STATUS PAGE
PROTOCOL **12-488**

Closed To New Accrual

Closure Effective Date: 03/29/2017
Reason: Study Accrual Goal Met

No new subjects may be enrolled in the study- as described above. Any questions regarding this closure should be directed to the study's Principal Investigator

DFCI Protocol No: 12-488

Front Sheet

Report Generated: 05/09/2018 02:06 PM

Title: Exploring the Utility of a Novel BRAF Test in Patients with Melanoma

Overall Institution: Massachusetts General Hospital

Overall Principal Investigator	Email	Institution
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Sponsor Name	Sponsor Protocol No	Roles	Grant Number(s)
DF/HCC Investigator		Regulatory	
Dana-Farber/Harvard Cancer Center		Funding	
ASCO Foundation		Funding	

Total Study-Wide Enrollment Goal: 220 **Total DF/HCC Estimated Enrollment Goal:** 220

Phase: Not Applicable

Age: Adults

Age Ranges: 18+

Will all subjects be recruited from pediatric clinics?

CTEP Study: No

Management Group(s): BIDMC Biologics (Kidney/Melanoma/Cutaneous)
DF/HCC Melanoma
DFCI/BWH Cutaneous Oncology
MGH Melanoma
MGH Regulatory Coordinators

Primary Management Group: DF/HCC Melanoma

Investigational Drug? This study uses a Drug/Biologic, but an IND was not submitted.

Drug(s), Biologic(s):

Investigational Device? This study uses an Investigational Device, but an IDE was not submitted.

Device Name:

IRB of Record:

Risk Category: Minimal Risk

Protocol Involves: Human Material Banking; Human Material Collection; Medical Record Review

Date Range: (Medical Record Review and Specimen Collection studies)

Participating Sites under the DFCI IRB

Institution: Beth Israel Deaconess Medical Center
Dana-Farber Cancer Institute
Massachusetts General Hospital

Participating Institutions Under Other IRB

None

Protocol Number: 12-488

Approval Date: 03/11/13 (IRB meeting date when protocol/consent approved or conditionally approved)

Activation Date: 05/15/13 (Date when protocol open to patient entry)

Approval signatures are on file in the Office for Human Research Studies, tel. 617-632-3029.

Date Posted	Revised Sections	IRB Approval Date	OHRS Version Date
06/19/13	Front Sheet replaced due to Amendment #1	06/18/13	N/A
09/30/13	Delayed Activation Alert Page removed: DFCI now ready for activation (note: previously activated at MGH & BWH on 05/15/13)	N/A	N/A
02/11/14	Study renewal/ Consent Form footer replaced due to Continuing Review #1	02/10/14	N/A
11/06/14	Consent Form and Front Sheet replaced due to Amendment #2	10/30/14	10/31/14
02/11/15	ON HOLD: All research must stop due to lapsed Continuing Review. Study expired 02/10/15	N/A	N/A
02/11/15	Remove Hold. Study renewal/ Consent Form footer replaced due to Continuing Review #2	01/14/15	N/A
Date Posted	**Revised Sections**	**IRB Approval Date**	**OnCore Version Date**
01/13/16	Study renewal/ Consent Form footer replaced due to Continuing Review #3	01/10/16	01/13/16
04/27/16	Protocol, Eligibility Checklist, Consent Form and Front Sheet replaced due to Amendment #3	04/07/16	04/27/16
Date Posted	**Revised Sections**	**Approved Date**	**Version Date (OnCore)**
01/05/17	Study renewal/ Consent Form footer replaced due to Continuing Review #4	12/21/2016	12/21/2016
03/30/17	Study closure – accrual goal met	03/29/17	N/A
12/13/17	Study renewal/Consent Form footer replaced due to Continuing Review #5	12/11/17	12/11/17
03/05/2018	No change to online documents per Amendment #4	03/05/2018	n/a
05/10/2018	Front Sheet, Protocol replaced per Amendment #5	04/30/2018	N/A
11/23/2018	Study renewal/Consent Form footer replaced per Continuing Review #6	11/21/2018	11/21/2018

DF/HCC Protocol #: 12-488

Title: Exploring the Utility of a Novel BRAF Test in Patients with Melanoma

Coordinating Center: Massachusetts General Hospital

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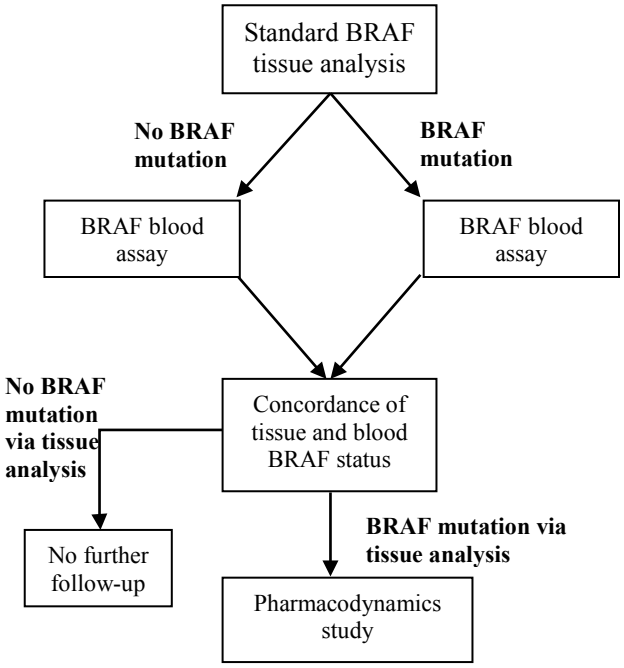
Agent(s): No therapeutics to be administered under this protocol

Protocol Type / Version # / Version Date: Version 4 / April 17, 2018

SCHEMA

Primary Objective

Advanced Melanoma



Stage II/resected Stage III

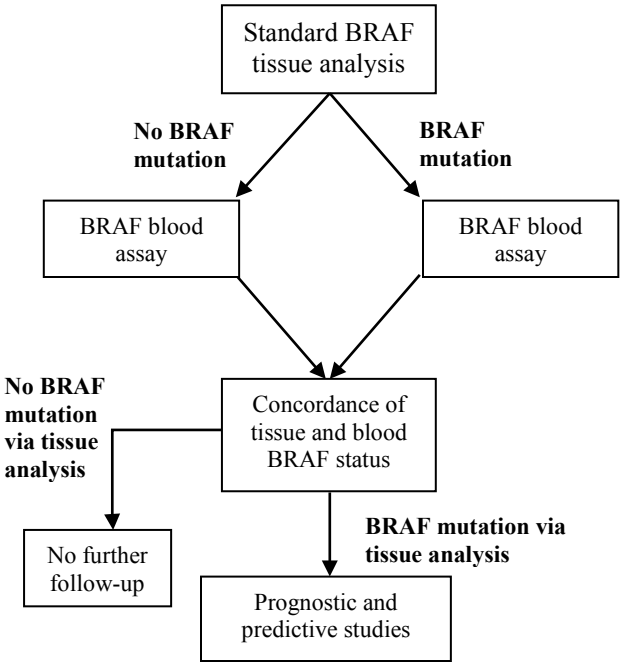


TABLE OF CONTENTS

1. OBJECTIVES

Primary

To determine the specificity and sensitivity of the blood based assay vis-à-vis tissue based BRAF analysis in patients with advanced and high-risk melanoma.

Secondary

1. To explore the pharmacodynamic effects of MAPK pathway inhibitors (including selective BRAF inhibitors, MEK inhibitors, and ERK inhibitors) utilizing pre- and on-treatment peripheral blood $BRAF^{V600E}$ mutational testing in patients with advanced melanoma (non-resectable Stage III or Stage IV).
2. To define the prognostic value of peripheral blood $BRAF^{V600}$ mutational testing in patients with Stage II and resectable Stage III melanoma.

1.1 Study Design

For the purposes of serum and plasma isolation optimization, patients who have agreed to participate in the study will donate no more than 20cc of peripheral blood (hereafter referred to as 1 sample) per timepoint. 10-20 ml blood will be acquired via standard venipuncture for serum and peripheral blood lymphocyte extraction at baseline and at varying intervals of time for each patient cohort. Amount of blood collected will follow the DF/HCC *Guidance for Maximum Blood Draw for Research Purposes.*

Any patient may refuse a research blood draw at any time while on study, or may refuse to continue donating samples at any time and may withdraw consent from participation. Subjects will receive no compensation for participation in this study. All unused collected blood will be saved for possible use in future related analyses.

1.2 Primary Objectives

To determine the sensitivity and specificity of peripheral blood $BRAF^{V600}$ mutation detection in patients with melanoma, we will analyze samples from two distinct cohorts of patients: 1) Patients with advanced melanoma; and 2) Patients with resected, Stage II or Stage III disease. A total of 110 patients in each cohort will be recruited to participate. Peripheral blood lymphocytes (PBL) and serum will be isolated from blood. RNA will be isolated from tissue and PBL/serum and $BRAF^{V600}$ analysis will be performed. Patients will be identified as being either positive or negative for the $BRAF^{V600}$ mutation based on the results of the tissue testing. The tumor tissue (either primary tumor or nodal/systemic metastasis) from each patient with advanced disease, or from all patients with Stage II or resectable Stage III disease with adequate tissue, will be analyzed in a CLIA-approved laboratory for $BRAF^{V600}$ sequencing analysis, either at the MGH TRL or at Clarient Laboratories using the FDA-approved Cobas® test. The results of the tissue-based and peripheral blood test will be compared and percent concordance will be determined.

1.3 Secondary Objectives

1. To explore the pharmacodynamic effects of MAPK-directed therapy on peripheral blood $BRAF^{V600}$ mutation detection, we will test isolated PBL and serum from samples obtained from patients in the cohort with advanced melanoma prior to receiving treatment with MAPK-directed therapy or immunotherapy (either commercially or on protocol). Patients will have been previously identified as being positive for a $BRAF^{V600}$ mutation based on the results of the testing of their tumor tissue. PBL and serum will be isolated from blood. RNA will then be isolated from tissue and PBL/serum and $BRAF^{V600}$ analysis will be performed as described above. Peripheral blood samples obtained prior to each cycle of MAPK-directed therapy or immunotherapy (either every three weeks or every four weeks depending on treatment protocol) will also be tested for $BRAF^{V600}$ RNA. Input RNA normalization will be carried out by using primers to a ribosomal protein, S-9, following the reverse transcription. Pre- and on-treatment $BRAF^{V600}$ levels will be compared, and a blood-based response will be defined as a 50% or more decrease in signal based on acrylamide gel analysis. A blood-based progression will be defined as a doubling in signal based compared with nadir level on acrylamide gel analysis. A comparison of blood-based response or progression ($\geq$50% improvement vs. <50% improvement; or 100% increase vs. < 100% increase) with clinical response (CR/PR by RECIST) and clinical progression (PD) will then be performed to assess concordance between changes over time with our assay and with imaging.

2. To explore the prognostic and predictive ability of peripheral blood $BRAF^{V600}$ mutation detection in the cohort of patients with Stage II or resected Stage III disease, we will test isolated PBL and serum from samples obtained from patients following complete resection of their disease but prior to receiving adjuvant therapy or embarking on expectant monitoring. To control for potential variations in circulating BRAF levels at various post-operative time points, patients must have their blood draw at least 4 weeks but no more than 8 weeks following their last surgery. Additionally, all patients with a thick primary melanoma (defined by AJCC as Breslow depth greater than 4 mm) or a clinically apparent/macroscopic involved lymph node, satellite lesion, or in-transit lesion will have tissue-based BRAF analysis performed either at the MGH TRL, at Clarient Laboratories using the FDA-approved Cobas® test, or in MGH pathology using $BRAF^{V600}$ stain. PBL and serum will be isolated from blood collected in two 10 mL green top tubes on each patient and will then be placed in Trizol Reagent prior to RNA isolation. RNA will then be isolated from tissue and PBL/serum and $BRAF^{V600}$ analysis will be performed as described above.

2. BACKGROUND

2.1 Study Disease

Metastatic melanoma is currently the 5th and 6th most common cancer in American men and women, respectively, and remains one of the few cancers with a rising incidence.(1) Over 9000 people are expected to die in the United States in 2012 from this dreaded disease. Recent treatment advances have been made which has led to the FDA approval of both vemurafenib, a BRAF inhibitor, and ipilimumab, an immunotherapy, for the treatment of patients with advanced

melanoma.(2-4) Unfortunately, the overwhelming majority of these patients still will die of their disease in spite of receiving these therapies.

2.2 IND Agent

No therapeutics to be administered under this protocol

2.3 Rationale

Strategies are underway to improve the effectiveness of BRAF-directed therapy and immune-based therapy, and similar efforts are needed to develop biomarkers with which we may better select treatment for melanoma patients and follow patients while they are being treated. With BRAF-targeted therapy now established as a standard therapy for metastatic melanoma, the development of highly-sensitive, blood-based assays have the potential to greatly improve the care of patients with BRAF-mutant melanoma in four major ways.

First, less than half of all patients with cutaneous melanoma have a tumor that harbors an oncogenic BRAF mutation.(5) In practice, current BRAF mutational analytic techniques are only capable of testing archival tissue and have limited sensitivity. Additionally, test results typically take two to three weeks, when factoring tissue acquisition and shipping issues, which delays the initiation of therapy for typically ill patients. Lastly, very often a patient who previously was diagnosed with an early stage melanoma who recurs will have a fine needle aspiration performed to confirm the diagnosis, and performing BRAF mutational analysis can be challenging due to the small amount of tissue that is obtained during such procedures. We believe that analyzing blood for the BRAF mutation will prove to be a more efficient method of determining a patient's BRAF status and possibly a more reliable one.

Second, tumor resistance to BRAF-directed therapy typically develops within six to eight months following initial tumor regression, but with a range of 2 months to 2 years.(2, 6-9) Importantly, each described mechanism involves the retention of the initiating BRAF mutation.(10-20) As the mechanisms of resistance are just now being elucidated, we feel that diagnostic assays which may identify developing resistance at an earlier time-point than standard clinical or radiographic assessments will have the ability to assist clinicians and researchers in determining the best next treatment option for patients whose disease is progressing on BRAF–directed therapy. This is particularly important due to the fact that a number of patients treated with BRAF inhibitors progress quite quickly following initial disease regression.(7) In these patients, it is typically difficult to transition to other therapies, such as ipilimumab, due to the rapid pace of growth in these resistant tumors.(21) While there is no current data which specifically supports such a strategy, it is thought that more advanced notice of disease progression when disease growth is more modest would allow for a more timely shift in treatment and improved benefit of next line therapy. Essentially, identifying a blood-based assay that can detect more subtle changes in disease burden than standard cross-sectional imaging would be requisite towards implementing this type of an approach. In addition, earlier detection of disease progression would also predictably allow investigators to analyze tumors or circulating tumor cells in these patients with a goal of determining the specific mechanism of resistance for an individual patient. This would have the added value of predicting which next therapy would be most appropriate for any

individual patient.

Third, over the next 12 months, it is expected that multiple trials will open to test the effectiveness of BRAF-directed therapy in patients with resected, high-risk disease. In patients with resected disease, the current standard of care for adjuvant therapy is interferon alpha, which has reproducibly been shown to improve disease-free survival but not overall survival.(22) Further, while the development of serologic or clinical evidence of autoimmunity has been associated with a marked improvement in overall survival in patients treated with adjuvant interferon, there has never been a reliable pre-treatment biomarker that predicts which patients are more likely to benefit from this therapy.(23) It is critical that trials of adjuvant BRAF-directed therapy test potential predictive biomarkers of benefit. One such biomarker would surely be a detectable BRAF mutation in tissue, as it is clear that patients with advanced melanoma only benefit from BRAF-directed therapy if there they have a tumor with a detectable BRAF mutation, however assessment of BRAF mutations in primary melanoma samples may be complicated by substantial tumor heterogeneity.(24) This may lead to both more false negatives and false positives, as it may not be clear which of the heterogeneous clones will ultimately establish metastasis. While it is uncertain whether detectable or high levels of circulating BRAF will be prognostic of a poor outcome or predictive of benefit to adjuvant BRAF-directed therapy, it is critical to evaluate both the predictive and prognostic qualities of blood-based BRAF assays in patients enrolled in adjuvant trials of BRAF inhibitors, as it is more likely that circulating cells of the subpopulations of primary melanomas are more reflective of the cells that will recur.

Fourth, the recommended optimal follow up of patients who are currently rendered disease free with surgery for their melanoma is unknown. Namely, the current NCCN guidelines do not recommend any blood analysis for such patients and only concede that considerations be made towards routine imaging in patients with higher risk disease (stage IIB-resected stage IV).(25) The development and validation of a blood-based prognostic biomarker would offer the potential to improve these guidelines and potential help direct radiographic imaging. As an example, it is conceivable that the positive predictive value of cross-sectional imaging would be much higher in the context of a rising biomarker, much the way it is in patients with concerning symptoms, then when imaging is performed at randomly selected intervals.

In summary, a blood-based BRAF mutational analysis has the potential to:

1. Enhance and potentially replace tissue based BRAF mutation detection.
2. Detect disease progression at an earlier time-point than imaging which may improve the effectiveness of next line therapy (given the lower tumor burden), and mechanism of resistance analysis for patients with BRAF mutant disease progressing on BRAF-directed therapy.
3. Predict which patients respond to adjuvant, BRAF-directed therapy.
4. Serve as a prognostic biomarker for patients with resected melanoma that may be followed serially and help clinicians choose which patients to perform diagnostic imaging.

Mutational testing for $BRAF^{V600}$ in tumor tissue has been well described using techniques such as bidirectional direct fluorescent sequencing and allele-specific polymerase chain reaction. The sensitivity of these assays, however is limited in that the assays in current use are only able to

detect the mutation if the tumor cells constitute >5-10% of the specimen submitted for genetic analysis.(26) While this degree of sensitivity is typically sufficient to detect the presence of the BRAF mutation in a homogenous tumor nodule, this is likely not sensitive enough to detect a few tumor cells in the background of a high percentage of stromal or lymphatic elements, infiltrating lymphocytes, or peripheral blood cells.

Recently, we have developed an RT-PCR-based technique that takes advantage of a unique restriction enzyme site present in wild-type (WT) but not the mutant BRAF to enrich the sample for the mutant form initially and then inserts a restriction site into the mutant but not WT BRAF to allow for detection and quantification of mutant BRAF.(27) As outlined in our original description of this technique and Figure 1, the protocol involves a series of PCR amplifications and restriction digestions that discriminate between WT and mutant BRAF at amino acid position 600. An initial RT-PCR is followed by digestion with TspR1 that preferentially digests the WT product but not the $BRAF^{V600}$ PCR product. A nested PCR using the digested material follows. This PCR product is subjected to sequencing using a nested oligonucleotide. The PCR product is then subjected to a third PCR (product 140 bp) using a unique nested forward oligonucleotide that creates aXba 1 restriction site in the amplified product only within the mutant sequence. After an Xba 1 digest, the products (108 and 32 bp) are separated on 15% acrylamide gels. After ethidium bromide staining the band at 108 bp is quantitated by densitometry.

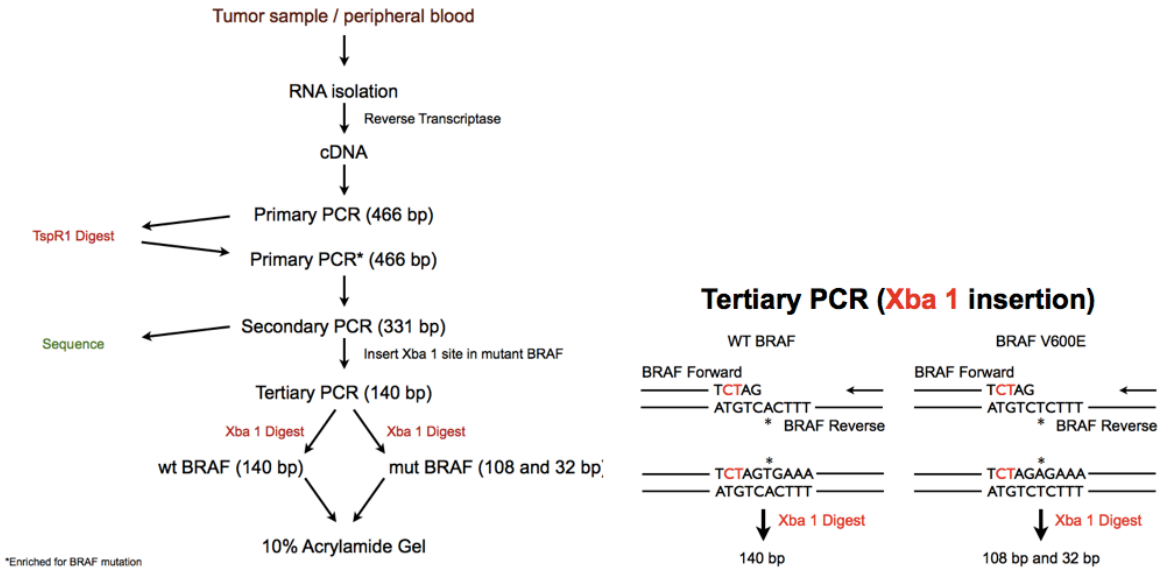


Figure 1: Schematic of BRAF Assay (left) and Xba 1 insertion and digestion products (right)

In tissue, we utilized this assay to detect mutation at a two log-fold greater sensitivity (1:999) than standard assay and, with a slight modification of the protocol, are now able to increase the sensitivity to one-mutant cells in a million non-mutant cells (1:400,000). This level of detection is theoretically high enough to detect the mutation in circulating tumor cells (CTCs) which are reportedly found in patients with melanoma at a range of from 1-100 per million peripheral blood lymphocytes.(28) As a proof of principle, we initially detected the mutation in both the blood of patients with known tissue $BRAF^{V600}$ mutation prior to their enrollment on the Phase 2

trial of vemurafenib and then in the blood of patients with resected stage III melanoma which was collected as part of the Harvard Skin SPORE blood and tissue banking protocol prior to the commencement of adjuvant therapy.

The next step in the development of this assay is optimizing the quantification process and then validating its use in relevant patient cohorts. One of the limitations of the current assay is that the insertion of the Xba1 one site during the tertiary PCR step only allows for assaying the $BRAF^{V600E}$ mutation specifically. While this is the most common oncogenic $BRAF^{V600}$ mutation, other mutations such as V600D and V600K make up 2-20% of $BRAF^{V600}$ mutations.(2, 5, 7, 29, 30) As a result, we are currently looking into other methods of analyzing the data. Specifically, a second-generation assay will maintain the initial TspR1 digestion step, as this is critical to maintaining the sensitivity of the assay. As has been mentioned, direct sequencing is possible following the second PCR step. While this step is unnecessary for detection and quantification, by applying next generation sequencing approaches, we believe that this will allow for the detection of non-V600E BRAF mutations while at the same time, possibly improve quantification of BRAF mutation levels.

We propose to develop and standardize a test which can reliably detect the $BRAF^{V600E}$ mutation in patients with known tissue $BRAF^{V600E}$ mutation in the blood of patients with melanoma. We aim to correlate testing with worse clinical outcome when disease is resected and to correlate blood testing with disease response to MAPK pathway inhibitors in the advanced, as well as potentially the adjuvant, setting.

3. PARTICIPANT SELECTION

Participants must meet the following criteria on screening examination to be eligible to participate in the study:

3.1 Eligibility Criteria

3.1.1 All participants must have biopsy-proven advanced (unresectable stage IIIC or Stage IV) or high-risk (Stage II or Stage III) malignant melanoma

3.1.2 Participants must be 18 years of age or older

3.1.3 Participant must have the ability to understand and willingness to sign a written informed consent document

3.2 Exclusion Criteria

3.2.1 Participants with a history of different malignancy are ineligible except for the following circumstances.

- Non-metastatic prostate cancer, cervical cancer *in situ*, and basal cell or squamous cell carcinoma of the skin.
- Individuals with a history of other malignancies are eligible if they have been disease-free for at least 2 years and are deemed by the investigator to be at low risk for recurrence of that malignancy.

3.2.2 Participants with a known history of a different BRAF-mutant malignancy

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial

4. REGISTRATION PROCEDURES

Participation is strictly voluntary. Patients coming to the participating sites that meet the eligibility criteria will be identified by physicians, study coordinator, research nurse, or other study team member. Potential participants will receive a description of the study, including the nature of participation, and the phone numbers for study contacts. Participants may be enrolled prospectively or retrospectively.

Written informed consent will be obtained by a physician or other licensed study team member prior to enrollment into the study. All participants (except those who have previously signed consent to enroll in either the 02-017 or 11-181 protocols) must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. Study coordinators will be allowed to obtain consent, as outlined in the November 2014 Guide to Human Research Activities, after receiving training from the principal investigator or by a sub-investigator following approval by the principal investigator.

The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and dated by the participant or the participant's legally authorized representative, and by the person obtaining the consent. The participant must be given 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.

This study has minimal to no risk to the study participants.

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registration must occur within 24 hours of the first blood sample being drawn. Any participant not consented to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

Following registration, participants may continue protocol interventions. If a participant does not receive protocol interventions following registration, the participant's registration on the study must be canceled. Registration cancellations must be made in OnCore as soon as possible.

4.2 Registration Process for DF/HCC Institutions

DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) must be followed.

4.3 General Guidelines for Other Investigative Sites

Not Applicable.

4.4 Registration Process for Other Investigative Sites

Not Applicable.

5. STUDY DESIGN

5.1 Sample Collection

Advanced Disease Cohort:

Blood samples will be obtained on participants following signed informed consent. For patients in the advanced melanoma cohort, a one-time blood sample will be obtained prior to the commencement of systemic therapy at a time when standard-of-care blood will be drawn. While it is ideal to obtain samples prior to any systemic therapy, participants who have previously received therapy, have progressed, and are in need of additional therapy will be considered suitable for participation. Participants with known BRAF mutation (determined by standard of care tissue testing) will undergo blood draws every 4 weeks (+/- 1 week) and at the time of disease progression.

BRAF WT (tissue): One- time, pre-treatment blood draw

BRAF Mut (tissue): Pre-treatment blood draw then every 4 weeks (+/- 1 week). A

blood draw at time of progression will also be obtained.

Stage II/ III Disease Cohort:

Blood samples will be obtained on participants following signed informed consent. For patients in the high-risk stage II/III cohort, a blood draw will be performed 4-8 weeks after the completion of surgical management at the time of standard of care blood work. In participants who sign informed consent prior to definite surgical management, a pre-operative blood draw may be obtained at the time that standard of care pre-op blood work is performed. Blood samples will then be obtained every three months following the initiation of either adjuvant therapy or routine, close follow-up for up to two years.

Pre-op consent: Pre-op blood draw, 4-8 week post-op blood draw, then every 3 month blood draw following initiation of adjuvant therapy/ observation for up to 2 years.

No pre-op consent: 4-8 week post-op blood draw, then every 3 month blood draw following initiation of adjuvant therapy/observation for up to 2 years.

Of note, retrospective samples will be analyzed in participants who have previously signed informed consent to the melanoma tissue banking protocols, DFHCC study 02-017 and 11-181. These participants will not need to sign the consent form accompanying this protocol.

5.2 Data Collection

The sources of the data will be hospital/electronic medical records provided by the BIDMC Caregroup and Partners networks. Participants will be enrolled prospectively as part of the evaluation for the use of $BRAF^{V600E}$ specific inhibitors either for experimental protocols or as standard of care (assuming the FDA-approval of one or more of these agents) in patients with melanoma. Data regarding response, progression, and survival either will be collected as part of the study and be made accessible to the study team or will be collected as part of routine clinical care in patients treated with standard of care BRAF inhibitors.

The data collected will be demographic data (age, gender, vital status), coded encounter data (diagnoses, dates), and clinic/office notes. The records will be reviewed electronically through the online medical record system at the three institutions accessed by the study team. The team will use a password protected computer and online medical record system maintained by the BIDMC, DFCI, and MGH. All study team members have HIPAA, confidentiality, and CITI training provided by the BIDMC, DFCI, and MGH at hiring.

The database is also stored in a password protected computer at the MGH. Identifiable information to researchers will not be released outside the MGH study team. Unlinked information will be accessible through a password protected computer and database. After the data is retrieved from patient records, the results will be entered into the database unlinked to any protected health information. Any analyses, reports, or presentations of the data will not reveal

patient information. No risks are involved for individuals whose information will be used in this study. Private/confidential information will be available only to the study coordinator and the study team on the front sheet.

5.3 Criteria for Taking a Participant Off Study

Participants will be removed from study when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent for data submission
- Death

Participants will be removed from study if they request to do so. The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF).

For Centralized Subject Registrations, the research team submits a completed Off Treatment/Off Study form to ODQ when a participant comes off study. This form can be found on the ODQ website or obtained from the ODQ registration staff.

For Decentralized Subject Registrations, the research team updates the relevant Off Treatment/Off Study information in OnCore.

5.4 Duration of Follow Up

Patients will be followed until disease recurrence, death, or until closure of the study. It is estimated that this study will remain open for five years.

6. ADVERSE EVENTS

6.1 Expected Toxicities

Slight transient discomfort at the puncture site as the needle is inserted is expected. Minimal bleeding that can be controlled by temporary pressure and bruising at the needle site are potential sequelae.

Toxicity Management is per phlebotomy lab standards

6.2 Adverse Event Reporting

No particular adverse events are expected from participation in this protocol. The participants will be exposed to the minor increased risk accrued from repeated peripheral blood sampling.

7. MEASUREMENT OF EFFECT

Not applicable in this protocol, as response to therapy will be judged per protocol specific to the therapy being received, which will vary from patient to patient.

8. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 6.0 (Adverse Events: List and Reporting Requirements).

8.1 Data Reporting

8.1.1 Method

The QACT will collect, manage, and perform quality checks on the data for this study.

8.1.2 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the QACT according to the schedule set by the QACT.

8.2 Data Safety Monitoring

Involvement in this study as a participating investigator implies acceptance of potential audits or inspections, including source data verification, by representatives designated by the DF/HCC Overall Principal Investigator (or Protocol Chair) or DF/HCC. The purpose of these audits or inspections is to examine study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol, institutional policy, Good Clinical Practice (GCP), and any applicable regulatory requirements.

All data will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of participant registration and will continue during protocol performance and completion.

8.3 Protocol Review and Amendments

This protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) and any other necessary documents must be submitted, reviewed and approved by a properly constituted IRB governing each study location.

Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB. The DF/HCC Overall Principal Investigator (or Protocol Chair) will disseminate

protocol amendment information to all participating investigators.

All decisions of the IRB concerning the conduct of the study must be made in writing.

8.4 Ethics and Good Clinical Practice (GCP)

This study is to be conducted according to the following considerations, which represent good and sound research practice:

- E6 Good Clinical Practice: Consolidated Guidance
  www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM129515.pdf
- US Code of Federal Regulations (CFR) governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki
  - Title 21 Part 11 – Electronic Records; Electronic Signatures
    www.access.gpo.gov/nara/cfr/waisidx_02/21cfr11_02.html
  - Title 21 Part 50 – Protection of Human Subjects
    www.access.gpo.gov/nara/cfr/waisidx_02/21cfr50_02.html
  - Title 21 Part 54 – Financial Disclosure by Clinical Investigators
    www.access.gpo.gov/nara/cfr/waisidx_02/21cfr54_02.html
  - Title 21 Part 56 – Institutional Review Boards
    www.access.gpo.gov/nara/cfr/waisidx_02/21cfr56_02.html
  - Title 21 Part 312 – Investigational New Drug Application
    www.access.gpo.gov/nara/cfr/waisidx_02/21cfr312_02.html
- State Laws
- DF/HCC research policies and procedures
  http://www.dfhcc.harvard.edu/clinical-research-support/clinical-research-unit-cru/policies-and-procedures/

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. In such case, the deviation must be reported to the IRB according to the local reporting policy.

8.5 Study Documentation

The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research participant. This information enables the study to be fully documented and the study data to be subsequently verified.

Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinical charts, laboratory and pharmacy records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

8.6 Records Retention

All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.

9. STATISTICAL CONSIDERATIONS

9.1 Study Design/Endpoints

Primary Objective: To determine the sensitivity and specificity of the peripheral blood $BRAF^{V600}$ mutation detection.

Two cohorts of melanoma patients will be assessed using the blood-based assay: patients with advanced disease (non-resectable Stage III or Stage IV), and patients with Stage II and resectable Stage III disease. Assessments of diagnostic accuracy, such as sensitivity, specificity, and positive predictive value (PPV) of the blood-based assay, will be compared separately for the two cohorts, and will use paired binary data. Sample size estimates are based on the binomial distribution and joint 90% confidence regions for sensitivity and specificity[1,2] to adjust for the two comparisons being performed. Positive and negative expression will be determined using optimized cut points for the assay that have already been established.

We are seeking evidence that the assay has the ability to detect both the presence of BRAF mutation in melanoma (sensitivity) and accurately indicate when a BRAF mutation is not present (specificity) in order to provide BRAF-directed therapy when indicated. We propose a sample of 110 patients with advanced melanoma to test the performance of the assay, and would require sensitivity of at least 0.80 and specificity of at least 0.90. We set our target sensitivity to be 0.90 and specificity to 0.95. With a sample size of 110 patients (approximately 55 with BRAF mutation and 55 without) and the assumed target sensitivity, the lower bound of the joint 90% confidence region will be greater than 0.82; the lower bound of the 90% joint confidence region for the specificity will be approximately 0.90.

We will also estimate the PPV of the assay to evaluate the practical utility of the test. Based on a sample size of 110 patients, Table 1 summarizes the estimated PPV and corresponding 90% confidence intervals over a range of prevalence rates of BRAF mutation, using the minimum and target levels of sensitivity and specificity. With a sample of 110 patients, the lower bound of the 90% confidence interval for PPV will be no less than 0.74.

Table 1 – Estimates of PPV and 90% Confidence Intervals

Prevalence Rate	Sensitivity	Specificity	PPV	90% Confidence Interval for PPV
0.4	0.80	0.90	0.84	(0.74 to 0.91)
	0.90	0.95	0.92	(0.83 to 0.97)
0.5	0.80	0.90	0.89	(0.80 to 0.94)

[1] Alonzo TA, Pepe MS, Moskowitz CS. Sample size calculations for comparative studies of medical tests for detecting presence of disease. *Statist. Med* 2002; 21:835-852.
[2]Obuchowski NA. Sample size calculations in studies of test accuracy.*Stat Methods in Medical Res* 1998; 7: 371-392.

	0.90	0.95	0.95	(0.87 to 0.98)
0.6	0.80	0.90	0.92	(0.85 to 0.96)
	0.90	0.95	0.96	(0.90 to 0.99)

The cohort of patients with Stage II and resectable Stage III melanoma is an exploratory cohort. We cannot predict the sensitivity and specificity of the assay in patients with Stage II or resected Stage III disease. In addition, only patients in this cohort with T4 disease or macrometastatic lymph nodes will have adequate tissue for assessment. However, we believe it is important to include this cohort since the ability to detect BRAF in this patient population may provide a basis for proposing further studies correlating BRAF detection with disease recurrence. The sensitivity and specificity of the assay will be calculated and presented with 90% joint confidence regions. Based upon a cohort size of 110 patients, the joint confidence regions for the sensitivity and specificity will be no wider than ±0.13.

Secondary Objectives

1. **Exploration of the pharmacodynamic effects of MAPK pathway inhibitors on pre- and on-treatment peripheral blood $BRAF^{V600}$ mutational testing.**

The exploration of pharmacodynamic effects will be based on the cohort of patients with advanced, BRAF-mutated disease confirmed by tissue analysis. We would expect that 50% of the cohort would have BRAF-mutated disease and that 90% of those patients will agree to participate in the pharmacodynamic studies. This results in a sample of size 50.

Our first investigation will correlate blood-assay results with pre-treatment radiographic measures of disease burden. We hypothesize that the cases with the lowest tumor burden would be most likely to have a negative blood assay result. We will use the sum of the longest diameter of tumor target lesions as the measure of disease burden using RECIST criteria (version 1.1). For this analysis, tumor burden will be a continuous outcome, and the blood assay will be classified as positive or negative. We will summarize baseline tumor burden descriptively and compare by assay result using the Wilcoxon rank-sum test. If the blood-assay is positive in 80% of the samples (N=40) and negative in 20% (N=10), a Wilcoxon-rank sum test, with a two-sided type-I error of 10%, will have 80% power to detect a difference in disease burden that is 0.95 times the common standard deviation.

The behavior of the blood-based assay with respect to early changes in disease will be assessed at the time of the first restaging. Since there are no prior data to suggest how assay levels will change in response to treatment, analyses will be primarily descriptive. Two analyses are proposed to explore the relationship between the performance of the assay and early clinical response. In both analyses, clinical response will be classified according to RECIST criteria (i.e., CR/PR vs. SD vs. PD vs. not assessable).

At the time of the first restaging, pre- and on-treatment blood-assay levels will be compared. Fold-changes in assay response will be calculated (post/pre). Fold-changes will be used as a continuous measure and will also be classified according to 0.5 or less (assay-based response), 2 or greater (assay-based progression) and between 0.5 and 2 (intermediate). Fold changes in the assay will be compared across RECIST responses using the Kruskal-Wallis test. In addition, for

assay-based response or progression, the proportions of patients with CR/PR, SD, or PD will be presented with exact 90% confidence intervals. We will further characterize the behavior of the assay by comparing assay fold changes with fold changes in tumor burden (post/pre); the relationship will be summarized graphically and using the Spearman rank correlation.

Assay performance data will also be divided into four groups defined by pre-treatment expression and at the first restaging (i.e., +/+, +/-, -/+, -/-) and compared with response. The proportions will be summarized and compared using exact confidence intervals in all four assay combinations and also in the two subgroups with a positive assay at pre-treatment.

The behavior of the blood assay over time will be summarized graphically. The status of the assay (+/-) will be shown at each assessment time for each patient. Graphics will note changes in disease status and times of disease progression and will provide an opportunity examine patient follow-up for patterns of change in the assay.

2. To investigate the prognostic and predictive ability of peripheral blood $BRAF^{V600}$ mutational testing in patients with Stage II or resectable Stage III melanoma.

The investigation of the prognostic and predictive ability of the assay will be based on the cohort of patients with Stage II and resected Stage III disease. Some of these patients will have a tissue-based BRAF assessment and a pre-treatment blood-based BRAF assay, and some of the patients will have the blood-based assay only.

We would expect that 50% of the cohort would have BRAF-mutated disease and that 90% of those patients will have follow-up data from which to assess the predictive ability of the assay. This results in a sample of size 50. BRAF status will be classified as positive or negative based on the post-resection, blood-based assay, and where applicable, for the tissue-based assessment.

<u>Prognostic Ability of the Assay</u>

Baseline assay results will be categorized as positive or negative and also be described on a continuous scale. The proportion of patients with a positive baseline assay will be presented with a 90% exact binomial confidence interval. Based on a cohort size of 50, the confidence interval will be no wider than 0.25. Confidence intervals will also be presented for the subgroups of patients with intermediate- or high-risk disease. We will also summarize continuous baseline assay levels using descriptive statistics for the complete cohort and according to subgroups.

Preliminary assessments of prognostic ability will be conducted using Kaplan-Meier (K-M) product-limit estimates for relapse or survival and stratified log-rank tests. K-M estimates will be stratified by AJCC stage and assay results to explore assay performance across different prognostic groups. Relapse-free survival and overall survival estimates at pre-specified time points will be presented with 90% confidence intervals estimated using log(-log(survival)) methodology. A secondary analysis will be conducted in the cohort with blood- and tissue-based assessments according to the four groups defined by their combined positive/negative status to look for differences in prognosis.

The relative effect of the blood-based assay with respect to relapse or survival will be estimated using Cox regression models. Since the AJCC staging incorporates prognostic factors such as number of involved nodes, tumor burden, ulceration, and tumor thickness, the Cox models will be stratified by AJCC stage to allow for differences in the baseline hazard of relapse or death. Models will include as covariates age, site of the primary melanoma, sex, and baseline serum LDH in addition to the blood-assay results. We will use aggregated data for the primary models; secondary models will be fit separately for the intermediate- and higher-risk cohorts. Hazard ratios for the assay will be presented with 90% confidence intervals.

Predictive Ability of the Assay

The behavior of the blood-based assay over time will be summarized graphically by AJCC stage or according to melanoma treatment. The status of the assay will be shown at each assessment time for each patient, in addition to time of disease progression. Of particular note will be assessment times where a previously undetectable assay becomes detectable, or times where there is a doubling in the assay relative to baseline. Treatment will be classified into four groups: immunotherapy, BRAF/MEK inhibitors, other translational signal transduction/targeted therapies, or chemotherapy (cytotoxic therapy).

The first assessment of assay performance over time will take place at 3 months and will be based on fold-changes in the quantified assay relative to baseline. This early look will minimize the number of patients lost due to rapid progression of disease. Based on 18-month progression rates reported in the literature, we estimate that approximately 11% of patients will have progressed by the time of the 3-month assessment. For an initial cohort size of 50 patients, and assuming that 45 patients will have 3-month assay, a Wilcoxon-signed-rank test, comparing the observed fold-change with one, with a two-sided type-I error of 10%, will have 80% power to detect a difference in fold-change that is 0.41 times the standard deviation.

In more exploratory analyses, we will model the behavior of the assay over time using repeated measures, random-effects regression with the continuous assay measure as the dependent variable. Models will include as covariates AJCC stage and treatment classification, in addition to other prognostic factors. To investigate the relationship between the assay and PFS (or OS), we will use the extended Cox (EC) model with assay performance as the time-dependent covariate. Models will be stratified by AJCC stage, and will include treatment group and prognostic factors as predictors. In separate EC models, the assay will be modeled (a) on a continuous scale, (b) based on fold-changes from baseline, and (c) based on an indicator of assay response (double the baseline level or change from non-detectable to detectable).

Characteristics of Study Sample

Patient demographic and disease characteristics will be presented in the aggregate and by cohort. Characteristics measured on a continuous scale, such as age, will be summarized using N, mean, standard deviation, median and range. Categorical characteristics will be summarized using N and percentage.

10. PUBLICATION PLAN

The results should be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study.

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