UCSF Helen Diller Family Comprehensive Cancer Center

Peripheral T cell determinants of response and resistance to pembrolizumab in melanoma

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Protocol Signature Page

1. I agree to follow this protocol version as approved by the UCSF Protocol Review and Monitoring Committee (PRMC), Institutional Review Board (IRB), and Data and Safety Monitoring Committee (DSMC).
2. I will conduct the study in accordance with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Good Clinical Practices (ICH-GCP) and the applicable IRB, ethical, federal, state, and local regulatory requirements.
3. I certify that I, and the study staff, have received the required training to conduct this research protocol.
4. I agree to maintain adequate and accurate records in accordance with IRB policies, federal, state and local laws and regulations.

UCSF Principal Investigator

_____
Printed Name

_____ _____
Signature Date

Abstract

Title	Peripheral T cell determinants of response and resistance to pembrolizumab in melanoma
Study Description	This is a non-therapeutic study assessing peripheral T cell determinants of response and resistance to immunotherapy in patients with advanced melanoma. Patients will be eligible to enroll if they have unresectable/metastatic stage III/IV melanoma and are immunotherapy naïve with an indication to start anti-PD1 therapy per NCCN guidelines. Tumor and peripheral blood mononuclear cells (PBMC) will be collected for correlative studies before and during treatment with pembrolizumab. A total of 28 subjects will be enrolled in this study.
Phase of Study	Non-therapeutic exploratory study
Study population	Patients will be eligible to enroll if they have unresectable/metastatic stage III/IV melanoma and are immunotherapy naïve with an indication to start anti-PD1 therapy per NCCN guidelines. Participants will be recruited from the Melanoma and Cutaneous Oncology Program at the University of California San Francisco Comprehensive Cancer Center. Patients must have accessible tumor for research biopsy.
Primary Objective	To understand how the systemic immune profile (T cell activation and expansion in the tumor microenvironment (TME)) changes in response to anti-PD1 therapy in patients with advanced or metastatic melanoma on pembrolizumab monotherapy.
Exploratory Objectives	1. To correlate the peripheral T cell profiles with the objective response rate (ORR) at 24 weeks in patients with advanced melanoma on pembrolizumab monotherapy. 2. To correlate the peripheral T cell profiles with progression free survival (PFS) in patients with advanced melanoma on pembrolizumab monotherapy. 3. To correlate the peripheral T cell profiles with overall survival (OS) in patients with advanced melanoma on pembrolizumab monotherapy. 4. To correlate the peripheral T cell profiles with toxicity profile 5. To determine the transcriptional and phenotypic features of tumor directed T cells in blood using a combination of phenotypic markers derived from COMET and cite-seq
Sample Size	28 patients
Duration of Study Treatment	Patients will be followed for 6 months from time of treatment initiation. After 6 months, patients do not need to be followed but standard of care scans and survival status can be assessed for up to 5 years.

Unique Aspects of this Study	A key question is whether systemic tumor directed CD8+ T cells can be identified and relatedly whether they can be used as a biomarker for response to immunotherapy. Integrating the local and systemic immune component into a model of PD-1 action is vital to a better mechanistic understanding of PD-1 response and resistance.

List of Abbreviations

AE	Adverse event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CBC	Complete blood cell (count)
CRF	Case report form
CT	Computerized tomography
CTLA-1	Cytotoxic T lymphocyte-associated protein 4
CTCAE	Common Terminology Criteria for Adverse Events
DSMC	Data and Safety Monitoring Committee
DSMP	Data and Safety Monitoring Plan
ECI	Events of Clinical Interest
ECOG	Eastern Cooperative Oncology Group
FDA	Food and Drug Administration
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HDFCCC	Helen Diller Family Comprehensive Cancer Center
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
ICF	Informed consent form
ICH	International Conference on Harmonization
IgG4	Immunoglobulin G4
irAE	Immune-related adverse event
IRB	Institutional Review Board
IV	Intravenous
MHC	Major histocompatibility complex
MRI	Magnetic resonance imaging
NCCN	National Comprehensive Cancer Center
ORR	Overall response rate
OS	Overall survival
PBMC	Peripheral blood mononuclear cell
PD-1	Programmed cell death 1
PDL-1	Programmed cell death ligand 1
PDL-2	Programmed cell death ligand 2
PI	Principal investigator
PFS	Progression free survival
PRC	Protocol Review Committee (UCSF)

List of Abbreviations

PT	Prothrombin time
RANO	Response Assessment in Neuro-oncology
RR	Response rate
TCR	T-cell receptor
TME	Tumor microenvironment
UCSF	University of California, San Francisco
WOCBP	Woman of childbearing potential

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1 Introduction

1.1 Background on Indication

1.1.1 The local and systemic immune response in checkpoint inhibitor therapy

Cancer immunotherapy has revolutionized the treatment of malignancy, leading to durable responses in some patients(1). The effectiveness of checkpoint inhibitor therapy depends on the presence of CD8+ T cells in the tumor microenvironment as well as the systemic immune response(2). “Exhaustion markers” on tumor infiltrating CD8+ cells such as programmed cell death protein-1 (PD-1) and cytotoxic T lymphocyte-associated protein 4 (CTLA-4) are required for effective PD-1 blockade(3–7). These “exhausted” T cells have a finite lifespan even with PD-1 blockade(3, 4) and appear to be replaced by fresh clonal proliferating T cells, a concept termed “clonal replacement”(8). Specifically, systemic immune cells undergo priming and infiltrate the tumor in response to PD-1 blockade, thus playing an important role in maintaining and generating a PD-1 response(9, 10). A key question is whether these systemic tumor directed CD8+ T cells can be identified and relatedly whether they can be used as a biomarker for response to immunotherapy. Integrating the local and systemic immune component into a model of PD-1 action is vital to a better mechanistic understanding of PD-1 response and resistance.

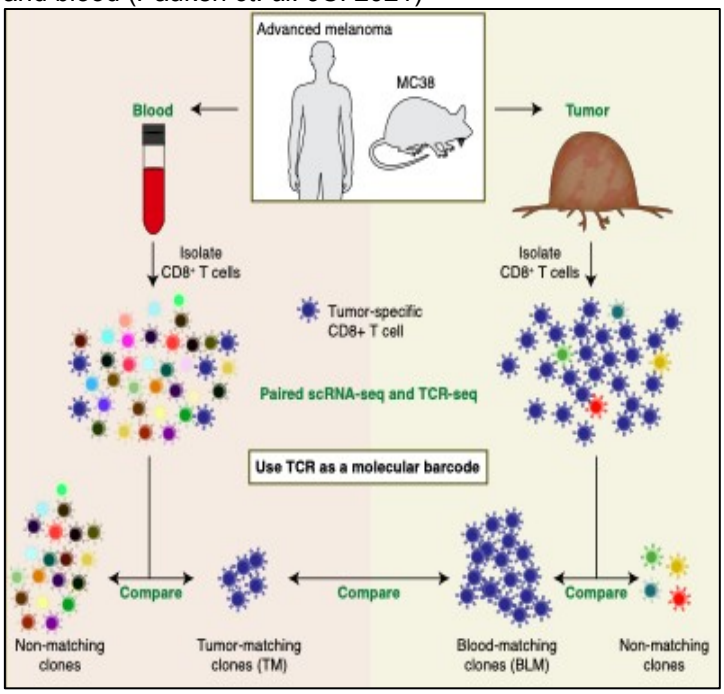
1.1.2 Identification and characterization of peripheral tumor-directed CD8+ T cells

The vasculature is a major site of $CD8^+$ T cell trafficking between primary tumors, secondary lymphoid organs, and metastatic sites(11), making the blood an ideal location to sample to investigate peripheral anti-tumor responses. Multiple recent studies have provided comprehensive profiling of T cells in the blood of patients with cancer, including patients on checkpoint blockade(12–17). However, the ability to identify T cell populations directed against tumor epitopes has been more challenging. Historically, it had been difficult to track tumor antigen-specific CD8+ T cells using conventional methods like peptide major histocompatibility complex (MHC) tetramers due to the limited number of defined tumor antigens and the limited number of MHC haplotypes that bind these antigens . Improved methods to identify T cell populations directed against tumor epitopes are needed to focus these analyses in order to better identify and characterize the minority of circulating T cell populations that have functional and prognostic relevance to the anti-tumor immune response.

1.1.3 Preliminary work

Recently, our group demonstrated that T cell receptor (TCR) analysis combined with single cell RNA sequencing can be used to detect tumor directed CD8+ T cells in mice with MC38 tumors and in humans with melanoma (**Figure 1**) (20). Tumor matching CD8+ T cells showed increased signs of activation compared to non-tumor matching cells in the blood but were less dysfunctional than corresponding clones in the tumor. This work provided both a technological advance as well as new insight into the biology of the tumor-matching T cells in the blood. However, a limitation of this work was that only four human subjects were included, and longitudinal samples were collected in only two participants. Therefore, there was limited ability to assess dynamic changes in peripheral tumor-directed CD8+ T cells and clinical response to immunotherapy.

Figure 1: Identifying T cell clones shared between tumor and blood (Pauken *et. al.* JCI 2021)



This figure depicts the method for peripheral tumor directed CD8+ T cell identification. The tumor-specific CD8+ T cells are shown in navy blue.

1.2 Rationale for the Proposed Study

To define the mechanistic basis of PD-1 action with particular interest in studying the peripheral T cell component. See background discussion above.

1.3 Rationale for the Dose Selection/Regimen

Pembrolizumab will be administered per standard of care, according to the NCCN guidelines. The planned dose of pembrolizumab for this study is 200 mg every 3 weeks (Q3W).

1.4 Rationale for Exploratory Biomarker Research

Tumor and blood samples will be processed to isolate T cells using 10X Chromium sequencing using standard methods. For each blood sample, in addition to the single-cell assay, we will generate bulk RNA-seq and freeze a subset of the sample for downstream validation assays. We will characterize the transcriptional landscape and subsequent differentiation state of the matching clones in blood and tumor using transcriptional signature analysis of the RNAseq data.

To test whether single-gene markers can be used to identify the CD8+ TM component with flow cytometry, we will apply COMET, a computational tool developed in the Singer lab that can help predict markers from single-cell RNA-seq data. We can use this program, as well as additional tailored code, to derive novel hypotheses (e.g. pathways enriched in clones that expand in responders) as well as to test hypotheses of interest (e.g. testing whether recently published cell states are enriched or depleted in responders when compared to non-responders).

2 Study Objectives

2.1 Hypothesis

We hypothesize that systemic T cells traffic into the tumor microenvironment (TME) and predict response and resistance to immunotherapy. These systemic tumor directed T cells can be defined by tumor/blood scRNA using TCR as a barcode and can help predict response to PD-1 therapy.

2.2 Primary Objective and Endpoints

Primary Objective	Endpoints	Time Frame
To understand how the systemic immune profile (T cell activation and expansion in TME) changes in response to pembrolizumab therapy in patients with advanced melanoma on pembrolizumab monotherapy.	• Tumor and blood T cell TCR + single cell RNA profile ○ pre-immunotherapy initiation in peripheral blood and tumor ○ post-immunotherapy initiation in peripheral blood (and optional post-immunotherapy tumor biopsy)	24 weeks

2.3 Exploratory Objectives and Endpoints

Exploratory Objective	Endpoints	Time Frame
To correlate the peripheral T cell profiles with the objective response rate (ORR) at 24 weeks in patients with advanced melanoma on pembrolizumab monotherapy.	• ORR at 24 weeks	24 weeks
To correlate the peripheral T cell profiles with progression free survival (PFS) in patients with advanced melanoma on pembrolizumab monotherapy.	• PFS	5 years
To correlate the peripheral T cell profiles with overall survival (OS) in patients with advanced melanoma on pembrolizumab monotherapy.	• OS	5 years
To correlate the peripheral T cell profiles with toxicity profile	• CTCAE v. 5	24 weeks
Transcriptional and phenotypic features of tumor directed T cells in blood using a combination of phenotypic markers derived from COMET and cite-seq	• Mean (95% CI) change in biomarker levels with treatment, by cycle	24 weeks

3 Study Design

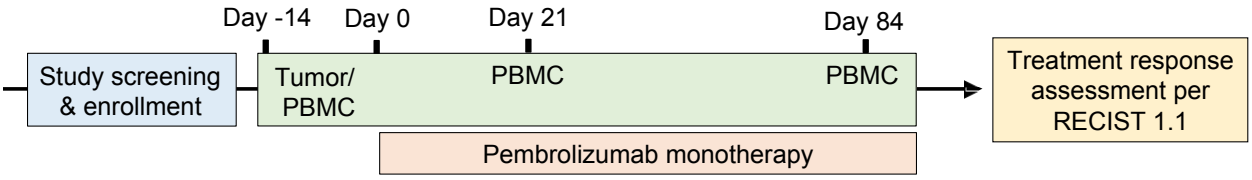
3.1 Characteristics

This is a non-therapeutic exploratory study assessing the peripheral T cell determinants of response and resistance to immunotherapy in melanoma. Patients will be enrolled if they have unresectable/metastatic stage III/IV melanoma and are immunotherapy naïve with indication to start anti-PD1 therapy per NCCN guidelines. Patients will be started on pembrolizumab monotherapy per standard of care. Tumor and peripheral blood mononuclear cells (PBMC) will be collected for correlative studies before and during treatment. If fresh tissue collection is not possible or not safe, archival tissue such as core or incisional biopsy or FNA cell block may be used. A total of 28 subjects will be enrolled in this study.

3.2 Trial Schema

Participants will be recruited from the Melanoma and Cutaneous Oncology Program at the University of California San Francisco Comprehensive Cancer Center. Patients must have accessible tumor for the research biopsy which needs to be a core, punch, excisional or incisional biopsy. A clinical research coordinator from our Melanoma and Cutaneous Oncology Program will be assigned to manage this trial and will assist with patient screening and consent. The trial will be reviewed at the Melanoma research site committee, which occurs twice per month. After enrolling in this study, participants will undergo a pre-treatment tumor core biopsy and PBMC collection. If fresh biopsy is not possible, archival tissue may be used. Then, patients will be started on pembrolizumab per standard of care and PBMCs will be collected every cycle (q3wk) (**Figure 2**).

Figure 2: Clinical trial schema



Shown is the study schema. Day 0 is defined as the first day of pembrolizumab monotherapy. “Tumor” indicates a research tumor biopsy, which must be a core biopsy (16-18 g needle X2), punch, excisional or incisional biopsy. If fresh biopsy is not possible, archival tissue may be used “PBMC” indicates collection of peripheral blood mononuclear cells. The baseline tumor/PBMC collection must be done within 28 days

3.3 Sample Size

Approximately 28 participants will be consented to achieve the target sample size of 28 evaluable subjects.

3.3.1 Participant Discontinuation Criteria

Participants may discontinue study activities at any time for any reason or be discontinued from the study activities at the discretion of the investigator should any untoward effect occur

3.4 Eligibility Criteria

3.4.1 Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

1. Patients must have histologically confirmed locally advanced or metastatic melanoma and be starting on standard of care pembrolizumab monotherapy. Patients may have received any or no prior anti-cancer therapy without limitation.
2. Must have one or more sites of disease amenable to biopsy (tumor, skin, lymph node, pleural fluid, peritoneal fluid, cerebral spinal fluid (CSF)).
3. Have measurable disease based on RECIST 1.1. Lesions situated in a previously irradiated area are considered measurable if progression has been demonstrated in such lesions.
4. Participants must be age 18 years or older on the day of signing informed consent.
5. Have the ability to provide written informed consent for the trial.
6. Be able and willing to comply with study procedures including provision of basic demographic information and medical history.
7. Be willing to receive periodic follow up phone calls to monitor health status and survival status.

3.4.2 Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

1. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent or with an agent directed to another stimulatory or co-inhibitory T-cell receptor (e.g., CTLA-4, OX-40, CD137).
2. Has received prior systemic anti-cancer therapy including investigational agents within the prior 2 weeks.
3. Has a diagnosis of immunodeficiency or is receiving chronic systemic steroid therapy (in dosing exceeding 10 mg daily of prednisone equivalent) or any other form of immunosuppressive therapy within 7 days prior to the first dose of study drug.
4. Has a contraindication to tissue biopsy for minimally-invasive research-procedure
5. Contraindication to phlebotomy (up to 40 mL per phlebotomy every three weeks).

3.5 Inclusion of Women and Minorities

3.5.1 Eligibility of Women and Minorities

Individuals of any sex/gender, race, or ethnicity are eligible for this study.

3.5.2 Recruitment of Minority Groups

The study recruitment strategy aims to achieve representation of minority groups that reflects the demographics of the affected population in the catchment area.

3.6 Accrual estimates

The study planned to accrue a total of 28 evaluable patients.

4 Specimen Collection and Processing

4.1 Types of specimens

At baseline prior to starting pembrolizumab, blood and tumor tissue will be collected. Repeat blood collection will occur every three weeks on study. Two-six weeks after treatment initiation, an optional repeat tumor biopsy and/or biopsy of a metastatic site can be performed at the discretion of the study provider if the patient is amenable.

4.1.1 Fresh Tissue Collection

Biopsy of the tumor tissue can be done via a core needle biopsy, excisional, or incisional biopsy. The baseline tissue biopsy will be collected in conjunction with a standard of care biopsy if possible. One to four additional passes of core needle, or FNAs (number determined by safe accessibility and size of lesion) will be collected for research purposes. If no standard of care biopsy is planned, a research-only biopsy can be performed. This can be done via core biopsy, excisional biopsy, or incisional biopsy at the discretion of the study provider. If fresh tissue biopsy is not possible, archival tissue may be used.

Two to six weeks after starting immunotherapy, an optional repeat tumor biopsy can be performed (research only). This can be done via core biopsy, excisional biopsy, or incisional biopsy at the discretion of the study provider with patient consent. In addition, an optional biopsy of a metastatic site (skin/subcutaneous tissue, lymph node FNA/core biopsy, tissue FNA/core biopsy, pleural fluid collection, peritoneal fluid collection, and/or cerebral spinal fluid collection) can also be performed at the discretion of the study provider at baseline and/or after initiation of immunotherapy. These will be done in conjunction with standard of care biopsies if possible or can be performed as research-only biopsies at the discretion of the study provider with patient consent.

4.1.2 Blood

Up to 40 mL of blood will be collected at baseline and every 3 weeks after starting pembrolizumab for at least the first eight cycles of pembrolizumab. Thereafter, additional research blood specimens can be collected at later timepoints at the discretion of the study PI and with patient consent. Blood draws will be performed during standard of care or clinical trial phlebotomy collections, whenever possible, to minimize patient risk. The amount of blood drawn will not exceed 100 mL in total.

4.2 Specimens Processing

4.2.1 Tissue Processing and Analysis

Tissue will be processed as per standard operating procedure for these sample types by a research technician or investigator.

4.2.2 Blood Processing and Analysis

Blood will be processed as per standard operating procedure for these sample types by a research technician or investigator.

4.3 Specimen Coding and Labeling

Specimens will be de-identified and assigned a study number for storage purposes. Specimens will be collected and managed using encrypted databases stored on the secure UCSF server,

using appropriate database software. Principal Investigator, Co-Investigators and lab personnel will have access to de-identified specimens.

4.4 Specimen Transport

The transportation, coding and storage systems will be performed under UCSF biohazardous specimen guidelines. The transportation of all tissues is handled by specially-trained research assistants employed by the Cancer Center or organ-based projects.

5 Study Timeline

5.1 Primary Completion

The study completion date is 60 months after study initiation.

5.2 Study Completion

The study completion date is 60 months after study initiation.

5.3 Study Calendar

Table 1- Schedule of Activities (SoA)

Study Period	Screening	Treatment cycle								EOT	Efficacy Follow-up	Survival Follow-Up
Visit Number/Title:	Screening	1	2	3	4	5	6	7	8	Discontinuation		
Scheduling window (days):	-28 to -1	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3		Every 9 or 12[6] Weeks(±7 days)	Every 12 Weeks[7]
Standard of Care[1]												
Pembrolizumab		X	X	X	X	X	X	X	X			
Administrative Procedures												
Informed Consent	X											
Inclusion/Exclusion Criteria	X											
Medical History	X											
Prior/Concomitant Medication Review	X											
AE assessment		X	X	X	X	X	X	X	X	X		
Tumor biopsy/correlative studies												
Tumor biopsy[3]	X		X[5]									
Correlative blood collection[4]	X	X	X	X	X	X	X	X	X		X	
Survival Status		←==========								==========→		X

[1] Standard of care Pembrolizumab 200 mg q every 3 weeks

[2] Tumor imaging is strongly preferred to be acquired by computed tomography (CT). For the abdomen and pelvis, contrast-enhanced magnetic resonance imaging (MRI) may be used when CT with iodinated contrast is contraindicated, or when local practice mandates it.

[3] Core biopsy (16-18 g needle X2) or a punch or excisional or incisional biopsy. Required biopsy prior to starting treatment.

[4] Up to 40 mL of whole blood collection for PBMC will be collected at baseline and prior to the first eight cycles of treatment with pembrolizumab. Blood can be collected at later timepoints at the discretion of the study PI and with patient consent.

[5] An optional repeat tumor biopsy will occur two-six weeks after treatment initiation at the discretion of the study PI and with the consent of the patient. This can be a biopsy of the primary tumor and/or a metastatic site, at the discretion of the study PI and with patient consent.

[6] Imaging will occur every 9-12 weeks per standard of care. Preferred imaging: PET/CT, CT CAP, or MRI at the discretion of the study provider

[7] Patients will be followed for 6 months on study, but then standard of care imaging and survival status can be tracked for up to five years

5.4 Participant Registration

A written, signed, informed consent form (ICF) and a Health Insurance Portability and Accountability Act (HIPAA) authorization must be obtained before any study-specific assessments are initiated. A copy of the signed ICF will be given to the subject and a copy will be filed in the medical record. The original will be kept on file with the study records.

All participants consented to the study will be registered in OnCore®, the UCSF Helen Diller Family Comprehensive Cancer Center Clinical Trial Management System (CTMS). The system is password protected and meets HIPAA requirements.

5.5 Schedule of Procedures and Assessments

5.5.1 Screening (prior to standard of care treatment)

The Screening procedures and assessments must be completed within 28 of initiating study treatment.

- Informed Consent
- Eligibility (inclusion and exclusion) - All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the trial.
- Demographics: Age, gender, medical comorbidities
- Cancer and medical history review - A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the Investigator. Details regarding the disease for the subject enrolled in this study will be recorded separately and not listed as medical history.
- Medication review - The investigator or qualified designee will review prior and concomitant medication that the subject use and will record the medications taken by the subject.
- Up to 40cc PBMC blood collection
- Tumor/tissue collection - Fine needle aspiration (FNA) or core needle biopsy of the tumor, portions of excisional biopsies or surgical resections
- Stagging imaging: PET/CT, CT chest/abd/pelvis, or MRI abd/pelvis
- Optional tissue collection form a metastatic site – Skin/subcutaneous tissue, lymph node FNA/core biopsy, tissue FNA/core biopsy, pleural fluid collection, peritoneal fluid collection, and/or cerebral spinal fluid collection.

5.5.2 Study Procedures during standard of care treatment

After initiation of pembrolizumab per standard of care, the following will occur each cycle:

- Up to 40cc PBMC blood collection every three weeks for the first eight weeks of pembrolizumab treatment, with optional PBMC collection during later cycles at the discretion of the study PI and with patient consent.
- 2-6 weeks after study initiation: Optional tumor/tissue collection – Fine needle aspiration (FNA) or core needle biopsy of the tumor, portions of excisional biopsies or surgical resections.
  2-6 weeks after study initiation: Optional tissue collection from a metastatic site - Skin/subcutaneous tissue, lymph node FNA/core biopsy, tissue FNA/core biopsy, pleural fluid collection, peritoneal fluid collection, and/or cerebral spinal fluid collection.

- Tumor imaging will be conducted per standard of care, approximately every 8-12 weeks. PET/CT, CT scan or MRI of chest, abdomen and pelvis is preferred. It is preferred if serial imaging is consistent for each participant.

5.5.3 Follow-up

Given that this is not an interventional trial, the clinical assessment and monitoring should occur per standard of care and at the discretion of the provider and are not specified by this trial.

5.5.4 Safety Follow-Up Visit

Given that this is not an interventional trial, the clinical assessment and monitoring should occur per standard of care and at the discretion of the provider and are not specified by this trial.

Each individual participant will be followed for up to 5 years post treatment to determine OS. They will not be followed beyond 5 years as part of this study.

5.5.5 Prohibited Medications

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

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy. Radiation therapy to symptomatic lesion(s) or to the brain may be allowed at the investigator's discretion.

Live vaccines within 30 days prior to the first dose of study treatment and while participating in the study. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (e.g., FluMist®) are live attenuated vaccines and are not allowed.

The use of steroids up to the equivalent of 10mg prednisone daily is allowed. Doses exceeding that is not allowed on this study.

Participants who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the study. All treatments that the Investigator considers necessary for a participant's welfare may be administered at the discretion of the Investigator in keeping with the community standards of medical care.

5.6 Response Criteria

Evaluation of Target Lesions

Complete Response (CR) .

Disappearance of all target lesions, determined by two separate observations conducted not less than 4 weeks apart. Any pathological lymph nodes (whether target or non-target) must

have reduction in short axis to <10 mm (the sum may not be “0” if there are target nodes). There can be no appearance of new lesions.

Partial Response (PR)

At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD. There can be no appearance of new lesions.

Progressive Disease (PD)

At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm (0.5 cm). (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD)

Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started.

Evaluation of Non-Target Lesions

Complete Response (CR)

Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (< 10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Incomplete Response/Stable Disease (SD)

Persistence of one or more non-target lesion(s) and/or 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.

Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The participant’s best response assignment will depend on the achievement of both measurement and confirmation criteria.

Table 2.1 Response Criteria for Participants with Measurable Disease

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.
Note: Participants with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

Table 2.2 Response Criteria for Participants with Non-Measurable Disease

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

Duration of Response

The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

Duration of stable disease

Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

Progression-Free Survival

Progression-free survival (PFS) is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

6 Statistical Considerations and Evaluation of Results

6.1 Sample Size Justification

This is a non-therapeutic clinical trial assessing peripheral T cell determinants of response and resistance to immunotherapy in melanoma. The proposed sample size of the study is not based on the alpha level and the power estimate for hypothesis testing, but rather on the assumed measurements of the TCR biomarkers profile under systemic immune environment during the PD-1 blockade with pembrolizumab in patients with melanoma. A total of 28 subjects will be accrued to develop the systemic immune biomarkers profile and to determine preliminary clinical evidence on the progression-free survival and objective response rate.

6.2 Statistical Analysis Plan Summary

6.2.1 Subject Demographic and Baseline Characteristics

Subject disposition (e.g., the number of subjects signing the informed consent, completed, and discontinued) along with the number of subjects in the analysis populations will be summarized. Descriptive statistics will be used to summarize subject demographics and baseline characteristics such as medical and surgical history. Additionally, demographics will also be presented for screen failure subjects.

6.2.2 Primary Analysis

We will analyze the bulk RNAseq data as described below:

Step 1: Identify genes predictive of response to anti-PD-1 therapy by testing for significant associations across expression rates of each gene and response/resistance, within a hurdle-Gaussian mixed-effect framework that accounts for variance across patients and technical noise present in single-cell data. The use of single-cell analysis to detect differentially expressed genes gives significant statistical power and our anticipated number of patients (n = 28, ≥300K total T cells) is sufficient to detect even subtle effects. Following identification of genes indicative of response to anti-PD-1 treatment, we will identify gene modules (gene sets that co-vary within the single-cell data using hierarchical clustering within the gene-space on batch corrected data). We will identify predicted functional attributes and central regulators for each module with statistical enrichment tests (e.g. GSEA17 and Enrichr18).

Step 2: Identify changes in subpopulation frequencies associated with response to anti-PD-1 therapy. We will identify T cell subpopulations in the tumor-directed component in blood whose relative frequency is indicative of response to anti-PD-1 therapy, using a negative binomial regression model. We will test for predictive differences in relative frequencies before and after treatment initiation separately, as well as when combining both timepoints with appropriate interaction terms.

Step 3: Identify differences in clonal expansion, distribution, and “transcriptional migration” of T cells associated with response to anti-PD-1 therapy. We will build a novel computational framework to identify T cell clonal behavior associated with response to anti-PD-1 therapy. We will use the profiling of TCR sequences at single-cell resolution to compare various clonal attributes (e.g. clonal size and expansion following treatment) in each of the subpopulations identified in Step 2 for their association with response to anti-PD-1 therapy. Additionally, we will search for “transcriptional migration” events, in which T cell clones change their transcriptional profile following treatment and will assess the predictive power of such events to the success of

anti-PD-1 therapy. Following clonal identification, analyses of clonal size, expansion, and change in transcriptional states are conducted using statistical modeling (mixed-effect generalized regression models with coefficients for response state while accounting for technical affects like batch effects and gene count in cells).Clinical outcomes will be evaluated base on the objective response (CR/PR) by RECIST 1.1 by Week 24. Descriptive statistics with frequency and proportions will be used to evaluate the ORR rate by Week 24. Descriptive statistics with mean, median, STD and 95%CI will be analyzed for the systemic immune biomarkers. The association between the systemic immune T cell levels and clinical response to anti-PD1 therapy will be analyzed using T-tests for significance.

6.2.3 Exploratory Analysis

Time to event variables (progression-free survival and overall survival) will be summarized using the Kaplan-Meier method, including graphical displays and incidence estimates at 12 and 24 weeks.

For immunologic and genomic correlative tests, the study endpoint will be determining changes in the expression with treatment. We will estimate mean (95% CI) changes in biomarker level with treatment. To examine trends, we will examine mean biomarker levels by cycle. If patients vary by amount of therapies received, we will model mean biomarker levels by dose & cycle. We will use generalized estimating equation models to determine if changes in immunologic and genomic biomarker levels correlate with patient response, for responses evaluated at the end of each treatment cycle and biomarkers evaluated at baseline and after cycles 3 and 6.

The exploratory analyses will evaluate systemic TCR matched newly activated effector CD8+ cells and intratumoral exhausted T cells as well as intratumoral Treg cells. In this analysis data will be summarized using descriptive statistics. For continuous variables, descriptive statistics will include the number of non-missing values, mean, standard deviation, median, min and maximum. For categorical variables, descriptive statistics will include counts and percentages per category. For association between biomarkers, Spearman correlations will be calculated to determine the significance of the association between the biomarkers of interest.

7 Study Management

7.1 Pre-study Documentation

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki as stated in 21 CFR §312.120(c)(4); consistent with GCP and all applicable regulatory requirements.

Before initiating this trial, the PI will have written and dated approval from the Institutional Review Board for the protocol, written informed consent form, subject recruitment materials, and any other written information to be provided to participants before any protocol related procedures are performed on any participants.

The PI must comply with the applicable regulations in Title 21 of the Code of Federal Regulations (21 CFR §50, §54, and §312), GCP/ICH guidelines, and all applicable regulatory requirements. The IRB must comply with the regulations in 21 CFR §56 and applicable regulatory requirements.

7.2 Institutional Review Board Approval

The protocol, the proposed informed consent form, and all forms of participant-facing materials related to the study (e.g. advertisements used to recruit participants) will be reviewed and approved by the IRB of record. Prior to obtaining IRB approval, the protocol must be approved

by the Helen Diller Family Comprehensive Cancer Center Site Committee and by the Protocol Review Committee (PRC). The initial protocol and all protocol amendments must be approved by the IRB prior to implementation.

7.3 Informed Consent

All participants must be provided a consent form describing the study with sufficient information for each participant to make an informed decision regarding their participation. Participants must sign the IRB -approved informed consent form prior to participation in any study specific procedure. The participant must receive a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

7.3.1 General Informed Consent

Consent must be documented by the participant's dated signature or by the participant's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion.

A copy of the signed and dated consent form should be given to the participant before participation in the trial.

The initial informed consent form, any subsequent revised written informed consent form and any written information provided to the participant must receive the IRB/ERC's approval/favorable opinion in advance of use. The participant or his/her legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the participant's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the participant's dated signature or by the participant's legally acceptable representative's dated signature.

Specifics about a trial and the trial population will be added to the consent form template at the protocol level.

The informed consent will adhere to IRB/ERC requirements, applicable laws and regulations and Sponsor requirements.

If the investigator recommends continuation of study intervention beyond disease progression, the participant or his/her legally acceptable representative will be asked to sign consent.

7.4 Changes in the Protocol

Once the protocol has been approved by the IRB, any changes to the protocol must be documented in the form of an amendment. The amendment must be signed by the PI and approved by PRC and the IRB prior to implementation.

If it becomes necessary to alter the protocol to eliminate an immediate hazard to participants, an amendment may be implemented prior to IRB approval. In this circumstance, however, the PI must then notify the IRB according to institutional requirements.

7.5 Case Report Forms (CRFs)

The PI and/or designee will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study. Study specific Case Report Forms (CRFs) will document safety and treatment outcomes for safety monitoring and data analysis. All study data will be entered into OnCore® via standardized CRFs in accordance with the

CTMS study calendar, using single data entry with a secure access account. Study personnel will complete the CRFs; the PI will review and approve the completed CRFs.

The information collected on CRFs shall be identical to that appearing in original source documents. Source documents will be found in the participant's medical records maintained by study personnel. All source documentation should be kept in separate research files for each participant.

In accordance with federal regulations, the PI is responsible for the accuracy and authenticity of all clinical and laboratory data entered onto CRFs. The PI will approve all completed CRFs to attest that the information contained on the CRFs is true and accurate.

The PI will be responsible for ensuring the accurate capture of study data. At study completion, when the CRFs have been declared to be complete and accurate, the database will be locked. Any changes to the data entered into the CRFs after that time can only be made by joint written agreement among the PI and the trial statistician.

All source documentation and CTMS data will be available for review/monitoring by the UCSF DSMC and regulatory agencies.

7.6 Oversight and Monitoring Plan

The UCSF Helen Diller Family Comprehensive Cancer Center DSMC will be the monitoring/auditing entity for this study. The UCSF DSMC will monitor or audit the study in accordance with the NCI-approved Data and Safety Monitoring Plan (DSMP). The DSMC will review study-related activities to ensure that the study is conducted in accordance with the protocol, local standard operating procedures, FDA regulations, and Good Clinical Practice (GCP). Significant results of the DSMC monitoring/auditing review will be communicated to the IRB and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, as applicable (see Appendix 1).

7.7 Record Keeping and Record Retention

The PI is required to prepare and maintain adequate and accurate case histories that record all observations and other data pertinent to the investigation on each participant. Case histories include the case report forms and supporting data including, for example, signed and dated consent forms and medical records including, for example, progress notes of the physician, the individual's hospital chart(s), and the nurses' notes. The case history for each individual shall document that informed consent was obtained prior to participation in the study.

Study documentation includes all CRFs, 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 participant 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.

8 Safety Parameters

8.1 Definition of Adverse Events

8.1.1 Adverse Event (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

All Grade 3-5 adverse events (AEs), whether or not considered expected or unexpected and whether or not considered associated with the study intervention or procedure, will be entered into OnCore®, UCSF's Clinical Trial Management System. Adverse events are graded according to the Common Terminology Criteria for Adverse Events (CTCAE) as developed and revised by the Common Therapy Evaluation Program (CTEP) of the National Cancer Institute. See Appendix 1.

8.1.2 Serious Adverse Event (SAE)

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

- Death
- Life-threatening adverse event
    - An adverse event is considered life-threatening if, in the view of either the investigator or sponsor, its occurrence places the participant at immediate risk of death. It does not include an adverse event that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life function
- Congenital anomaly/birth defect

Important medical events that may not result in death, are life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.1.3 Unanticipated Problem (UP)

An unanticipated problem (UP) is any incident, experience, or outcome that meets all of the following criteria:

1) unexpected (in terms of nature, severity, or frequency) given (a) the research procedures are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being study;

2) related or possibly related to participation in the research; and

3) suggests that the research places subjects or others at a greater risk ofharm (including physical, psychological, economic, or social harm) related to the research than was previously known or recognized.

Only a small subset of adverse events occurring in human subjects participating in research will meet these three criteria for an unanticipated problem. Furthermore, there are other types of incidents, experiences, and outcomes that occur during the conduct of human subjects research that represent unanticipated problems but are not considered adverse events. For example, some unanticipated problems involve social or economic harm instead of the physical or psychological harm associated with adverse events. In other cases, unanticipated problems place subjects or others at increased risk of harm, but no harm occurs.

8.2 Classification of Adverse Events

8.2.1 Severity

Adverse events are graded according to the National Cancer Institute Common Terminology Criteria for Adverse events (CTCAE) version 5.0.

8.2.2 Attribution

Adverse events are given an assignment of attribution or relationship to the investigational agent(s) or study procedure. Attribution categories are:

- Definite – The adverse event is clearly related to the investigational agent(s) or study procedure.
- Probable – The adverse event is likely related to the investigational agent(s) or study procedure.
- Possible – The adverse event may be related to the investigational agent(s) or study procedure.
- Unrelated – the adverse event is clearly not related to the investigational agent(s) or study procedure.

8.2.3 Expectedness

An adverse event is considered unexpected if the nature, severity, or frequency of the event is not listed in the study protocol, product inserts, investigator brochure or informed consent document.

8.3 Recording of Adverse Events

Refer to the Data Safety Monitoring Plan, located in Appendix 2.

8.4 Expedited Reporting

8.4.1 Reporting to the HDFCCC Data and Safety Monitoring Committee

If a death occurs during the treatment phase of the study or within 30 days after the last administration of the study drug(s) and it is determined to be related either to the study drug(s) or to a study procedure, the UCSF PI or his/her designee must notify the DSMC Chair (or qualified alternate) within 1 business day of knowledge of the event. The contact may be by phone or e-mail.

8.4.2 Reporting to Institutional Review Board

The UCSF PI must report events to the IRB according to institutional guidelines.

- UCSF IRB website for guidance in reporting adverse events: [redacted]

8.5 Follow-up of Adverse Events

All participants who experience adverse events will be followed with appropriate medical management until resolved or stabilized, as determined by the investigator. For selected adverse events for which administration of the study drug/intervention was stopped, a re-challenge of the subject with the study drug/intervention may be conducted if considered both safe and ethical by the investigator.

8.6 Adverse Events Monitoring

Refer to the Data Safety Monitoring Plan, located in Appendix 1.

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Appendix 1: Data and Safety Monitoring Plan for a Non-therapeutic Institutional Trial

1. Oversight and Monitoring Plan

The UCSF Helen Diller Family Comprehensive Cancer Center (HDFCCC) Data and Safety Monitoring Committee (DSMC) is responsible for monitoring data quality and participant safety for all HDFCCC institutional clinical trials. A summary of DSMC activities for this trial includes:

- Annual auditing
- Review of serious adverse events
- Minimum of biennial regulatory auditing

The UCSF HDFCCC Data and Safety Monitoring Committee (DSMC) is responsible for participant safety for all HDFCCC institutional clinical trials. Greater than minimal risk nontherapeutic studies are characterized as low risk studies due to the trial design, as there isn't administration of drugs or complementary therapy that puts the participants at significant risk.

2. Monitoring and Reporting Guidelines

Investigators will conduct a continuous review of data and participant safety at monthly site committee meetings where the status of each participant is discussed and documented in the site committee minutes.

For "greater than minimal risk" nontherapeutic trials, the assigned DSMC Senior Monitor/Auditor will audit three of the enrolled participants once per year, with a maximum of ten participant charts audited during the entire course of auditing this trial until IRB closure.

If blood or tissue banking trials are determined to be "greater than minimal risk", then only Serious Adverse Events (SAEs) recorded in OnCore will be reviewed at each DSMC meeting for these trials.

After completion of each auditing visit, the DSMC Monitor/Auditors will send a follow-up report to the study team within 20 business days after the auditing visit is complete for the PI and the study team to resolve all action items from this report within 20 business days. An abbreviated regulatory review (i.e., reviewing protocol and consent versions, SAEs, PVs, DOA logs, 1572 forms, etc.) will occur at each participant monitoring review; however, a full regulatory review will occur on a biennially basis by the DSMC for regulatory compliance.

Auditing of all enrolled participants in these trials will be complete after 10 enrolled participants have been audited. However, regulatory reviews of the trial, safety reviews (i.e., Serious Adverse Event (SAE) reviews and Protocol Violation (PV) reviews), and audit/inspection preparation (as applicable) will continue until the trial is closed by the IRB.

3. Review and Oversight Requirements

3.1 Adverse Event Monitoring

All Grade 3-5 adverse events (AEs), whether or not considered expected or unexpected and whether or not considered associated with the study intervention or procedure, will be entered into OnCore®, UCSF's Clinical Trial Management System.

Adverse events are graded according to the Common Terminology Criteria for Adverse Events (CTCAE) as developed and revised by the Common Therapy Evaluation Program (CTEP) of the National Cancer Institute. Adverse events are further given an assignment of attribution or relationship to study intervention or procedure. Attribution categories are:

- **Definite** – The adverse event is clearly related to the study intervention or procedure.
- **Probable** – The adverse event is likely related to study intervention or procedure.
- **Possible** – The adverse event may be related to study intervention or procedure.
- **Unrelated** – the adverse event is clearly not related to the study intervention or procedure.

All clinically significant adverse events entered into OnCore® will be reviewed on a monthly basis at the Site Committee meetings

3.2 Serious Adverse Event Reporting

By definition, an adverse event is defined as a serious adverse event (SAE) according to the following criteria:

- Death.
- Life-threatening (i.e., results in an immediate risk of death).
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Permanent or significant disability/incapacity.
- Gives rise to a congenital anomaly/birth defect, or cancer, or any experience that suggests a significant hazard, contraindication, side effect, or precaution that may require medical or surgical intervention to prevent one of the outcomes listed above.
- Event that changes the risk/benefit ratio of a study.
- Any other event the Principal Investigator judges to be serious or which would suggest a significant hazard, contraindication, side effect, or precaution.

Serious adverse event reporting will be in accordance with all IRB regulations. For trials conducted under an investigational new drug (IND) application, the SAE will be reported in accordance with Code of Federal Regulation Title 21 Part 312.32 and will be reported on a Med Watch form.

UCSF IRB website for guidance in reporting serious adverse events:
[redacted]

Med Watch forms and information:
www.fda.gov/medwatch/getforms.htm

All serious adverse events are entered into OnCore®, as well as submitted to the IRB. The SAEs are reviewed and monitored by the Data and Safety Monitoring Committee on an ongoing basis and discussed at DSMC meetings, which take place every six weeks. The date the SAE was sent to all required reporting agencies will be documented in OnCore®.
If a death occurs during the treatment phase of the study and is determined to be possibly, probably, or definitely related either to the study intervention or procedure, the Investigator or his/her designee must notify the DSMC Chair or Vice Chair and DSMC Director within one business day.

3.3 Review of Adverse Event Rates

If at any time the Investigator voluntarily holds enrollment or stops the study due to safety issues, the DSMC Chair (or Vice Chair) and the DSMC Director must be notified within one business day and the IRB must be notified as per IRB reporting requirements.

Data and Safety Monitoring Committee Contacts:

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