumor burden reduction by ≥ 10%.

coll ected if no melanoma lesion is felt to be accessible for tissue biopsy or if the physician deems the tissue biopsy inappropriate. Patients will then begin treatment and continue with their chosen immunotherapy every 2 or 3 weeks per standard of care (SOC) depending upon the selected immunotherapy agent (± 2 weeks).

Specimen Processing:

- De-identified tissue from one melanoma lesion will be collected (5-6 mm punch versus 1-3 mm core), placed in a collection tube containing sterile cold saline buffer or RLT buffer, and then transported to the Hanks Laboratory (LSRC, C207/C210) on ice where this tissue will be used for immediate RNA isolation. These de-identified RNA samples will be cryopreserved at -80 C and transported to the Duke Genomic Sequencing Center for RNAseq analysis.
- De-identified specimens containing 40 cc of whole blood will be stored on ice and shipped overnight to Acousys Biodevices Inc. (**http://www.acousysbio.com/**) for circulating melanoma cell (CMC) isolation using a photoacoustic flow cytometer. These CMCs will be shipped back to the Hanks Lab at Duke for further single cell qrt-PCR gene expression studies.
- De-identified specimens containing 20 cc of whole blood will be stored on ice and transported to the Hanks Laboratory (LSRC, C207/C210). Plasma will be isolated and stored at -80 C before it will be transported to the Nixon Laboratory (MSRB1, Room 394) for multi-analyte ELISA studies. The Buffy coat will be collected during this preparation for genomic DNA isolation and stored at -80 C in the Hanks Lab for future genomic DNA sequencing studies.
- De-identified specimens containing 10 cc of whole blood will be collected in Streck cell-free DNA preservative tubes and stored at room temperature before shipment to Epic Biosciences for CMC

isolation and characterization. Note that this tube must be collected following the specimens noted above. Please see sample shipping instructions to Epic Sciences in the study lab manual.

- Archived formalin-fixed paraffin-embedded tissues will be requested with consent from each participating patient for further Nanostring gene expression analysis and/or additional immunofluorescence/immunohistochemical microscopy studies
    - Up to 12 slides containing formalin-fixed paraffin-embedded tissue will be collected from available tissue blocks currently stored by the Duke BRPC
    - Outside archived tissue will be reviewed by dermatopathology and any available slides not required for clinical diagnostic purposes will be collected for the above studies.

Treatment - Week 3-6.

Patients will undergo a 10 cc blood draw for shipment to Epic Sciences as noted above. This lab draw should be performed following SOC lab draws on that day. Please see sample shipping instructions to Epic Sciences in the lab study manual.

Treatment - Week 12.

Patients will undergo a SOC week 12 (± 4 weeks) whole body PET CT or CT which will be assessed by a trained radiologist based on RECIST1.1 criteria. Superficial lesions will be assessed by caliper or ruler measurements.

Those patients found to have disease progression defined as a ≥ 20% increase in target lesions based on their week 12 re-staging will undergo a repeat blood draw (70 cc) and tissue biopsy if an accessible lesion can be identified (**Figure 3**). Those lesions previously biopsied will be prioritized for additional tissue biopsy when possible. Of note, any tissue subjected to radiation therapy will not be considered for further tissue sampling on this protocol. Tissue biopsies will involve a 5-6 mm punch biopsy on superficial lesions or an ultrasound-guided core needle biopsy on lesions that are deemed to be clinically appropriate if more accessible disease cannot be identified. Each patient's case will be reviewed between the study PI, a study co-investigator, and an independent interventional radiologist to determine if a lesion is appropriate for biopsy. Lesions that are felt to be at lower risk for complications and will be considered for biopsy include soft-tissue lesions and superficial hepatic lesions that are not near critical anatomic structures. Patients with only pulmonary nodules and/or deep hepatic lesions felt to be higher risk will not be considered for tissue biopsy on this study. An excisional biopsy rather than a punch biopsy will also be considered for smaller superficial lesions on a case by case basis. Both tissue and blood analysis will be conducted in the same manner as that performed prior to treatment.

Those patients with evidence of disease stability or treatment response defined as a reduction in target lesion size by ≥10% on week 12 measurements will undergo a repeat 30 cc blood draw (**Figure 3**). Disea se stability will therefore be defined as a tumor burden that does not increase greater than 20% and does not decrease greater than 10%.

In the event of an immune-related adverse event that necessitates treatment delay based on the opinion of the managing physician, the accrued patient will be monitored for up to 60 days thereafter. If the adverse event has not resolved sufficiently within 60 days to continue therapy, the patient will be removed from the study. In the event of treatment delay, the next imaging study after starting therapy will be defined as the week 12 imaging study.

Treatment - Week 24.

Patients will undergo a SOC week 24 (± 4 weeks) whole body PET CT or CT. Those patients with treatment response or with disease stability will undergo repeat blood draw (70 cc) and tissue biopsy when continued disease stability is noted on their week 24 imaging (**Figure 3**). Those patients exhibiting disease progression defined as a ≥ 20% increase in target lesions based on their week 24 imaging or based on caliper or ruler measurements of superficial lesions will also undergo repeat blood draw (70 cc) and tissue biopsy if an accessible lesion can be identified. Those lesions previously biopsied will be prioritized for additional tissue biopsy when possible. Of note, any tissue subjected to radiation therapy will not be considered for further tissue sampling on this protocol. Disease progression and treatment response will be based on the criteria described above. Tissue biopsies will involve a 5-6 mm punch biopsy on superficial lesions or an ultrasound-guided core needle biopsy on lesions that are deemed to be clinically appropriate if more accessible disease cannot be identified. Each patient's case will be reviewed between the study PI, a study co-investigator, and an independent interventional radiologist to determine if a lesion is appropriate for biopsy. Lesions that are felt to be at lower risk for complications and will be considered

for biopsy include soft-tissue lesions and superficial hepatic lesions that are not near critical anatomic structures. Patients with only pulmonary nodules and/or deep hepatic lesions felt to be higher risk will not be considered for tissue biopsy on this study. An excisional biopsy rather than a punch biopsy will also be considered for smaller superficial lesions on a case by case basis. Both tissue and blood analysis will be conducted in the same manner as that performed prior to treatment.

In the event of an immune-related adverse event that necessitates treatment delay based on the opinion of the managing physician, the accrued patient will be monitored for up to 60 days thereafter. If the adverse event has not resolved sufficiently within 60 days to continue therapy, the patient will be removed from the study. In the event of treatment delay, the next imaging study after starting therapy will be defined as the week 12 imaging study.

Extended Surveillance.

Patients' SOC whole body PET CT or CT imaging will be monitored up to 36 months (+/- 4 weeks) from the date of the treatment start. Those patients exhibiting disease progression defined as a ≥ 20% increase in target lesions based on their imaging or based on caliper or ruler measurements of superficial lesions will also undergo repeat blood draw (70 cc) and tissue biopsy if an accessible lesion can be identified. The blood draw and biopsy may be performed at any time during the surveillance period if disease progression occurs. Of note, any tissue subjected to radiation therapy will not be considered for further tissue sampling on this protocol. Disease progression will be determined based on imaging and/or measurements that occur between 24 (+/-4) weeks and 36 months (+/- 4 weeks). Those lesions previously biopsied will be prioritized for additional tissue biopsy when possible. Tissue biopsies will involve a 5-6 mm punch biopsy on superficial lesions or an ultrasound-guided core needle biopsy on lesions that are deemed to be clinically appropriate if more accessible disease cannot be identified. Each patient's case will be reviewed between the study PI, a study co-investigator, and an independent interventional radiologist to determine if a lesion is appropriate for biopsy. Lesions that are felt to be at lower risk for complications and will be considered for biopsy include soft-tissue lesions and superficial hepatic lesions that are not near critical anatomic structures. Patients with only pulmonary nodules and/or deep hepatic lesions felt to be higher risk will not be considered for tissue biopsy on this study. An excisional biopsy rather than a punch biopsy will also be considered for smaller superficial lesions on a case by case basis. Both tissue and blood analysis will be conducted in the same manner as that performed prior to treatment.

Arm 2 - Stage III/IV Adjuvant Melanoma Patients.

A. Presentation Prior to Surgery.

Within twenty-eight (28) days prior to undergoing surgery, patients will be consented for participation and 30 cc of blood will be collected (**Table 2**). Tumor and tumor-draining lymph node tissue will be acquired during the surgical procedure. Any tissue subjected to prior adjuvant radiation therapy will not be considered for further tissue sampling on this protocol. Only tissue considered to be in excess of that needed for diagnostic testing and analysis will be collected for this study and this will be determined by the surgeon and pathologist involved in the surgical procedure. Imaging by CT or PET CT will be used as a baseline tumor assessment and will be performed ≤ 42 days prior to their initiation on adjuvant anti-PD-1 antibody adjuvant immunotherapy. If at any point during surveillance patients exhibit evidence of disease recurrence, they will undergo blood draw (30 cc) and tissue biopsy if an accessible lesion can be identified. The blood draw and biopsy may be performed at any time during the surveillance period if disease progression occurs. Disease recurrence will be determined based on imaging and/or measurements that occur within the three years of surveillance. Tissue biopsies will involve a 5-6 mm punch biopsy on superficial lesions or an ultrasound-guided core needle biopsy on lesions that are deemed to be clinically appropriate if more accessible disease cannot be identified. Each patient's case will be reviewed between the study PI, a study co-investigator, and an independent interventional radiologist to determine if a lesion is appropriate for biopsy. Lesions that are felt to be at lower risk for complications and will be considered for biopsy include soft-tissue lesions and superficial hepatic lesions that are not near critical anatomic structures. Patients with only pulmonary nodules and/or deep hepatic lesions felt to be higher risk will not be considered for tissue biopsy on this study. An excisional biopsy rather than a punch biopsy will also be considered for smaller superficial lesions on a case by case basis. Both tissue and blood analysis will be conducted in the same manner as that performed prior to treatment. If available, archived formalin-fixed paraffin-embedded tissues may be acquired from each of these patients for further Nanostring gene expression analysis and/or additional immunofluorescence/immunohistochemical microscopy studies

B. Presentation Following Surgery.

If patient presents to Duke after surgery and before the start of adjuvant immunotherapy, documentation describing CT/PET CT imaging showing no evidence of disease will be required, archival tissue samples will be requested, and 30 cc of blood will be collected prior to start of adjuvant treatment. If at any point during surveillance these patients exhibit evidence of disease recurrence, they will undergo blood draw (30

cc) and tissue biopsy if an accessible lesion can be identified. The blood draw and biopsy may be performed at any time during the surveillance period if disease progression occurs. Disease progression will be determined based on imaging and/or measurements that occur within the three years of surveillance. Tissue biopsies will involve a 5-6 mm punch biopsy on superficial lesions or an ultrasound-guided core needle biopsy on lesions that are deemed to be clinically appropriate if more accessible disease cannot be identified. Each patient's case will be reviewed between the study PI, a study co-investigator, and an independent interventional radiologist to determine if a lesion is appropriate for biopsy. Lesions that are felt to be at lower risk for complications and will be considered for biopsy include soft-tissue lesions and superficial hepatic lesions that are not near critical anatomic structures. Patients with only pulmonary nodules and/or deep hepatic lesions felt to be higher risk will not be considered for tissue biopsy on this study. An excisional biopsy rather than a punch biopsy will also be considered for smaller superficial lesions on a case by case basis. Both tissue and blood analysis will be conducted in the same manner as that performed prior to treatment. If available, archived formalin-fixed paraffin-embedded tissues may be acquired from each of these patients for further Nanostring gene expression analysis and/or additional immunofluorescence/immunohistochemical microscopy studies

C. Presentation Following Prior Adjuvant Anti-PD-1 Antibody Immunotherapy.

If patient presents to Duke with disease recurrence/progression after prior adjuvant anti-PD-1 antibody immunotherapy, archival tissue samples will be requested, 30 cc of blood will be collected prior to start of additional systemic therapy, and a tissue biopsy will be performed if an accessible lesion can be identified Disease progression will be determined based on imaging and/or measurements that occur within the three years of surveillance. Tissue biopsies will involve a 5-6 mm punch biopsy on superficial lesions or an ultrasound-guided core needle biopsy on lesions that are deemed to be clinically appropriate if more accessible disease cannot be identified. Each patient's case will be reviewed between the study PI, a study co-investigator, and an independent interventional radiologist to determine if a lesion is appropriate for biopsy. Lesions that are felt to be at lower risk for complications and will be considered for biopsy include soft-tissue lesions and superficial hepatic lesions that are not near critical anatomic structures. Patients with only pulmonary nodules and/or deep hepatic lesions felt to be higher risk will not be considered for tissue biopsy on this study. An excisional biopsy rather than a punch biopsy will also be considered for smaller superficial lesions on a case by case basis. Both tissue and blood analysis will be conducted in the same manner as that performed prior to treatment. If available, archived formalin-fixed paraffin-embedded tissues may be acquired from each of these patients for further Nanostring gene expression analysis and/or additional immunofluorescence/immunohistochemical microscopy studies

Specimen Processing:

- De-identified tissue from one melanoma lesion will be collected (5-6 mm punch versus 1-3 mm core), placed in a collection tube containing sterile cold saline buffer or RLT buffer, and then transported to the Hanks Laboratory (LSRC, C207/C210) on ice where this tissue will be used for immediate RNA isolation. These de-identified RNA samples will be cryopreserved at -80 C and transported to the Duke Genomic Sequencing Center for RNAseq analysis.
- De-identified specimens containing 30 cc of whole blood will be stored on ice and transported to the Hanks Laboratory (LSRC, C207/C210). Plasma will be isolated and stored at -80 C before it will be transported to the Nixon Laboratory (MSRB1, Room 394) for multi-analyte ELISA studies. Buffy coat will be collected during this preparation for genomic DNA isolation and stored at -80 C in the Hanks Lab for future genomic DNA sequencing studies.
- Archived formalin-fixed paraffin-embedded tissues may be acquired from each participating patient for further Nanostring gene expression analysis and/or additional immunofluorescence /immunohistochemical microscopy studies
    - Up to 12 slides containing formalin-fixed paraffin-embedded tissue will be collected from available tissue blocks currently stored by the Duke BRPC
    - Outside archived tissue will be reviewed by dermatopathology and any available slides not required for clinical diagnostic purposes will be collected for the above studies.

Follow-up.

Retrospective personal health information (PHI) pertaining to the management of the patient and the patient's clinical course following this period will be assessable for future studies. Specifically, the following information will be reviewed and obtained for study from the medical health record: age, gender, pertinent laboratory studies, primary melanoma site, diagnosis of any other metastatic sites (ie. brain, liver, lymph nodes, lung, other), melanoma histology, date of diagnosis of primary site and metastases, date of treatments (including surgery, chemotherapy, and radiation), types of treatments, response to treatments

as indicated by follow-up imaging studies, date of progression or recurrence, date of death, and any other information pertinent to their diagnosis and treatment of their melanoma. The personal health information is an essential aspect of this study as it may determine the prognostic importance of the clusters of patients determined by genetic and protein biomarker analysis.

De-identified samples or data collected from this protocol may be shared to develop research collaborations. For example, genes identified to be associated with melanoma stability or response following checkpoint inhibitor therapy can potentially be commercialized to improve our ability to manage patients with advanced melanoma.

Table 1. Arm 1 - Unresectable Stage III or Stage IV Melanoma Patients Undergoing Checkpoint Inhibitor Immunotherapy

Procedure	Pre-Treatment Visit within 28 days of starting therapy	Prior to Initiating Immuno-therapy	Treatment Start Date	Week 3-6	Week 12 +/- 4 weeks	Week 24[b] +/- 4 weeks	Extended Surveillance
Informed Consent	X						

I/E[a]	X						
SOC Physical Exam and Labs	X		X	X	X	X	X
ECOG	X		X	X	X	X	X
PT/INR[d]		X[h]			X	X	X
Research Blood		X[h]		X[e]	X[f]	X[g]	X[i]
PET CT/CT	X[c]				X	X	X
Biopsy		X[h]			X[f]	X[g]	X[i]
Archive	X[j]						

d Tissue					X[j]	X[j]	X[j]

[a] *Adverse Events and Con meds are not captured on this trial.*

[b] *After week 24, patients are followed as clinically indicated but are not actively followed on trial.*

[c] *Pretreatment PET CT/CT must be performed to 42 days of treatment start-date.*

[d] *PT/INR will only be drawn on those patients being considered for potential image-guided biopsy.*

[e] *Research blood will be drawn at week 3-6.*

[f] *Research blood will be drawn at week 12 based on imaging responses. Week 12 tissue biopsy will be obtained in patients that have progressed on their week 12 PET CT imaging based on modified RECIST1.1 criteria.*

[g] *Research blood will be drawn at week 24 based on imaging responses. Week 24 tissue biopsy will be obtained in patients that have progressed or achieved disease stability after prior response based on modified RECIST1.1 criteria.*

[h] *Research blood, biopsy, and PT/INR can be drawn within 28 days of starting therapy or on the treatment start date prior to treatment initiation.*

[i] *Research blood and/or tissue biopsies will be obtained in patients that have progressed based on modified RECIST1.1 criteria during the three year follow-up period after the initiation of therapy.*

[j] *Archived formalin-fixed, paraffin-embedded tissue may be acquired for selected participating patients depending on their historical tissue biopsy and the development of disease progression.*

Table 2. Arm 2 - Stage III or Stage IV Melanoma Patients Undergoing Adjuvant Checkpoint Inhibitor Immunotherapy

Procedure	**Pre-Surgery**	**Surgery**	**Pre-Treatment**	**Disease Recurrence**
Informed Consent	X		X[b]	X[b]
I/E[a]	X		X	X

SOC Physical Exam and Labs	X		X	X
ECOG	X		X	X
PT/INR[d]	X			X
Research Blood[e]	X[e]		X[e]	X[e]
PET CT/CT			X[c]	X
Biopsy		X	X	X
Archived Tiss			X[f]	X[f]

u e[f]				

[a]*Adverse Events and Con meds are not captured on this trial.*

[b]*Those patients who underwent surgery and adjuvant immunotherapy prior to their presentation at Duke will be assessed and consented for participation in this study.*

[c]*Pretreatment PET CT/CT must be performed ≤42 days of treatment start-date.*

[d]*PT/INR will only be drawn on those patients being considered for potential image-guided biopsy*

[e]*Research blood will be drawn prior to the surgical procedure and at the time of disease recurrence. For patients who underwent surgery and adjuvant immunotherapy prior to their presentation will only undergo a blood draw at that time of disease recurrence.*

[f]*Archived formalin-fixed, paraffin-embedded tissue may be acquired for selected participating patients depending on their historical tissue biopsy and the development of disease progression.*

Future Research:

Depending on the results generated by the studies described above, some tissue specimens may be subjected to immunohistochemical studies and/or be used to generate cell lines in culture for future studies.

Selection of Subjects

- List inclusion/exclusion criteria and how subjects will be identified.

Arm 1 **Subject Selection:**

Eligible patients with stage IV/ unresectable stage III melanoma selected to undergo treatment with an anti-CTLA-4 antibody, an anti-P

Inclusion Criteria

- Patients with stage III or IV melanoma, with melanoma validated by histology or cytology
- Patients may participate with primary cutaneous melanomas of unknown primary site
- Age ≥ 18 years
- ECOG performance status of 0-2
- Life expectancy of at least 6 months
- Patients with active disease will be treated with either an anti-CTLA-4 antibody , an anti-PD-1 antibody, an anti-PD-L1 antibody, or a combination of an anti-CTLA-4 antibody/anti-PD-1 antibody (combined therapy regimens with any other agents are not allowed on this study).
- Patient must have a measurable systemic lesion defined as greater than or equal to 10 mm based on PET CT/CT/MRI imaging. Pretreatment PET CT/CT imaging must be performed ≤ 42 days prior to treatment initiation.
- Patients with target skin lesions must equal at least 10 mm when their longest diameters are aggregated. Target skin lesions (5 maximum) must be at least 5 mm in their longest diameter to be considered measurable by caliper or ruler.
- Those patients with a failed biopsy attempt or those with disease that is not amenable to biopsy will still be eligible for enrollment and will only undergo blood draws during the study protocol.
- Both men and women of all races and ethnic groups are eligible for this trial.
- Ability to understand and the willingness to sign a written informed consent document
- Patients with intra-cranial disease or disease involving the central nervous system are eligible

Exclusion Criteria

- Patients with a history of a systemic autoimmune disease (eg systemic lupus erythematosus) requiring active therapy
- Patients with a history of another malignancy within the last 5 years except for those patients felt by the treating physician to be cured of that malignancy
- Patients with a diagnosis of a mucosal or ocular melanoma
- Patients who have undergone adjuvant locoregional radiation therapy if less than 4 weeks prior to day of initial biopsy
- Patients who have had prior cytotoxic chemotherapy if less than 6 weeks prior to day of initial biopsy

- Patients who have had prior interferon therapy if less than 4 weeks prior to day of initial biopsy
- Patients who have had prior anti-CTLA-4 antibody or anti-PD-1 antibody or anti-PD-L1 antibody therapy if less than 4 weeks prior to day of initial biopsy
- Patients who have had prior IL-2 therapy if less than 4 weeks prior to day of initial biopsy
- Patients who have had prior BRAF inhibitor and/or MEK inhibitor therapy if less than 4 weeks prior to day of initial biopsy
- Patients who have received an immunotherapy agent on a previous clinical trial protocol if less than 4 weeks prior to day of initial biopsy
- Patients who are undergoing active steroid therapy if the dose exceeds physiologic steroid doses (equivalent of prednisone 10 mg po daily or less)
- Patients with ongoing or active infection
- Pregnant patients
- Patients with any laboratory test values or serious pre-existing medical condition, that in the opinion of the investigator, makes the patient unsuitable for the study
- Patients unable to comply with the requirements of the study protocol

Arm 2 Subject Selection:

The following patients will be asked to participate in the study by the Principal Investigator, co-In

Inclusion Criteria

- Patients with a current diagnosis of stage III or IV melanoma or a history of stage III or IV melanoma, with melanoma validated by histology or cytology
- Age ≥ 18 years
- ECOG performance status of 0-2
- Patients selected for future adjuvant anti-PD-1 antibody immunotherapy following resection of stage III or stage IV melanoma or patients who received prior adjuvant anti-PD-1 antibody immunotherapy for stage III or IV melanoma and have developed recurrent disease based on physical exam and/or CT/PET CT cross-sectional imaging
- Those patients with a failed biopsy attempt or those with disease that is not amenable to biopsy will still be eligible for enrollment and will only undergo blood draws during the study protocol. Of note, any tissue subjected to prior adjuvant radiation therapy will not be considered for further tissue sampling on this protocol.
- Both men and women of all races and ethnic groups are eligible for this trial.
- Ability to understand and the willingness to sign a written informed consent document
- Patients with intra-cranial disease or disease involving the central nervous system are eligible

Exclusion Criteria

- Patients with a history of a systemic autoimmune disease (eg systemic lupus erythematosus) requiring active therapy
- Patients with a history of another malignancy within the last 5 years except for those patients felt by the treating physician to be cured of that malignancy
- Patients with a diagnosis of a mucosal or ocular melanoma
- Patients who have had prior cytotoxic chemotherapy if less than 6 weeks prior to day of initial biopsy
- Patients who have undergone adjuvant locoregional radiation therapy if less than 4 weeks prior to day of initial biopsy
- Patients who have had prior interferon therapy if less than 4 weeks prior to day of initial biopsy
- Patients who have had prior anti-CTLA-4 antibody or anti-PD-1 antibody or anti-PD-L1 antibody therapy if less than 4 weeks prior to day of initial biopsy
- Patients who have had prior IL-2 therapy if less than 4 weeks prior to day of initial biopsy
- Patients who have had prior BRAF inhibitor and/or MEK inhibitor therapy if less than 4 weeks prior to day of initial biopsy
- Patients who have received an immunotherapy agent on a previous clinical trial protocol if less than 4 weeks prior to day of initial biopsy
- Patients who are undergoing active steroid therapy if the dose exceeds physiologic steroid doses (equivalent of prednisone 10 mg po daily or less)
- Patients with ongoing or active infection
- Pregnant patients
- Patients with any laboratory test values or serious pre-existing medical condition, that in the opinion of the investigator, makes the patient unsuitable for the study
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- Those patients with a failed biopsy attempt or those with disease that is not amenable to biopsy will still be eligible for enrollment and will only undergo blood draws during the study protocol. Of note, any tissue subjected to prior adjuvant radiation therapy will not be considered for further tissue sampling on this protocol.
- Both men and women of all races and ethnic groups are eligible for this trial.
- Ability to understand and the willingness to sign a written informed consent document
- Patients with intra-cranial disease or disease involving the central nervous system are eligible

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- Patients with a diagnosis of a mucosal or ocular melanoma
- Patients who have had prior cytotoxic chemotherapy if less than 6 weeks prior to day of initial biopsy
- Patients who have undergone adjuvant locoregional radiation therapy if less than 4 weeks prior to day of initial biopsy
- Patients who have had prior interferon therapy if less than 4 weeks prior to day of initial biopsy
- Patients who have had prior anti-CTLA-4 antibody or anti-PD-1 antibody or anti-PD-L1 antibody therapy if less than 4 weeks prior to day of initial biopsy
- Patients who have had prior IL-2 therapy if less than 4 weeks prior to day of initial biopsy
- Patients who have had prior BRAF inhibitor and/or MEK inhibitor therapy if less than 4 weeks prior to day of initial biopsy
- Patients who have received an immunotherapy agent on a previous clinical trial protocol if less than 4 weeks prior to day of initial biopsy
- Patients who are undergoing active steroid therapy if the dose exceeds physiologic steroid doses (equivalent of prednisone 10 mg po daily or less)
- Patients with ongoing or active infection
- Pregnant patients
- Patients with any laboratory test values or serious pre-existing medical condition, that in the opinion of the investigator, makes the patient unsuitable for the study
- Patients unable to comply with the requirements of the study protocol

Subject Recruitment and Compensation

- Describe recruitment procedures, including who will introduce the study to potential subjects. Describe how you will ensure that subject selection is equitable and all relevant demographic groups have access to study participation (per 45 CFR 46.111(a) (3)). Include information about approximately how many DUHS subjects will be recruited. If subjects are to be compensated, provide specific prorated amounts to be provided for expenses such as travel and/or lost wages, and/or for inducement to participate.

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Consent Process

- Complete the consent section in the iRIS Submission Form.

Subject's Capacity to Give Legally Effective Consent

- If subjects who do not have the capacity to give legally effective consent are included, describe how diminished capacity will be assessed. Will a periodic reassessment occur? If so, when? Will the subject be consented if the decisional capacity improves?

Subject competency will be assessed by meeting with the patient through dialogue, physical exam, talking with their care givers and families per federal guidelines and Duke IRB guidelines.

Study Interventions

- If not already presented in #4 above, describe study-related treatment or use of an investigational drug or biologic (with dosages), or device, or use of another form of intervention (i.e., either physical procedures or manipulation of the subject or the subject's environment) for research purposes.

see design and procedures

Risk/Benefit Assessment

- Include a thorough description of how risks and discomforts will be minimized (per 45 CFR 46.111(a) (1 and 2)). Consider physical, psychological, legal, economic and social risks as applicable. If vulnerable populations are to be included (such as children, pregnant women, prisoners or cognitively impaired adults), what special precautions will be used to minimize risks to these subjects? Also identify what available alternatives the person has if he/she chooses not to participate in the study. Describe the possible benefits to the subject. What is the importance of the knowledge expected to result from the research?

There is minimal risk of infection, bleeding, pain associated with any punch biopsy. There is no additional risk associated with the blood draw as this will be performed during standard of care blood draw procedures. Patients that are selected and consent to undergo an ultrasound-guided core biopsy will be subjected to the least invasive biopsy procedure to minimize any risk to the patient. Rare but potential risks associated with ultrasound-guided core biopsy are internal bleeding as well as bleeding, pain and/or infection at the needle entry site. Every possible measure will be taken to minimize these risks. There are no direct benefits to patients on this study. Results of these studies may benefit future patients with advanced melanoma and potentially other cancer types. Results of future studies performed with these samples may benefit future patients with advanced melanoma as well as other cancer types.

Study records will be identified by a unique code number. The key to the code will be kept in a separate locked file in Dr. Hanks' office. Subjects will not be identified by name, social security number, address, telephone number, or any other direct personal identifier in study records disclosed outside of Duke University Health System (DUHS). Research data will be kept until the completion of the study in a password-protected database that will be accessible only to Dr. Hanks or designated staff members.

Costs to the Subject

- Describe and justify any costs that the subject will incur as a result of participation; ordinarily, subjects should not be expected to pay for research without receiving direct benefit.

All costs regarding specimen collection will be covered by Dr. Brent Hanks' grant funding from private donors, Merck, and the Duke Cancer Institute.

Data Analysis & Statistical Considerations

- Describe endpoints and power calculations. Provide a detailed description of how study data will be analyzed, including statistical methods used, and how ineligible subjects will be handled and which subjects will be included for analysis. Include planned sample size justification. Provide estimated time to target accrual and accrual rate. Describe interim analysis including plans to stop accrual during monitoring. Phase I studies, include dose escalation schema and criteria for dose escalation with definition of MTD and DLT.

Sample Size:

Arm 1. Statistical requirements for the RNAseq analysis planned for this study will determine the required sample number necessary for the overall study given the intrinsic heterogeneity of this approach. For this portion of the study, we will be focusing our analysis on the differential expression of genes that encode soluble proteins since we believe that these soluble proteins are more likely to impact the immune microenvironment of the tumor via paracrine signaling mechanisms (5, 6). Since approximately ~10% of the human genome encodes soluble proteins, we estimate that analysis will focus on the differential expression of ~3,000 genes (18). Based on previous IHC-based predictive studies to anti-CTLA-4 immunotherapy, we estimate needing specimens from approximately 40 patients (19). Based on simulation studies, ~44 paired samples will have high power (>80%) to detect differentially expressed genes from RNASeq experiments, in particular, when using DeSeq2 and edgeR software, which are based on a negative binomial distribution. Considering potential pitfalls associated with tissue processing and RNA isolation as well as the mean response rate reported for the checkpoint inhibitors, we will target 120 patients for paired specimen tissue acquisition. We currently initiate an average of one advanced melanoma patient every 10 days on a checkpoint inhibitor immunotherapy regimen. We estimate that approximately 75% of our patient population that we routinely treat with checkpoint inhibitor therapy will be eligible for this study (see inclusion criteria above). We therefore foresee screening approximately 160 patients for this study protocol.

Arm 2. Statistical requirements for the RNAseq analysis planned for this study will determine the required sample number necessary for the overall study given the intrinsic heterogeneity of this approach. For this portion of the study, we will be focusing our analysis on the differential expression of genes that encode soluble proteins since we believe that these soluble proteins are more likely to impact the immune microenvironment of the tumor via paracrine signaling mechanisms (5, 6). Since approximately ~10% of the human genome encodes soluble proteins, we estimate that analysis will focus on the differential expression of ~3,000 genes (18). Based on previous IHC-based predictive studies to anti-CTLA-4 immunotherapy, we estimate needing specimens from approximately 40 patients (19). Based on simulation studies, ~44 paired samples will have high power (>80%) to detect differentially expressed genes from RNASeq experiments, in particular, when using DeSeq2 and edgeR software, which are based on a negative binomial distribution. Considering potential pitfalls associated with tissue processing and RNA isolation as well as the mean response rate reported for the checkpoint inhibitors, we will target 120 patients for paired specimen tissue acquisition. We currently initiate an average of one melanoma patient on adjuvant anti-PD-1 antibody immunotherapy every 15 days. We estimate that approximately 75% of our patient population that we routinely treat with checkpoint inhibitor therapy will be eligible for this study (see inclusion criteria above). We therefore foresee screening approximately 160 patients for this study protocol.

Statistical analysis plan:

Analysis of paired differential expression from pre-treatment to week 24 or until time of disease progression during extended surveillance will be completed using the R package DESeq2 and edgeR (20, 21). The top set of differentially expressed genes will be identified based on these analyses, and further downstream analyses of potential pathways will be investigated. Simulation studies reveal that edgeR and DeSeq2 had the highest power to detect differential expression (22). We also plan an analysis comparing progressive disease versus stable disease utilizing the top set of differentially expressed genes based from the paired analysis in a logistic regression model. Given the potential for a large number of available genes as covariates, we will utilize variable selection techniques, such as lasso or ridge regression. This secondary analysis is more exploratory, but should provide preliminary evidence of the drivers of immune-response and subsequent disease events.

Data & Safety Monitoring

- Summarize safety concerns, and describe the methods to monitor research subjects and their data to ensure their safety, including who will monitor the data, and the frequency of such monitoring. If a data monitoring committee will be used, describe its operation, including stopping rules and frequency of review, and if it is independent of the sponsor (per 45 CFR 46.111(a) (6)).

not in original Research Summary

Privacy, Data Storage & Confidentiality

- Complete the Privacy and Confidentiality section of the iRIS submission form.

Describe Role of External Personnel:

n/a

Study Scope

Does the subject population contain >50% malignant hematology or oncology patients, or their caregivers?

(•) Yes ( ) No

Are you using a drug, biologic, food, or dietary supplement in this study?

( ) Yes (•) No

Are you using a medical device, an algorithm (whether computer based or not), an in vitro diagnostic test, or using samples to look for biomarkers in this study?

(•) Yes ( ) No

Does this study involve a Humanitarian Use Device (HUD)?

( ) Yes (•) No

Does this study employ magnetic resonance, including imaging (MRI), spectroscopy (MRS), angiography (MRA) or elastography (MRE) beyond the standard of care?

( ) Yes (•) No

Does this study specify or require the performance of diagnostic procedures using ionizing radiation (x-rays, DEXA, CT scans, nuclear medicine scans, etc.) that are beyond the standard of care?

( ) Yes (•) No

Does this study specify or require the performance of therapeutic procedures using ionizing radiation (accelerator, brachytherapy or systemic radionuclide therapy) that are beyond the standard of care?

( ) Yes (•) No

Will the participant be subjected to increased or decreased ambient pressure?

( ) Yes (•) No

Do you plan to recruit subjects from Duke Regional Hospital (DRH)?

○ Yes ◉ No

Do you plan to recruit subjects from Duke Raleigh Hospital (DRAH)?

○ Yes ◉ No

Does this study utilize the Duke Early Phase Clinical Research Unit (DEPCRU)?

○ Yes ◉ No

Are you using the Duke logo in any advertisements?

○ Yes ◉ No

Is this study retrospective, prospective, or both?

"Retrospective" means that data or samples already in existence (collected prior to the study submission) will be used.
"Prospective" means there will be data or samples collected in this study for research purposes.

○ Retrospective
◉ Prospective
○ Retrospective and Prospective

Does this protocol include any research using botulinum toxin, including the FDA-approved clinical product (Botox)?

○ Yes ◉ No

Does this protocol involve the administration of any of the following materials to humans?

•Any viral vector or plasmid
•Any cells that have been modified by a viral vector
•Any other genetically-modified cells
•Any genetically-modified virus, bacterium, or other agent
•Any other recombinant or synthetic nucleic acid

○ Yes ◉ No

Cancer Protocol Committee Section

Is this trial:

◉ Interventional
○ Observational
○ Other

B. Select the time perspective(s): (see Appendix A in help icon to the right for definitions)

☑ Prospective
☐ Retrospective
☐ Cross-sectional
☐ Other:

Provide the NCT number in clinicaltrials.gov (if this is an applicable clinical trial) for this submission:

NCT

Supply the *estimated primary completion date* :

07/01/2020

Supply the *estimated study completion date* :

07/01/2020

Is there an independent Data Monitoring Committee (DMC/DSMB) for this protocol?

○ Yes ◉ No

Is this a Duke investigator-initiated multi-site trial?

○ Yes ◉ No

PROJECTED ACCRUAL **Projected accrual should be based on PI input, Disease Group Leader input, protocol priority, and availability of the subject population. Future CPC decisions on renewal or termination will be based primarily on accrual projections provided below.**

Time from IRB Initial Review Date (Total number of subjects will calculate once you save section)

Projected # of subjects who are not screen failures	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7	Year 8	Total
At DUHS	7	14	10	35	34				**100.00**

Projected # of subjects who are not screen failures	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7	Year 8	Total
At non-DUHS sites:									**0.00**

COMPETING STUDIES **List all studies that compete for the subject population being recruited for this study.**

eIRB #	Principal Investigator	Protocol Title
No records have been added		

Which oncology CRU disease group would this protocol belong to?

Melanoma

Will the study use the services of the DCI Monitoring Team?

○ Yes ◉ No

Will this protocol use the DCI Safety Surveillance Team for adverse event tracking and reporting?

○ Yes ◉ No

Will this protocol require the services of the DCI-IT team to help develop a study database?

○ Yes ◉ No

Subject Population Groups and Enrollment

Population Groups (Select targeted population groups only):

Note:

- If Minors are included, the study will be routed to the Department of Pediatrics for Pediatric Risk Assessment.
- Students and Employees over whom Key Personnel have a supervisory role may not be enrolled in this study.
- Healthy Controls must be given a Notice of Privacy Practices.

☑ Adults
☐ Minors who are Wards of State
☐ Minors
☑ Duke Patients
☐ Pregnant Women
☐ Fetuses
☐ Prisoners
☐ Adults incapable of giving consent
☐ Adults with diminished capacity
☐ Handicapped subjects
☐ Students
☐ Employees
☐ Healthy Controls
☐ Deceased subjects
☐ Blanket Protocol

Please select any population groups excluded from participation in this study:

☑ Pregnant women

Will you administer a pregnancy test to eligible female subjects prior to the start of study activities?

○ Yes ○ No

Maximum number of subjects to be consented at Duke:

Enter a single number. If you anticipate consenting a range of subjects, enter the **upper** limit of the range. The number should represent the maximum number of subjects for the life of the study.

160

Maximum number of subjects to be consented at all sites:

Enter a single number. If you anticipate consenting a range of subjects, enter the **upper** limit of the range. The number should represent the maximum number of subjects for the life of the study.

160

Subject Procedures and Costs

Biobank - Does this study involve the collection, use, tracking, banking (storage) or distribution of human biological specimens?

Human biological specimens include blood or its components, healthy or diseased tissue, bodily fluids, DNA/RNA or human stem cells.

(•) Yes ( ) No

Procedures

Check all the apply:

- [ ] Genetic Testing
- [ ] Gene Transfer
- [ ] DNA Banking
- [ ] Testing for Reportable Infectious Diseases
- [x] Human Cell Banking
- [ ] *Use of Human Embryonic Stem Cells
- [ ] *Use of Human-induced Pluripotent Stem Cells
- [ ] *Use of Other Cells Derived from Human Embryos
- [ ] *Use of Human/Animal Chimeric Cells
- [ ] *Specialized Cell Populations for Cell Therapy
- [x] Use of Human Tissue
- [ ] Use of Bodily Fluids
- [x] Use of Blood (or its components)
- [ ] Not Applicable

Will blood be drawn in this study for research purposes?

(•) Yes ( ) No

Maximum amount to be drawn in any 8 week period (ml):

80

Number of blood draws per week:

1

Will the Operating Room be used in this study?

Include only research time, not clinical care time.

( ) Yes (•) No

Will there be extra costs to subjects or insurance as a result of the research (e.g. tests, hospitalization)?

( ) Yes (•) No

Will there be Subject Compensation?

( ) Yes (•) No

Pathology Specialty Committee Form

Purpose of Approval:

To document that the proposed study will not interfere with pathologic evaluations of tissues needed for routine current or future clinical care, and will not interfere with the routine patient care activities of the pathology department. Please note: Use of blood does not need approval.

Please describe briefly what tissues (fresh tissue, frozen tissue, paraffin blocks, slides, smears) will be needed for this study:

Biopsy may be taken of accessible lesions at pre-treatment, and at 12 weeks if disease progression occurs. If progression does not occur at 12 weeks, then tissue will be collected from accessible lesions at 24 weeks if stable disease or disease progression. If disease progression does not occur at 24 weeks, then an accessible lesion may be biopsied with disease progression within the next 30 months.

Archival tissue will be requested if available.

For adjuvant patients, archival tissue will be requested. If patient recurs within 3 years, accessible lesions may be biopsied.

Dr. Rami Al-Rohil is the pathologist for this study.

Qualification for automatic pathology approval:

Part A:

If this study requires access to clinical archival paraffin human tissue, will all requested material be obtained through the Pathology BRPC Shared Resource, under their fee-for-service model? If your study does not require access to clinical archival paraffin human tissue, select "Not Applicable".

- ○ Yes
- ◉ No
- ○ Not Applicable

Does your study also require collection of new or non-clinical archival tissue specimens?

◉ Yes ○ No

Part B:

Is all of the tissue for this study on the Surgical Pathology Tissue and Medical Devices Exceptions List?

- (See DUHS intranet, search "pathology" at URL **https://egrc.duhs.duke.edu**).

○ Yes ◉ No

Is (or was) all of the tissue obtained solely for research purposes?

◉ Yes ○ No

- All criteria a-e must be true to answer "yes".
- If any criterion is not met, or if you are unsure if it meets criteria, answer "no"

a) The biopsy or surgery is an additional procedure performed for the sole purpose of collecting tissue for the study?

◉ Yes ○ No

b) The patient will have an established diagnosis at the time of the additional biopsy?

◉ Yes ○ No

c) No routine pathologic evaluation of the tissue from the biopsy will be performed at Duke?

(•) Yes ( ) No

d) No routine (non-experimental) clinical care will be determined by evaluation of the research tissue?

(•) Yes ( ) No

e) Patients will be consented to the additional biopsy including the lack of any pathologic evaluation of the tissue?

(•) Yes ( ) No

Will all tissues be obtained via the Duke Brain Tumor Center Biorepository?

( ) Yes (•) No

Will all tissues be obtained via the Duke BioRepository and Precision Pathology Center (BRPC)?

( ) Yes (•) No

Your study qualifies for automatic pathology approval. Please note that the PI (or the existing tissue bank or biorepository) will be solely responsible for collecting, processing, storing, shipping, and disposing of any tissues obtained for research.

Subject Recruitment Materials

For each document to be reviewed, use the table below to provide the following information:

Attach a copy of each advertisement that you will be using with this study in the Initial Submission Packet. If any Ad will have multiple wording variations, attach a copy of each version of the Ad.
All materials that will be used to advertise the study in order to recruit subjects must be approved by the IRB.

Types of subject recruitment materials include, but are not limited to, the following:

Direct Advertising
Posters
Billboards
Flyers
Brochures

Media Advertising
Newspaper Ads
Magazine Ads
Radio Ads
TV commericals / Video
Internet website
Social Media

Other Types of Advertising
Newsletter
Email
Postcards / Letters

(Note: Doctor-to-Doctor letters do not require IRB approval)

	Document name	Material category	Location material displayed	Has this material previously been approved by the IRB?	
No records have been added					

Consent Process

Attach draft consent forms in the Initial Review Submission Packet.

Consent forms must be MS Word documents and follow the specific format outlined by the IRB. **Click here** to download a copy of the consent form template.

Note: Please do not edit the section of the footer that contains the Protocol ID, Continuing Review and Reference Date fields. Those fields will be used to stamp the final consent form when it is approved by the IRB. If you want to add an internal version date, please put it in the header.

Who will conduct the consent process with prospective participants?

Give the person's role in this study (PI, Study Coordinator, etc.):

Dr. Hanks or one of his adequately trained study team members will consent (PI or Study Coordinator)

Who will provide consent or permission?

(Select all that apply):

- [x] Participant
- [ ] Parent(s) or Legal Guardian(s)
- [ ] Legally Authorized Representative (LAR)

How much time will the prospective participant (or legally authorized representative) have between being approached about participating in the study and needing to decide whether or not to participate?

If you are not giving the person overnight to consider whether or not to participate, please justify.

Subjects will be given as much time as they would like to consider participation in this trial. They will be allowed to go home and think about teh study should tehy desire and we will ensure that all questions are thoroughly answered before they sign the form.

Where will the consent process occur?

Clinic 3-2 behind closed doors to ensure privacy.

What steps will be taken in that location to protect the privacy of the prospective participant?

We will only discuss the study with them in a confidential location and make srue we are the only ones present. Paper will be secured.

How much time will be allocated for conducting the initial consent discussion, including presenting the information in the consent document and answering questions, with each prospective participant?

We usually alot for an hour for discussion of the study and the ICF documentation however subjects are always given as much time as needed to fully read, understand and ask questions regarding consent and study.

What arrangements will be in place for answering participant questions before and after the consent is signed?

We will ask them if they have any questions, the study team is properly trained regarding the protcol and Dr. Haks is alwyas available should he be needed for questions.

Describe the steps taken to minimize the possibility of coercion or undue influence.

We will offer them all alternatives for the treatment of their current stage of disease and tell them that this is one choice and allow them to make the decision.

What provisions will be in place to obtain consent from participants who do not read, are blind or who do not read/understand English?

We will not be consenting non English speaking participants.

Do you plan to obtain written consent for the conduct of research?

(●) Yes ( ) No

Protected Health Information (PHI)

Indicate how you intend to use potential subjects' Protected Health Information (PHI):

( ) I will review, but not record, PHI prior to consent.
(●) I will record PHI prior to consent.
( ) I do not intend to use PHI prior to consent.
( ) I will record PHI without consent. (decedent research, database repository, chart review)

Request for Waiver or Alteration of Consent and/or HIPAA Authorization

Will the population include deceased individuals?

( ) Yes (●) No

This waiver request applies to the following research activity or activities:

☑ Scheduling of research activities in MaestroCare and/or the recording of PHI via telephone for screening purposes prior to obtaining written consent for the research. Scheduling of research activities in MaestroCare and/or the recording of PHI via telephone for screening purposes prior to obtaining written consent for the research. (If you check this box, please complete all sections below.)
☑ Ascertainment (identification, selection) and/or recruitment of potential subjects while recording identifiable private information, such as protected health information (PHI), prior to obtaining the subject's consent. (If you check this box, please complete sections B and C below.)
☐ Conduct of the research project without obtaining verbal or written consent and authorization. (If you check this box, please complete sections B and C below.)

Note: Answer the questions below as they pertain solely to PHI collected prior to consent.

Provide the following information:

List the elements of informed consent and/or HIPAA authorization for which waiver or alteration is requested:

- Provide the rationale for each.

We request a waiver of all core elements of consent and HIPAA to ascertain subjects for this study. To perform this study, we will need to review medical records of the patients on the Clinic and OR schedules of Investigators on this study to look for eligible participants. Potential subjects will be stored on an excel spreadsheet on a secure server until they are asked about participation. The rationale for this is that it is not practical to consent every patient, to review their records, for eligibility for this study.

The attached phone script may be used to gain permission to schedule research activities pre-consent. This is necessary to meet protocol criteria for screening windows.

List the specific protected health information (PHI) to be collected and its source(s):

- (Note: PHI = health information + identifiers)

We will collect Name & MRN prior to consent.

Criteria for Waiver: The DUHS IRB may waive the requirement for informed consent and authorization if all of the following criteria are met:

- Please respond to each item in the space below using protocol-specific language to provide justification:

a) The research or clinical investigation involves no more than minimal risk to subjects:

The research will involve minimal risk

b) The waiver or alteration will not adversely affect the rights and welfare of the subjects. Include a description of any measures to be taken to ensure that the rights and welfare of subjects will be protected:

This wavier will not adversely affect the rights and welfare of the subjects because the study team will ensure that all information is kept on our secure server with limited access.

c) Whenever appropriate, the subjects will be provided with additional pertinent information after participation:

Subjects will be consented at the first opportunity.

d) If this research activity relates to research involving deception, explain how subjects will be provided with additional pertinent information after study participation and what information will be provided. Otherwise indicate "not applicable":

n/a

e) The use or disclosure of protected health information involves no more than minimal risk to the privacy of individuals, based on, at least, the presence of the following elements (e1. and e2.)

Demonstrate that the use or disclosure of PHI involves no more than minimal risk to the privacy of subjects by describing the plans requested below:

e1) An adequate plan to protect the identifiers from improper use and disclosure.
Describe the plan (how protection will be accomplished) and indicate where the PHI will be stored and who will have access:

PHI will be stored on secure servers maintained by the Department of Medicine with access limited to the PI and Melanoma Research study team

e2) An adequate plan to destroy the identifiers at the earliest opportunity consistent with conduct of the research, unless there is a health or research justification for retaining the identifiers or such retention is otherwise required by law.

Describe the plan (how and when identifiers will be destroyed and by whom). If there is a health or research justification for retaining the identifiers or such retention is otherwise required by law, provide the reason to retain identifiers:

Once patients are deemed ineligible or decline participation, the study coordinator will immediately remove their information from the excel spreadsheet and/or cancel pre-scheduled appointments

e3) Adequate written assurances that the protected health information will not be reused or disclosed to any other person or entity except (i) as required by law, (ii) for authorized oversight of the research study, or (iii) for other research for which the use or disclosure of PHI would be permitted by the HIPAA Privacy Rule. By electronically signing this submission, the PI provides this written assurance:

not a question

f) The research could not practicably be conducted or carried out without the waiver or alteration:

- Explain why informed consent/authorization can not be obtained from subjects.

In order to perform this research, we must be able to screen potential patients for eligibility.

g) The research could not practicably be conducted or carried out without access to and use of the protected health information:

If patients are not pre-screened, the study team will not have the ability to enroll a sufficient number of patients.

h) For research using biospecimens or identifiable information, the research could not practicably be carried out without access to and use of the protected health information:

If patients are not pre-screened, the study team will not have the ability to enroll a sufficient number of patients.

Waiver of Documentation of Consent and HIPAA Authorization for Scheduling in MaestroCare and/or the recording of PHI via telephone for screening purposes:

These research activities prior to obtaining written consent for the study presents no more than minimal risk of harm to subjects:

- (•) True
- ( ) False

These are procedures for which written consent is normally not required outside of the research context:

- (•) True
- ( ) False

An IRB-approved phone script will be used to obtain verbal consent from subjects for scheduling and/or screening prior to obtaining written consent for the study:

- (•) True
- ( ) False

Devices

Include all devices being evaluated in this study:

Include all devices being evaluated in this study to determine their safety or effectiveness, and include information about a humanitarian use device where requested.Also dd devices without an IDE here, including any Humanitarian Use Device that does not require an IDE because it is to be used according to its FDA approved product labeling and its safety or effectiveness is not being evaluated.

Complete an IDE Billing Notice as applicable. This can be attached with the Brochure in the Initial Submission Packet.

View Details	Device Name	Is the Device FDA Approved	Will the device to be evaluated or the Humanitarian Use Device be manufactured at Duke?	IDE /Compassionate Use Request Number
⊟	N/A	No	Yes	

Device Source	n/a
CMS Category	☐ A ☐ B
Is the device provided to subject free of charge?	Yes
Is this a HUD (HDE)?	No
HDE Number	
Is the Device FDA Approved	No
Will the device to be evaluated or the Humanitarian Use Device be manufactured at Duke?	Yes
Do you have an IDE number for this device?	No

IDE/Compassionate Use Request Number	
IDE Holder	N/A
IDE Details	
In the opinion of the sponsor, select the level of risk associated with this device	No Significant Risk

Who will be responsible for the storage, inventory and control of the device to be evaluated or the Humanitarian Use Device?

Where will the device to be evaluated or the Humanitarian Use Device be stored?

Who will be responsible for giving or administering the device to be evaluated or the Humanitarian Use Device to the research subject?

From where will the device to be evaluated or the Humanitarian Use Device be dispensed?

At the completion of this research study, what will be done with the unused or returned device or the Humanitarian Use Device?

Privacy and Confidentiality

Explain how you will ensure that the subject's privacy will be protected:

Consider privacy interests regarding time and place where subjects provide information, the nature of the information they provide, and the type of experience they will be asked to participate in during the research.

Subjects will be approached by their provider and once they agree to speak with a member of a research team the study will be discussed in a closed room.

Describe how research data will be stored and secured to ensure confidentiality:

How will the research records and data be protected against inappropriate use or disclosure, or malicious or accidental loss or destruction? Records and data include, for example, informed consent documents, case report forms or study flow sheets, survey instruments, database or spreadsheets, screening logs or telephone eligibility sheets, web based information gathering tools, audio/video/photo recordings of subjects, labeled specimens, data about subjects, and subject identifiers such as social security number.

All data are stored on password protected servers and locked file cabinets with limited access.

Application Questions Complete

Please click Save & Continue to proceed to the Initial Submission Packet.

The Initial Submission Packet is a short form filled out after the protocol application has been completed. This is an area to attach protocol-related documents, consent forms, and review the application.