**NCI Protocol #:** N/A

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**TITLE:** A phase II trial of abbreviated MAPK targeted therapy plus pembrolizumab in patients with unresectable or metastatic melanoma

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**NCI-Supplied Agent(s):** N/A
**Other Agent(s):** Pembrolizumab – Merck
Dabrafenib plus trametinib – Commercial
Trametinib – Commercial

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**IND Sponsor:** Ryan J. Sullivan, MD

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## Synopsis

### Rationale for Study:

Over the past decade, amazing progress has been made culminating in the development of effective molecularly targeted and immune targeted therapies for the treatment of solid tumors. The two diseases that have been at the forefront of this progress are melanoma and non-small cell lung cancer (NSCLC). In each disease, molecular subsets have been identified (BRAF and NRAS mutant in melanoma, EGFR and ALK mutant in NSCLC) that define a patient population associated with high response rates to targeted agents. Additionally, monoclonal antibody targeting of the program death 1 (PD1) receptor and its ligand (PDL1) has shown impressive activity in both diseases with response ranging from 15-30% in NSCLC and 30-50% in melanoma. As the field looks to improve upon this activity, one strategy has been to consider combining molecular-targeted and immune therapies.

The preponderance of data supporting this approach is from the melanoma field. In particular, a number of groups have shown that MAPK inhibition leads to increased melanoma antigen expression, decreased immunosuppressive cytokines, and increased trafficking of CD8+ T-lymphocytes. Additionally, it appears that BRAF inhibitors with or without MEK inhibitors are capable of triggering this phenomenon, though these findings dissipate in the setting of acquired, BRAF-inhibitor resistance. It has been well described that a common tissue and tumor response to lymphocytic infiltration is the upregulation of PDL1 that then inhibit these invading T-cells through interaction with their PD1 receptor. In melanoma, the BRAF-inhibitor associated infiltration is associated with increased tumor/stromal PDL1 expression. Dual inhibition of BRAF and PD1 has been shown to be associated with more profound and durable responses in animal models of melanoma.

We propose to test the hypothesis that induction molecularly targeted therapy triggers a favorable tumor microenvironment to augment the effects of maintenance PD1 targeting with pembrolizumab in two cohorts: BRAF mutant and BRAF-wild-type melanoma. This proposal represents a unique opportunity to test the effectiveness of combined immune and molecularly targeted therapy in two distinct subsets based on preclinical and clinical data. The successful completion of this study will:

1. Determine the immunologic effects of oncogene targeted therapy on tumor microenvironment
2. Prove the concept that abbreviated molecularly targeted therapy will augment the effectiveness of pembrolizumab maintenance therapy

### Study Objectives:

<u>Primary Objective:</u>

Document the rate of clinical benefit (SD/PR/CR per RECIST) of abbreviated

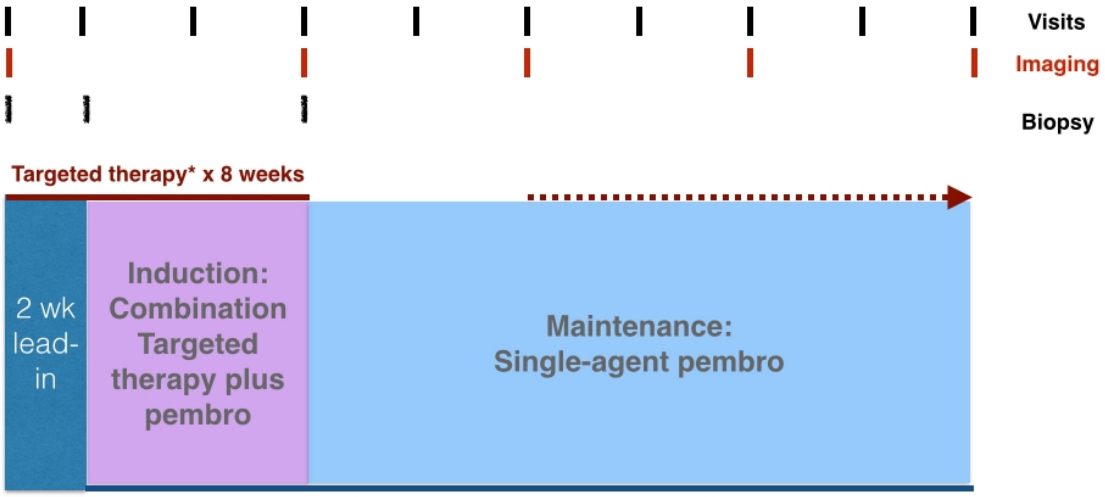
molecularly targeted therapy in combination with pembrolizumab in patients with unresectable and/or metastatic melanoma. A subject will have achieved clinical benefit if there is evidence of CR, PR, or SD at the time of the 24-week scan and the subject has not resumed targeted therapy.

Secondary Objectives:

1. To describe progression free survival (PFS) and 1 year overall survival (OS) of abbreviated molecularly targeted therapy in combination with pembrolizumab in patients with metastatic melanoma.
2. To define the effects of targeted and the combination of immune and molecularly targeted therapy on tumor microenvironment

**Study Design:**
A Simon 2-stage design will be implemented for each disease cohort. Patients will be treated with two-week lead-in of molecularly targeted therapy (MTT), followed by two 3 week cycles of combined targeted therapy (MTT) and pembrolizumab (at dose of 200 mg IV every three weeks), followed by maintenance single-agent pembrolizumab in all patients with RECIST defined stable disease (SD), partial response (PR), and complete response (CR). MTT *only will be resumed* if patients develop symptomatic or radiographic evidence of progression. If patients do not develop evidence of progressive disease (PD), then they will continue maintenance single-agent pembrolizumab for up to two years. In each cohort, patients will be accrued to first stage of the Simon design. If a prespecified number of patients maintain SD/PR/CR off MTT but on pembrolizumab at the 24-week time point, then the second stage of the two-stage design will be opened. (Please see details in statistical section) Biopsies will be performed on all patients enrolled to the first stage of the Simon two-stage for each cohort. These will be obtained at baseline and after the two-week lead-in. A third biopsy obtained after the completion of induction therapy will be obtained if feasible. An optional biopsy at time of disease progression will also be performed.

## SCHEMA




| Targeted therapy* | Disease |
|---|---|
| Trametinib | BRAF WT Melanoma |
| Dabrafenib/trametinib | BRAF MT Melanoma |

1. 2-week lead-in of targeted therapy (TT)
2. 6-week "induction"of combination TT plus pembrolizumab
3. All SD/PR/CR at week 6 per RECIST go off TT
4. TT resumes in any patient with radiographic or symptomatic PD
5. Off pembrolizumab if:
    1. Unacceptable toxicity
    2. 6 months of therapy
    3. PD on 2 successive scans

WT = wild-type; MT = mutant

**Key Eligibility:**

- Participants must have histologically confirmed metastatic or unresectable melanoma.
- Participants must have BRAFV600-mutation status known.
- Participants must have measurable disease.
- Age greater than or equal to 18 years.
- Participants must have disease amenable to and be willing to undergo serial core or excisional biopsies of a tumor lesion(s).
- ECOG performance status ≤1 (see Appendix A).
- Participants must have normal organ and marrow function as defined below:
- Participants not previously treated with BRAF inhibitors (vemurafenib, dabrafenib, encorafenib), MEK inhibitors (selumetinib, trametinib, binimetinib, cobimetinib), and/or anti-PD1/PDL1 monoclonal antibodies for metastatic or unresectable disease. Any other prior therapy *will be* allowed (including ipilimumab, adjuvant anti-PD1 therapy, high-dose IL-2).
- Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment.

- Participants with symptomatic brain metastases will be excluded from this clinical trial. Subjects with asymptomatic, stable brain metastases and/or who have been previously treated for these conditions that are asymptomatic in the absence of corticosteroid therapy are allowed to enroll. Brain metastasis must be stable with verification by imaging (brain MRI completed at screening demonstrating no current evidence of progressive brain metastases).
- No symptomatic or untreated leptomeningeal disease.

**Number of Patients & Centers**

Total number of patients: 50

**Sample Size Justification:**
This trial will provide preliminary estimates of toxicity and efficacy and will be based on parallel Simon two-stage designs according to disease cohort. The rate of clinical benefit (CBR), defined as the rate of patients who have CR/PR/SD at week 24 while remaining off targeted therapy after induction, will be the primary efficacy endpoint. For each disease cohort, the Simon two-stage design is based on a type-I error rate of 0.1, at least 85% power, and a total sample size of 25 subjects. A complete safety assessment is planned to coincide with the review of clinical benefit at the end of the first stage.

For both cohorts, the null hypothesis of a true CBR of 0.50 will be tested against a one-sided alternative CBR of 0.75. The benchmark CBR rate of 0.50 is based on the results of the Keynote 001 trial in patients with refractory or first-line malignant melanoma treated with pembrolizumab (Robert, ASCO 2016) that reported a CBR of 0.51 regardless of BRAF mutation status. A short course of MAPK-targeted therapy in addition to pembrolizumab would be considered worthy of further investigation if there is evidence of an improvement in CBR to 0.75. Fourteen (14) subjects that are evaluable for the primary endpoint (defined as receiving at least 1 dose of dabrafenib/trametinib/pembrolizumab combination therapy prior to treatment discontinuation for any reason.) will be enrolled in the first stage, and if 8 or fewer subjects with clinical benefit are observed, then enrollment will stop. If there are 9 or more subjects with clinical benefit, then an additional 11 subjects will be enrolled, for a total of 25. The null hypothesis will be rejected if a total of 16 or more subjects with clinical benefit are observed in 25 subjects. This design yields power of 86% (target type II error of 0.15) when the CBR rate is 0.75. If the null hypothesis is true, the probability is 0.79 that enrollment to a cohort will stop at the end of the first stage. This high probability of stopping under the null was chosen to require strong evidence of benefit before opening the second stage.

Clinical benefit rates will be presented with 90% confidence intervals based on the method of Atkinson and Brown, which allows for the two-stage design.

All reported adverse events will be summarized as part of the early safety review at the

end of the first stage. With 14-15 subjects per disease cohort, there is high probability of observing at least one event if a toxicity has an incidence of at least 10%. If the true incidence of an unexpected or severe toxicity is 10% or greater, the probability is at least 0.75 that one or more subjects will experience the toxicity during the early safety monitoring period.

The distribution of secondary endpoints of PFS and OS will be based on the method of Kaplan-Meier. ORR within each disease cohort will be estimated by the number of subjects with a best response of CR or PR (per RECIST) and presented with exact 90% confidence intervals. For sample sizes of 25 per disease cohort, the confidence intervals will be no wider than 0.35.

Correlative studies will be based on tumor biopsies and will assess changes in biomarkers due to OTT. PD-L1 expression will be classified as positive or negative according to IHC. Pre- versus post-OTT PD-L1 expression will be compared using McNemar's test. Changes in T cell subtypes and in co-stimulatory molecules will be summarized using descriptive methods

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# 1. OBJECTIVES

## 1.1 Study Design

A Simon 2-stage design will be implemented for each disease cohort. Patients will be treated with two-week lead-in, followed by a 6-week combined targeted therapy (TT) and pembrolizumab, followed by maintenance single-agent pembrolizumab in all patients with RECIST defined stable disease (SD), partial response (PR), and complete response (CR). TT will be resumed if patients develop symptomatic or radiographic evidence of progression. If patients do not develop evidence of progressive disease (PD), then they will continue maintenance single-agent pembrolizumab for up to two years. In each cohort, patients will be accrued to first stage of the Simon design. If a prespecified number of patients maintain SD/PR/CR off TT but on pembrolizumab, then the second stage of the two-stage design will be opened. (Please see details in statistical section) Biopsies will be performed on all patients enrolled to the first stage of the Simon two-stage for each cohort. These will be obtained at baseline and after the two-week lead-in. A third biopsy obtained after the completion of induction therapy will be obtained if feasible. An optional biopsy at time of disease progression will also be performed.

## 1.2 Primary Objectives

Document the rate of clinical benefit (SD/PR/CR per RECIST) of abbreviated molecularly targeted therapy in combination with pembrolizumab in patients with unresectable and/or metastatic melanoma. A subject will have achieved clinical benefit if there is evidence of CR, PR, or SD at the time of the 24-week scan and the subject has not resumed targeted therapy.

## 1.3 Secondary Objectives

1.3.1. To describe progression free survival (PFS) and 1 year overall survival (OS) of abbreviated molecularly targeted therapy in combination with pembrolizumab in patients with metastatic melanoma.
1.3.2. To define the effects of targeted and the combination of immune and molecularly targeted therapy on tumor microenvironment

# 2. BACKGROUND

## 2.1 Study Disease(s)

The incidence of melanoma has dramatically risen over the past several decades and in 2012, an estimated 74,0000 new cases and nearly 10,000 deaths are expected in the United States. (Seigel CA J 2015) In fact,

melanoma is the 5th and 7th most common malignancy in the US among men and women respectively. The prognosis for patients with metastatic melanoma has been traditionally poor, though is dramatically different if the disease is limited to subcutaneous and/or lymph nodes (M1a), if the lungs are the only site of visceral metastasis (M1b), or if other sites of visceral metastasis are identified and/or if the lactate dehydrogenase (LDH) level is elevated (M1c). In particular, per the AJCC database the 1-year survival rates of M1a, M1b, and M1c are approximately 70%, 60%, and 40% respectively. Still, melanoma typically disseminates widely, and frequently involves sites that are uncommon in other cancers, such as the GI tract and the skin. (Balch J Clin Oncol 2009)

Over the past decade, amazing progress has been made culminating in the development of effective molecularly targeted and immune targeted therapies for the treatment of melanoma. In particular, molecular subsets have been identified (BRAF, NRAS, and CKIT mutant) that define a patient population associated with response to targeted agents. Additionally, monoclonal antibody targeting of the program death 1 (PD1) receptor and its ligand (PDL1) is associated with responses in 25-45% of patients, and more importantly, has demonstrated a survival advantage compared to ipilimumab (in the front-line setting) and chemotherapy (in front-line and post-ipilimumab settings). As the field looks to improve upon this level of activity, combination regimens have emerged that are associated with improved efficacy compared with single-agent therapy.

The first therapeutic regimen to demonstrate superiority over single-agent therapy is the combination of a BRAF inhibitor (BRAFi) and MEK inhibitor (MEKi). In fact, there are now three “positive” randomized trials of combination BRAF/MEK inhibitors showing improved response, progression free survival, and overall survival compared to single-agent BRAF inhibitors. As a result of this data, the FDA approved the dabrafenib (BRAFi) plus trametinib (MEKi) combination in early 2014 and is evaluating the combination of vemurafenib (BRAFi) plus cobimetinib (MEKi) for regulatory approval. Further, there is no longer any dispute that combined targeted therapy is the preferred BRAF-targeted therapy approach. Unfortunately, only a small subset of patients have durable benefit and remain on therapy for more than two or three years.

Immunotherapy combination therapy is another approach. The most advanced data with this approach is with the combination of ipilimumab and nivolumab. In particular, Phase I-III trials show that this regimen is associated with high response rates (exceeding 60%) and improved PFS (median ~12 months) at the cost of high toxicity (grade 3 or 4 treatment-related adverse events ~55+%). Given high rate of severe and life-threatening toxicity, this regimen is unlikely to serve the backbone upon which to rationally build three and four drug combinations.

A second approach to improve upon the effectiveness of anti-PD1 therapy is to develop patient selection strategies to prospectively differentiate patients most

likely to experience long-term benefit from single-agent therapy from those who require combinations. Recently, a number of predictive biomarkers of response to anti-PD1 antibodies in melanoma and other malignancies have been reported. These include tumor factors (such as mutational burden and tumor neoantigen expression) immunologic factors (including cytotoxic T-lymphocyte infiltration with functional capacity to recognize tumor antigens, so-called T-cell diversity), and tumor-immune microenvironmental factors like PD-L1 expression. With each new report, a clear picture is emerging that tumors with high mutational burden and neoantigen expression, increased T-cell diversity, and elevated PD-L1 expression are primed for immune destruction in the setting of single-agent anti-PD1 antibodies. Conversely, tumors with low mutational load and neoantigen expression, low T-cell diversity, and low PD-L1 expression are less susceptible to immune-mediated tumor cell destruction. One of the next great challenges in the field is to develop strategies that convert an immunologically non-responsive tumor microenvironment into one primed to respond to anti-PD1 therapy.

## 2.2 **IND Agents**

### 2.2.1 Pembrolizumab

#### 2.2.1.1 Mechanism of action

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

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor

tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma (MEL). This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Keytruda™ (pembrolizumab) has recently been approved in the United Stated for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor.

2.2.1.2 Summary of nonclinical and clinical studies.

A phase 1/2, multipart study is being conducted to evaluate the combination of the approved doses of pembrolizumab and dabrafenib and trametinib for *BRAF*V600E/K-mutant advanced melanoma. Results presented at the American Society of Clinical Oncology (ASCO) Annual Meeting June 3-7, 2016 on the nonrandomized phase 1, dose-finding part of KEYNOTE-022 showed Median days on therapy: 115 (range, 4-524). DLTs were reported in 3 out of 14 DLT-evaluable patients. Overall, 4 (26.7%) patients discontinued because of treatment-related AEs; there were no treatment-related deaths. Potentially immune-mediated AEs were reported in 6 (40.0%) patients, and included 2 cases of hyperthyroidism (1 grade 1, 1 grade 2), 2 cases of hypothyroidism (grade 1), 2 cases of uveitis (1 grade 1, 1 grade 2), and 1 case each of autoimmune hepatitis (grade 3), severe skin reactions (grade 3), and pneumonitis (grade 2). Based on observed DLTs and the AE profile, no additional dose levels were opened; pembrolizumab 2 mg/kg Q3W + dabrafenib 150 mg BID + trametinib 2 mg QD was the MTD.

2.2.1.3 Nonclinical and clinical pharmacokinetics

An open-label Phase I trial (Protocol 001) is being conducted to evaluate the safety and clinical activity of single agent MK-3475. The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of MK-3475 showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No MTD has been identified to date. 10.0 mg/kg Q2W, the highest dose tested in PN001, will be the dose and schedule utilized in Cohorts A, B, C and D of this protocol to test for initial tumor activity. Recent data from other clinical studies within the MK-3475 program has shown that a lower dose of MK-3475 and a less frequent schedule may be sufficient for target engagement and clinical activity.

PK data analysis of MK-3475 administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life (refer to IB). Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days).

This early PK and pharmacodynamic data provides scientific rationale for testing a Q2W and Q3W dosing schedule.

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of MK-3475 were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. MK-3475 has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg Q3W body weight based regimen are anticipated to remain well within the established exposure margins of 0.5 – 5.0 for MK-3475 in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W vs. the proposed dose regimen of 2 mg/kg Q3W (i.e. 5-fold higher dose and exposure). The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

The rationale for further exploration of 2 mg/kg and comparable doses of pembrolizumab in solid tumors is based on: 1) similar efficacy and safety of pembrolizumab when dosed at either 2 mg/kg or 10 mg/kg Q3W in melanoma patients, 2) the flat exposure-response relationships of pembrolizumab for both efficacy and safety in the dose ranges of 2 mg/kg Q3W to 10 mg/kg Q3W, 3) the lack of effect of tumor burden or indication on distribution behavior of pembrolizumab (as assessed by the population PK model) and 4) the assumption that the dynamics of pembrolizumab target engagement will not vary meaningfully with tumor type.

2.2.1.4 Potential for drug-drug interaction

Given the absence of interaction with the Cytochrome P450 enzyme system, the potential for drug-drug interaction is quite low with pembrolizumab.

2.2.1.5 Safety Profile

The most common adverse reactions (reported in ≥ 20% of patients) of pembrolizumab include fatigue, cough, nausea, pruritus, rash, decreased appetite, constipation, arthralgia, and diarrhea.

Pembrolizumab leads to the development of severe or life-threatening immune related adverse events in 10-15% of patients treated. The following toxicities are of particular interest: Immune-mediated pneumonitis (occurring in approximately 3% of the 411 melanoma patients treated on the phase I trial of pembrolizumab), immune-mediated colitis (1%), immune-mediated hepatitis (0.5%), immune-mediated hypophysitis (0.5%), immune-mediated nephritis (0.7%), immune-mediated hyper- (1.2%) and hypothyroidism (8.3%).

2.2.1.6 Rationale for proposed initial dose

The choice of the 200 mg Q3W as an appropriate dose for the switch to fixed dosing is based on simulations performed using the population PK model of pembrolizumab showing that the fixed dose of 200 mg every 3 weeks will provide exposures that 1) are optimally consistent with those obtained with the 2 mg/kg dose every 3 weeks, 2) will maintain individual patient exposures in the exposure range established in melanoma as associated with maximal efficacy response and 3) will maintain individual patients exposure in the exposure range established in melanoma that are well tolerated and safe.

A fixed dose regimen will simplify the dosing regimen to be more convenient for physicians and to reduce potential for dosing errors. A fixed dosing scheme will also reduce complexity in the logistical chain at treatment facilities and reduce wastage.

There are extensive data that have been generated in the pembrolizumab development program supporting that a fixed dose of 200 mg is suitable for use in subjects with advanced melanoma as a replacement for weight-based dosing of 2 mg/kg. Details of these supporting data were were published recently (Freshwater T, Kondic A, Ahamadi M, Li CH, de Greef R, de Alwis D, Stone JA.

Evaluation of dosing strategy for pembrolizumab for oncology indications. J Immunother Cancer. 2017 May 16;5:43. doi: 10.1186/s40425-017-0242-5. eCollection 2017. PubMed PMID: 28515943; PubMed Central PMCID: PMC5433037). The results presented in this paper demonstrate that fixed dosing of pembrolizumab 200 mg Q3W maintains exposures comparable with or slightly increased relative to those from 2 mg/kg Q3W. Fixed dosing eliminates the waste generated by weight-based dosing, improves compliance, and might also reduce the risk of dosing errors by reducing dosing complexity.

Based on these data, trials using pembrolizumab monotherapy or in combination with other therapies have utilized a fixed dosing regimen for pembrolizumab and have resulted in FDA approvals for NSCLC, HNSCC, cHL, urothelial carcinoma, and MSI-H cancer using 200 mg Q3W.

With respect to melanoma, on May 17, 2017, the FDA approved to replace the 2 mg/kg Q3W dose for melanoma patients with 200 mg Q3W.

### 2.2.2 Dabrafenib Mesylate (GSK2118436B)

The RAS/RAF/MEK/ERK pathway is a critical proliferation pathway in many human cancers. This pathway can be constitutively activated by molecular alterations including BRAF activating mutations. Approximately 90% of all identified BRAF mutations in human cancer consist of a T1799 transversion mutation in exon 15, which results in a V600 E/D/K(T1799A) amino acid substitution. This mutation appears to mimic regulatory phosphorylation and increases BRAF activity approximately 10-fold compared to wild type (wt). RAF is a validated target in BRAF V600E-containing melanoma. In August 2011, the FDA approved vemurafenib (PLX4032, Zelboraf®), an ATP-competitive selective RAF inhibitor for the treatment of late-stage $BRAF^{V600E}$ melanoma. In the pivotal phase III trial of vemurafenib *vs.* dacarbazine (Chapman *et al.,* 2011), vemurafenib demonstrated significant improvement in overall survival (OS) (6-month OS of 84% *vs.* 64%, hazard ratio [HR]=0.37; *P*<0.001), progression-free survival (PFS) (estimated median PFS of 5.3 months *vs.* 1.6 months (HR=0.26; *P*<0.001]), and overall response rate (ORR) (48% *vs.* 5%). However, in patients with colorectal cancer (CRC) bearing the BRAF V600E mutation, there was only one partial response (PR) among 20 patients treated (ORR 5%) and four minor responses (Kopetz *et al.,* 2010).

2.2.2.1 Mechanism of action

Dabrafenib mesylate (GSK2118436B), a 4-(3-aminosulfonylphenyl)-5-(pyrimidin-3-yl) thiazole, is an ATP-competitive, selective inhibitor of RAF kinase. Dabrafenib potently inhibits all RAF isoforms, with the strongest potency against the V600 mutant, as compared to its activity against wt BRAF and CRAF (see below). In a panel of 270 kinases tested outside RAF isoforms, only seven kinases (LIMK1, ALK5, NEK11, SIK, SIK2, PKD2, and BRK) were inhibited at a 50% inhibitory concentration (IC50) <100 nM (Investigator's Brochure, 2012a).

**Inhibitory activity of dabrafenib on RAF**

| | $BRAF^{V600E}$ | $BRAF^{V600K}$ | $BRAF^{V600D}$ | wt BRAF | CRAF |
|---|---|---|---|---|---|
| $IC_{50}$ | 0.65 nM | 0.50 nM | 1.84 nM | 3.2 nM | 5.0 nM |

In a panel of 110 human tumor cell lines with known BRAF mutational status, dabrafenib potently inhibited proliferation of a majority (73%) of $BRAF^{V600E}$ mutant cell lines with growth $IC_{50}$ ($gIC_{50}$) <100 nM. In contrast, there was poor or no activity in other BRAF mutants or wt BRAF cell lines.

Dabrafenib given orally (PO) for 14 days at doses ranging from 0.1-300 mg/kg administered once daily (QD), twice daily (BID), or three times daily (TID) inhibited tumor growth in mice bearing BRAFV600E A375P F11s or Colo205 tumor xenografts. The effect was generally dose dependent up to 10 mg/kg/day (A375P F11s) or 30 mg/kg/day (Colo205), yielding 90-120% tumor reduction relative to untreated animals. However, cessation of treatment was associated with regrowth of the tumors. In A375P F11s melanoma xenografts, inhibition of pERK by >50% in the tumor was seen at doses of ≥3 mg/kg. Based on the single-dose studies, ~100 nM (52 ng/mL) dabrafenib in blood at 6 h post-dosing was needed for effective pharmacodynamic biomarker inhibition in the tumor. At repeated dosing of 30 mg/kg/day, the tumor pERK levels were reduced by >50% at 8 h after dosing (69% on Day 1 and 53% on Day 14). Levels of pERK returned to baseline 24 h post-dosing. Similar ↓pERK effects were seen in the ES-2 ovarian xenograft model, but pERK inhibition was weaker in the Colo205 xenograft model. Of note, concentrations of dabrafenib showing pharmacodynamic activity in xenografts did not cause a reduction in pERK/tERK levels in the normal intact brain.

2.2.2.2 Clinical Pharmacokinetics (PK) and Activity of Dabrafenib

GSK has sponsored several dabrafenib monotherapy studies, mostly in patients with BRAF V600 mutant melanoma; and trials for combinations with a MEK inhibitor.

*FTIH Phase 1 Trial of Dabrafenib Monotherapy (BRF112680)*
BRF112680 is the FTIH study of dabrafenib (gelatin capsule) monotherapy, administered at escalating doses at different schedules (12-600 mg PO QD, BID, or TID). A total of 184 patients who had BRAF mutations (V600E or V600K) in their tumors were treated (Investigator's Brochure, 2012a).

*PK and metabolism of dabrafenib:*
In the single-dose studies, plasma dabrafenib concentrations peaked at a median time of 2 h ($T_{max}$) and bi-exponentially declined thereafter. The median terminal half-life ($t_{1/2}$) was approximately 8 hours after a single dose. Increases in $C_{max}$ and AUC were generally dose proportional up to 300 mg. However, on repeat daily dosing, the drug exposure decreased with time. At the recommended phase 2 dose (RP2D) of 150 mg PO BID, $C_{max}$ and AUC were about 40% lower on Day 15 vs. Day 8 (geomean $C_{max}$ of 1.35 vs. 0.81 mcg/mL and geomean AUC of 2.62 vs. 4.17 mcg•hr/mL), although the AUC remained stable over subsequent administrations. The decreased exposure with time may be a result of GSK2118436-mediated induction of its own metabolism.

Three metabolites of dabrafenib were characterized and may contribute to activity. GSK2285403 (hydroxy-metabolite [M7]) PK paralleled that of dabrafenib, while the carboxy- (GSK2298683 [M4]) and desmethyl- (GSK2167542 [M8]) metabolites exhibited a longer $t_{1/2}$ and accumulated following repeat dosing. Similar to dabrafenib concentrations, exposure for all metabolites showed a less than dose proportional increase with repeat dosing.

Fecal excretion was a major route of dabrafenib elimination in humans, accounting for 71.1% of the dose administered, and renal excretion accounted for about 20% of drug elimination.

Administration of dabrafenib with a high-fat, high-calorie meal reduced the oral bioavailability of dabrafenib when compared to the fasted state with a decrease in $C_{max}$ and AUC of 51% and 31%, respectively, and delayed its absorption. Therefore, the current recommendation is to administer dabrafenib under fasting conditions, either 1 h before or 2 h after a meal.

*Drug-drug interactions:*
As a CYP3A4 inducer, dabrafenib, when administered 150 mg BID in combination with midazolam (a substrate of CYP3A4), significantly reduced the midazolam exposure by 74% (the ratios of midazolam with or without dabrafenib were 0.39 for $C_{max}$ and 0.26 for AUC). Dabrafenib thus induces CYP3A4 metabolism and may induce other enzymes such as CYP2B6, CYP2C8, CYP2C9, and CYP2C19. Co-administration of dabrafenib and medicinal products which are affected by the induction of these enzymes such as hormonal contraceptives, warfarin, or dexamethasone may result in decreased concentrations and loss of efficacy.

Based on preclinical in vitro studies, dabrafenib was shown to be primarily metabolized by CYP2C8 and CYP3A4. Co-administration of ketoconazole (CYP3A4 inhibitor) increased the AUC of dabrafenib by 57%. Medicinal products that are strong inhibitors or inducers of CYP2C8 or CYP3A are likely to increase or decrease, respectively, dabrafenib concentrations. Alternative agents should be considered during administration with dabrafenib when possible. Use caution if strong inhibitors (*e.g.*, ketoconazole, nefazodone, clarithromycin, ritonavir) or inducers (*e.g.*, rifampin, phenytoin, carbamazepine, phenobarbital) of CYP2C8 or CYP3A4 are coadministered with dabrafenib.

*Pharmacodynamic effect of dabrafenib:*
Median tumor pERK inhibition was 83.9% (range: 38.0 to 93.3%) in BRAF mutant melanoma subjects receiving doses of 70 to 200 mg BID. The relationship between exposure and % pERK inhibition was characterized using a maximum response ($E_{max}$) model with 100% maximum inhibition and $IC_{50}$ of 134 ng/mL (95% CI: 92.7, 155) based on the sum of the potency-adjusted parent and active metabolite concentrations. A dose-related decrease in pERK was predicted with total daily doses <200 mg (100 mg BID) dabrafenib, with a plateau occurring beyond total daily doses of 200 mg thereafter.

*Selection of the RP2D:*
The single-agent MTD for dabrafenib was not reached. A dose of 150 mg BID was selected for further single-agent development, based on the following PK/pharmacodynamics, safety, and activity: a) dose increases beyond 150 mg BID yielded no increase in $C_{max}$ and <50% increase in AUC; b) incidence and severity of AEs was similar at 100-300 mg BID; c) pERK target suppression was >80%; and d) the tumor response rate (RR) was 50% at 150 mg BID.

*Antitumor Activity of Dabrafenib Monotherapy*

*Activity in patients with BRAF V600E or V600K melanoma:*
The FTIH monotherapy study (BRF112680) enrolled 114 enrolled patients with BRAF V600 mutant melanoma in the dose escalation phase (Part 1), and 70 patients at the RP2D (150 mg BID) in Part 2. Within this study, a cohort of 10 patients with previously untreated asymptomatic brain metastasis was evaluated for intracranial response to dabrafenib (Long *et al.,* 2011). All patients had decreases in the size of the brain metastasis; three patients achieved complete radiographic resolution of brain lesions (bCR) as well as reduction in extracranial disease. The response rates in patients treated at 150 mg BID are shown below.

**FTIH monotherapy study (BRF112680) response rates in melanoma patients**

| | Subgroup | Patient # | ORR |
|---|---|---|---|
| Part 1 | V600E | 77 | 50% |
| | V600K | 14 | 20% |
| Part 2, Cohort A | V600E/K with brain mets | 10 | 40% |
| | V600E/K without brain mets | 20 | 55% |

When dabrafenib was used at 50 mg BID (Part 2, Cohort C) in patients with BRAF V600E mutant melanoma, the response rate was only 17%.

*Correlative studies in the phase 1 monotherapy trial:*
Preliminary genomic analysis was performed on 37 patients with melanoma, using a Sequenom mutation analysis for 11 genes (AKT, BRAF, CDK4, CDKN2A, GNAQ, GNA11, Kit, MEK1, MEK2, and NRAS), and PTEN analysis by sequencing, comparative genomic hybridization (CGH), and multiplex ligation-dependent probe amplification (MPLA) (Nathanson *et al.,* 2011). Nine patients (24%) had PTEN genetic alterations including mutation, hemi-/homozygous deletion. PTEN deficiency was associated with lower responses (ORR of 11% and 54% in patients with and without PTEN alteration, respectively).

*Phase III trial of dabrafenib versus chemotherapy in patients with advanced BRAFV600 mutant melanoma (BREAK3 Trial):*
Patients with previously untreated, unresectable stage III or IV $BRAF^{V600E}$-mutated melanoma were randomized (3:1) and stratified by stage to dabrafenib (150 mg PO BID) or dacarbazine (DTIC) (1000 mg/$m^2$, IV, every 3 weeks [Q3W]). Of 250 patients enrolled, 187 were randomized to dabrafenib and 63 to DTIC from February

to September 2011. At the time of the primary analysis, there were 118 events (77 dabrafenib and 41 DTIC). The hazard ratio for PFS was 0.30 (95% CI: 0.18-0.53; *P*<0.0001), with median PFS of 5.1 months for dabrafenib and 2.7 for DTIC. OS data were immature, with 30 deaths reported. Confirmed RR was 53% for dabrafenib and 19% for DTIC. Benefits in PFS and RR were observed in all subgroups evaluated.

*Activity in BRAF V600E mutant tumors other than melanoma:*
Efficacy data for dabrafenib in non-melanoma tumors are limited to 18 patients enrolled in the phase 1 trial. Among them, seven had CRC, nine had thyroid cancer, and one each had NSCLC and ovarian cancer, respectively. Confirmed partial responses (PRs) were seen in one patient with CRC, and in 2 patients with thyroid cancer; the patient with NSCLC had an unconfirmed PR at 6 weeks followed by progressive disease (PD) at 12 weeks. Eleven patients (six with thyroid cancer and five with CRC) had stable disease (SD) as their best response; the ovarian cancer patient had SD before progressing at approximately 36 weeks.

2.2.2.3 Safety Profile

A comprehensive list of adverse events is included in Section 7 of the protocol.

As of February 20, 2012, among 184 patients treated on the FTIH phase 1 trial, 99% experienced at least one adverse event (AE) (any grade). The most common (>20% of all subjects) AEs of any grade across all dosing cohorts in Part 1 and Part 2 were fatigue (42%), pyrexia (37%), headache (35%), nausea (34%), hyperkeratosis (33%), diarrhea (27%), arthralgia (25%), pain in extremity (25%), decreased appetite (24%), alopecia (23%) and rash (23%).

Serious AEs (SAEs) were reported in 39% of patients, including SCC (12%), pyrexia (7%), and urinary tract infection (3%). Sixty-four patients are reported to have had study drug interrupted due to the occurrence of AEs. Pyrexia was the AE that led most frequently to a dose interruption. Fifteen patients reported a dose reduction due to the occurrence of AEs. There were no instances of discontinuation of study treatment due to AEs and no fatal AEs reported in the study among patients who received at least one dose of dabrafenib.

**AEs of special interest:**
The following events observed in dabrafenib monotherapy studies

are discussed in detail because they may be a class effect of BRAF inhibitor compounds, have occurred at high frequency, and/or are potentially life-threatening.

Dermatologic effects: Rashes and other skin lesions, from hyperkeratosis to SCC, have been observed at the frequencies below in FTIH study BRF112680.

| AE Term | Any Grade | Grade ≥3 |
|---|---|---|
| Hyperkeratosis | 61 (33) | 0 |
| Skin papilloma | 46 (25) | 1 (<1) |
| Rash | 42 (23) | 1 (<1) |
| Skin lesion | 26 (14) | 0 |
| Actinic keratosis | 21 (11) | 0 |
| Squamous cell carcinoma | 22 (12) | 21 (11) |
| Pruritis | 22 (12) | 0 |
| Seborrheic keratosis | 25 (14) | 0 |
| Acrochordon | 19 (10) | 0 |
| Melanocytic nevus | 19 (10) | 0 |
| Rash pruritic | 11 (6) | 0 |

Pre-malignant and malignant skin lesions: Cutaneous SCC and keratocanthoma were reported in 11% and 2%, respectively, of patients treated with dabrafenib in FTIH study BRF112680. SCC and proliferative skin toxicities are considered a class effect of BRAF inhibitors such as vemurafenib and sorafenib (Long *et al.,* 2011). SCC was treated with local excision, and treatment with dabrafenib was continued. Most SCCs of the skin have been localized and generally treated with curettage, and have been without significant clinical sequelae. Only one patient required a dose reduction in response to the event. The median onset of the first SCC occurred on Day 67 (range: Day 9-217).

Other treatment-emergent malignancies: Other treatment-emergent cutaneous malignancies such as basal cell carcinoma and new primary melanoma have been reported with BRAF inhibitors (Zelboraf, 2011).

Pyrexia: Across dabrafenib studies, all SAEs of pyrexia, influenza-like illness, cytokine release syndrome, systemic inflammatory response syndrome underwent clinical review for serious events of pyrexia complicated by hypotension, dehydration, circulatory collapse, severe rigors, or renal failure in the absence of another identifiable etiology (*i.e.*, infection).

Abnormal ejection fraction: Left ventricular ejection fraction (LVEF) changes were not observed in the early safety reviews of

dabrafenib. However, these events are included in AEs of special interest because they are known side effects of several kinase inhibitors including imatinib, sunitinib, and lapatinib (Force *et al.*, 2007).

Cardiac valvular abnormalities: Data from preclinical studies suggested that dabrafenib has the potential to cause cardiac valve abnormalities. In a 28-day dog toxicology study, high doses (50 mg/kg/day; approximately 40-fold over the therapeutic dose) of dabrafenib in 1 dog (n=10) resulted in hypertrophy of the right atrio-ventricular valve (tricuspid valve). Therefore, this was monitored in clinical trials with echocardiograms.

Uveitis: Uveitis was reported at a frequency of 3.8% as an AE of vemurafenib in previously treated patients with BRAF V600 mutation-positive metastatic melanoma (Chapman *et al.*, 2011; Sosman *et al.*, 2010), and has been observed in patients receiving dabrafenib (incidence in study BRF112680 was 1%).

Renal failure: Cases of renal failure have been identified on clinical trials with a possible causal relationship to dabrafenib.

2.2.2.4 Clinical Experience with the Combination of **Dabrafenib + Trametinib**

Data on 247 patients with metastatic melanoma and BRAF V600 mutations participating in the phase 1/2 study of dabrafenib and trametinib, BRF113220, have been published (Flaherty *et al.*, 2012 #2).

*PK*
Repeat-dose trametinib (2 mg QD) had no effect on the PK of single-dose dabrafenib (75 mg), but the plasma levels of dabrafenib after repeated dosing were higher in combination with trametinib as compared to that with previously reported PK with monotherapy. Geometric mean Day 15 AUC of dabrafenib in combination ranged from 3539 to 5187 ng•hr/mL, while the AUC observed in the monotherapy study was 2619 ng•hr/mL. The data suggest that trametinib may have a minor inhibitory effect on dabrafenib clearance (Flaherty *et al.*, 2012 #2).

Preliminary results showed that trametinib accumulated with repeat dosing; Day 21 $AUC_{0-24}$ was 314 ng•hr/mL in combination with dabrafenib at 75 mg BID and 335 ng•hr/mL in combination with dabrafenib at 150 mg BID (Investigator's Brochure, 2012b).

*Safety and the RP2D for the combination of trametinib and*

*dabrafenib*

In the dose escalation portion (Part B) of study BRF113220, one DLT of a recurrent grade 2 neutrophilic panniculitis occurred, among the 24 patients treated at the highest dose level (150 mg dabrafenib BID + 2 mg trametinib QD) (150/2) (Flaherty *et al.*, 2012). The MTD of the combination was not reached, and the RP2D was therefore 150/2. Pyrexia was common in the 150/2 group, occurring in 88% of patients.

In the randomized, open-label, phase 2 portion (Part C) of the study, the incidence of certain AEs were lower with the combination as compared to single agent dabrafenib: cutaneous squamous-cell carcinoma (including keratoacanthoma) (19 vs. 7%), and rash (27 vs. 36%) (Flaherty *et al.*, 2012 #2). On the other hand, the frequencies of pyrexia appeared increased (39 vs. 14%). The combination was also associated with an increased prevalence of MEK inhibitor-associated acneiform dermatitis (16 vs. 4%, with no grade 3 or 4 events reported). There was one death from sepsis in the 150/1 combination group, and there were three deaths in the combination 150/2 group (two from brain hemorrhage and one from pulmonary embolism); none of these events were considered to be related to a study drug. The incidences of main AEs (all grades) observed in Part C of the trial are presented in the table below as patients (percent).

| Adverse Event | Dabrafenib Monotherapy (n=53) | Combination 150/1 (n=54) | Combination 150/2 (n=55) |
|---|---|---|---|
| Pyrexia | 14 (26) | 37 (69) | 39 (71) |
| Nausea | 11 (21) | 25 (46) | 24 (44) |
| Vomiting | 8 (15) | 23 (43) | 22 (40) |
| Diarrhea | 15 (28) | 14 (26) | 20 (36) |
| Rash | 19 (36) | 11 (20) | 15 (27) |
| Hyperkeratosis | 16 (30) | 3 (6) | 5 (9) |
| Cutaneous squamous cell carcinoma | 10 (19) | 1 (2) | 4 (7) |
| Alopecia | 18 (34) | 5 (9) | 3 (5) |
| Skin papilloma | 8 (15) | 4 (7) | 2 (4) |
| Decreased ejection fraction | 0 | 2 (4) | 5 (9) |
| Cardiac failure | 0 | 1 (2) | 0 |
| Hypertension | 2 (4) | 2 (4) | 5 (9) |
| Chorioretinopathy | 0 | 0 | 1 (2) |
| Neutropenia | | | (11) |
| Acneiform dermatitis | (4) | (11) | (16) |

*Activity*

Efficacy analyses were performed in the intention-to-treat

population of the phase 2 portion (Part C) of study BRF113220, with a median follow-up of 14.1 months (Flaherty *et al.*, 2012). All major efficacy endpoints were improved, including PFS, 12-month PFS, ORR, and duration of response. Endpoint values as assessed by site investigators for this portion of the study are presented in the table below.

| End Point | Dabrafenib Monotherapy (n=54) | Combination 150/1 (n=54) | Combination 150/2 (n=54) |
|---|---|---|---|
| Progression-free Survival – months Median (95% CI) | 5.8 (4.6-7.4) | 9.2 (6.4-11.0) | 9.4 (8.6-16.7) |
| Progression-free Survival at 12 mo. % (95% CI) | 9 (3-20) | 26 (15-39) | 41 (27-54) |
| CR or PR Patients (% [95% CI]) | 29 (54 [40-67]) | 27 (50 [36-64]) | 41 (76 [62-86]) |
| Duration of response Median months (95% CI) | 5.6 (4.5-7.4) | 9.5 (7.4-NA) | 10.5 (7.4-14.9) |

#### 2.2.2.5 Rationale for proposed starting dose

The safe and efficacious dose of dabrafenib is 150 mg PO twice-daily as reflected by the FDA approval of this dose. Further, this dose is safe and effective in combination with trametinib. Given the known survival advantage with this therapy taken at this dose and schedule, we believe it is a priority to administer this dose of dabrafenib as the backbone of the proposed combination therapy.

### 2.2.3 Trametinib Dimethyl Sulfoxide (GSK1120212B)

The RAF-MEK-ERK pathway plays a critical role in multiple cellular functions. Activation of the pathway can result from activation/mutations of the upstream receptor tyrosine kinases (RTKs) and RAS, or upregulation/mutations in RAF and MEK. Upon activation, RAF acts as the MAPK kinase and activates MAPKK (MEK1/2), which in turn catalyze activation of the effectors ERK1/ERK2. Once activated, ERK1/2 translocate into the nucleus and phosphorylate a number of effector proteins and transcriptional factors that regulate cell proliferation, motility, differentiation, and survival. Trametinib is one of the several MEK inhibitors in clinical development.

Experience to date indicates that MEK is a valid target. In a phase III trial comparing trametinib with dacarbazine or paclitaxel in patients with BRAF V600E or V600K mutant metastatic melanoma,

trametinib demonstrated a significantly better response rate, progression-free survival, and overall survival (Flaherty *et al.*, 2012 #1). However, single agent activities are limited. Extensive research is underway to identify the patient selection markers and develop rational combination strategies. Preclinical studies have provided strong rationale and proof of principle for combination of MEK inhibitors with RTK inhibitors (EGFR or IGF-1R) (Gopal *et al.,* 2010; Ebi *et al.,* 2011), PI3K/AKT inhibitors (Engelman *et al.,* 2008; Hoeflich *et al.,* 2009), and mTOR inhibitors. On the other hand, the optimal dose/schedule and patient selection criteria for combination regimens have not been defined. Phase 1 results for a number of combinations have been reported, including AZD6244 + MK2206 (Tolcher *et al.,* 2011) and GDC-0973 + GDC-094 (MEK + PI3K inhibitor) (Bendell *et al.,* 2011).

#### 2.2.3.1 Mechanisms of Action and Preclinical Data with Trametinib

Trametinib is a dimethyl sulfoxide (DMSO) solvate compound (ratio 1:1) with potent, allosteric and ATP non-competitive inhibition of MEK1/2 ($IC_{50}$ of 0.7 and 0.9 nM against MEK1 and MEK2, respectively) (Gilmartin *et al.,* 2011). Trametinib inhibited MEK1/2 kinase activity and prevented RAF-dependent MEK phosphorylation (S217 for MEK1), producing prolonged pERK1/2 inhibition. Trametinib showed better potency against unphosphorylated MEK1/2 (u-MEK1/2) when compared with preactivated diphosphorylated MEK (pp-MEK), suggesting that u-MEK affords a higher affinity binding site for trametinib than does pp-MEK.

The specificity of trametinib was confirmed against a panel of 183 kinases, including MEK5 (the closet kinase homolog to MEK1/2), CRAF, BRAF, ERK1, and ERK2 (Yamaguchi *et al.,* 2011). Trametinib demonstrated equal potency against activated MEK1- and MEK2-mediated phosphorylation of ERK (sequence identity of 85% across the whole protein and 100% in the active site for humans). Trametinib demonstrated preferential inhibition of RAF-mediated MEK1 activation ($IC_{50}$ = 0.60 nM) over pMEK1 kinase activity ($IC_{50}$ = 13 nM) (Investigator's Brochure, 2012a).

BRAF-mutant Colo205, A375P F11s, and HT-29 human tumor xenograft mouse models showed the most significant mean tumor growth inhibition (TGI) (80% to 87%) at 3.0 mg/kg trametinib, with multiple complete and partial tumor regressions. In the Colo205 model, tumor regression was observed even at a dose of 0.3 mg/kg (Yamaguchi *et al.,* 2011). Two KRAS-mutant xenograft models, HCT-116 and A549, also showed significant TGI (83% and 75%)

but without significant tumor regressions (Gilmartin *et al.,* 2011). As predicted by cell proliferation assays, tumor xenograft lines with wild-type (wt) RAF/RAS (PC3, BxPC3, and BT474) were much less sensitive, showing only modest TGI (44-46%) with no tumor regressions.

Pharmacodynamic studies were performed in mice treated with trametinib for 14 days (Gilmartin *et al.,* 2011). In the A375P F11s xenograft model, the first dose of trametinib (3 mg/kg) significantly reduced pERK for more than 8 hours on Day 1. pERK inhibition was more sustained (over 24 hours) after the Day 7 dose, probably due to an increase in the steady-state levels of trametinib after repeated doses. The average $C_{max}$ in blood was 1,410 nM on Day 7, with an estimated half-life ($t_{1/2}$) of 33 hours. In addition, immunohistochemistry (IHC) also confirmed inhibition of cell proliferation (reduced Ki67) and G1 cell cycle arrest (elevated p27Kip1/CDKN1B) following 4 days of treatment.

2.2.3.2 Clinical Pharmacokinetics (PK) and Activity of Trametinib

*FTIH Phase 1 Trial of Trametinib Monotherapy (MEK111054)*
There are 3 parts in this ongoing study. Part 1: The dose-escalation portion involves administration of trametinib (repeat doses of 0.125 mg to 4.0 mg) to patients with solid tumors or lymphoma in one of three schedules - (1) QD for 21 days followed by 7 days without drug, (2) loading dose on Day 1 or Day 1-2, followed by QD with the designated dose, or (3) QD dosing without a drug holiday. Part 2: cohort expansion at the recommended phase 2 dose (RP2D) for pancreatic cancer, melanoma, NSCLC, CRC, or any BRAF mutation-positive cancer. Part 3: expansion to characterize the biologically active range of trametinib via analysis of pharmacodynamic biomarkers (biopsies or FDG-PET).

The dose escalation part and some of the cohort expansion components have been completed. The MTD of trametinib was established as 3 mg QD, but the recommended phase 2 dose (RP2D) was chosen at 2 mg QD based on tolerability of repeated cycles (Infante *et al.,* 2010).

*PK and metabolism of trametinib:*
PK measurements were conducted under fasting conditions. After a single dose (Day 1), $AUC_{0-24}$ and $C_{max}$ values were dose-proportional up to 6 mg, lower than dose proportional following 8 mg, and greater than dose proportional following the 10 mg dose. Median $T_{max}$ was 1.5 hours.

After repeat doses (Day 15), trametinib accumulated with a mean accumulation ratio of 6.6 at the RP2D of 2 mg QD. Between-subject variability in exposure ranged from 27-50% for $C_{max}$ and 20-41% for $AUC_{0-24}$ across all dosing regimens. The effective $t_{1/2}$ was approximately 4.5 days, and steady state was reached by approximately Day 15. Trametinib had a small peak:trough ratio of ~2 (Infante *et al.,* 2010). At 2 mg QD on Day 15, mean $AUC_{0-24}$ was 376 ng•h/mL and $C_{max}$ 23 ng/mL, and the mean trough concentrations ranged from 10.0 to18.9 ng/mL. The long half-life and small peak:trough ratio of trametinib allowed constant target inhibition within a narrow range of exposure.

*Drug-drug interactions:*
Trametinib is metabolized predominantly via deacetylation (non-cytochrome P450 [CYP450]-mediated) with secondary oxidation or in combination with glucuronidation biotransformation pathways (Investigator's Brochure, 2012a). The deacetylation is likely mediated by hydrolytic esterases, such as carboxylesterases, or amidases. Based on *in vitro* studies, trametinib is not an inhibitor of CYP1A2, CYP2A6, CYP2B6, CYP2D6, and CYP3A4. Although trametinib was found to be an *in vitro* inhibitor of CYP2C8, CYP2C9, and 2C19; inducer of CYP3A4; and inhibitor of transporters (OATP1B1, OATP1B3, P-glycoprotein [P-gp], and breast cancer resistance protein [BCRP]), its low efficacious dose, and low clinical systemic concentration (22.2 ng/mL or 0.04 mcM at 2 mg) relative to the *in vitro* inhibition/induction potency suggests an overall low potential for drug-drug interactions.

*Pharmacodynamic effect and biomarkers:*
The relationship between dose and tumor biomarkers such as pERK, Ki67, and p27, were evaluated in patients with BRAF or NRAS mutation-positive metastatic melanoma (Investigator's Brochure, 2012a). In general, increasing exposures and/or doses provided greater pharmacodynamic effects. The median change observed at a dose of 2 mg QD was 62% inhibition of pERK, 83% inhibition of Ki67, and a 175% increase in p27.

*Antitumor Activity of Trametinib Monotherapy*
In the FTIH phase 1 trial, 14 patients with BRAF-mutant melanoma received trametinib at 2 mg QD (2 mg/day continuously, or 2 mg for 21 days followed by a 1 week break). The overall objective response rate (ORR) was 43% (6/14), including 2 complete responses (CRs) (Investigator's Brochure, 2012a). In 9 patients with BRAF wt melanoma, 2 patients achieved a partial response (PR), and 3 stable disease (SD) (Infante *et al.,* 2010). In 26 evaluable pancreatic cancer patients, there were 2 PRs (1 PR was

KRAS mutation-positive) and 11 SD (2 achieved ≥20% tumor reduction) (Messersmith *et al.,* 2011). Among the 27 CRC patients (without selection of RAS or RAF mutations), 8 SD were observed.

In a phase 3 trial, patients with unresectable stage IIIC or IV cutaneous melanoma with a BRAF V600E or V600K mutation were randomized (2:1) to trametinib (2 mg, PO, QD) or chemotherapy (dacarbazine or paclitaxel) (Flaherty *et al.*, 2012). There were 322 patients in the intention-to-treat (ITT) population, of whom 273 (85%) were in the primary efficacy population (patients with $BRAF^{V600E}$-positive cancer who did not have brain metastases at baseline). In the ITT analyses, the ORR was 22% in the trametinib group and 8% in the chemotherapy group; the median duration of PFS was 4.8 months in the trametinib group as compared with 1.5 months in the chemotherapy group; and the 6-month OS rate was 81% in the trametinib group and 67% in the chemotherapy group.

*Antitumor Activity of Trametinib in Cancer Other Than Melanoma*
In a phase 1/2 monotherapy study, acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) patients were given trametinib at dose levels from 1-2 mg QD. Drug-related AEs in 45 patients were similar to that observed in patients with solid tumors, and 2 mg PO QD was selected for further investigation in this patient population. Twelve patients (23%) withdrew due to an AE, including cardiac failure (2) and infection (2). Efficacy was reported in 39 patients (Borthakur *et al.,* 2010). The best response in 13 patients with KRAS or NRAS mutations included 3 CRs (23%), 7 SD (54%), and 1 PD (progressive disease) (5%). In 26 patients with wild-type RAS or an unknown mutation, there were 2 PRs (8%).

#### 2.2.3.3 Trametinib Safety Profile

A comprehensive list of adverse events is included in Section 7 of the protocol.

Due to limited experience in human subjects, there is currently incomplete information available about the relationship of AEs and administration of trametinib. Based on available AE data from clinical studies involving trametinib to date, the most common toxicities are rash and diarrhea. Rash and diarrhea are common, class-effect toxicities for MEK inhibitors. In addition, visual impairment and left ventricular ejection fraction (LVEF) reduction, although observed at lower frequencies, are also considered class-effect toxicities as they have been observed with trametinib as well as other MEK inhibitors.

**AEs of special interest:**
Rash, diarrhea, visual disorders, hepatic disorders, cardiac-related AEs, and pneumonitis are considered AEs of special interest because they are either known class effects (*i.e.*, have been observed with other MEK inhibitors) or are potentially life-threatening (Investigator's Brochure, 2012a).

Rash: Rash was a common AE observed across different dose levels and in different combinations. The majority of rash observed with trametinib was acneiform and appeared to occur most frequently on the face, scalp, chest, and upper back. At the 2 mg dose, rash was seen in 48% to 91% of patients in different trials. The majority of rash AEs were grades 1 or 2 (68% to 80%); 1% to 18% of patients experienced grade 3 rash AEs, and one patient had a grade 4 rash AE.

Diarrhea: At the 2 mg monotherapy dose, 28% to 58% of patients in three trials had diarrhea. Of 219 patients with diarrhea at this dose, the majority of diarrhea AEs were grade 1 or 2 in severity (28% to 56% of all study patients); 6 patients had grade 3 diarrhea, and none had grade 4 diarrhea.

Visual disorders: At the 2 mg monotherapy dose, 6% to 21% of the patients in three trials experienced visual disorders. Of the 62 total patients experiencing visual disorders at this dose level, the majority of visual disorders were grades 1 or 2 (6% to 20% of all study patients); five patients experienced grade 3 visual disorders, and one patient experienced a grade 4 visual disorder.

*Central serous retinopathy (CSR):* CSR is a class side effect of MEK inhibitors. As of 22 May 2012, 13 cases of CSR have been reported amongst approximately 1,600 patients treated with trametinib, either as monotherapy or in combination with other anti-cancer agents: two cases of grade 1, eight cases of grade 2, and three cases of grade 3. All 13 resolved.

*Retinal vein occlusion (RVO):* As of 22 May 2012, four cases of RVO have been observed with trametinib. All four cases occurred in one eye only, and study drug was stopped at time of diagnosis in all cases. There was a decrease in visual acuity in two patients with central RVO (CRVO), while the other two patients experienced no meaningful decrease in visual acuity. Three of the four cases were considered related to study treatment by the investigators.

Hepatic disorders: Abnormalities of liver enzymes and bilirubin have been observed with administration of trametinib. However, assessment of these cases was often confounded by co-morbid conditions (such as biliary obstruction), concomitant use of other

potentially hepatotoxic drugs, and liver metastases. At the 2 mg monotherapy dose, 10% to 19% of patients in three trials had hepatic disorders. Of the 56 total patients experiencing hepatic disorders, the majority were grade 1 or 2 in severity (7% to 15% of all study patients); 12 patients had grade 3 hepatic disorders, and 3 patients had grade 4 hepatic disorders.

Cardiac-related AEs: At the 2 mg monotherapy dose in three trials, 3% to 21% of patients had cardiac-related AEs. Of the 43 total patients experiencing cardiac-related AEs, the majority were grade 1 or 2 in severity (4% to 16% of all study patients); six patients at this trametinib dose level had grade 3 cardiac-related AEs (three left ventricular dysfunction, two decreased LVEF, and one ventricular dilatation), and one patient experienced a grade 4 cardiac-related AE (cardiogenic shock). One patient died of acute cardiac failure, with evidence of massive tumor invasion of the heart; this AE was considered not drug-related by the investigator.

In the phase 3 trial of trametinib vs. chemotherapy in patients with melanoma (MEK114267), patients were monitored by serial echocardiogram or MUGA scans. As of 23 June 2012, among 211 patients on the trametinib arm, 17 cardiac-related AEs were reported and included: decreased LVEF (ten grade 1-2, and two grade 3), left ventricular dysfunction (two grade 2, and two grade 3), and one grade 3 cardiac failure. No cardiac-related AEs have been observed on the chemotherapy arm of the study. Cardiac-related AEs leading to permanent discontinuation of study drug included decreased LVEF (n=2), left ventricular dysfunction (n=2), cardiac failure (n=1), myocardial infarction (n=1), and tachycardia (n=1). There was also one death due to cardiogenic shock secondary to ischemic heart disease, but it was not considered related to trametinib.

Pneumonitis: As of the Investigator Brochure's cut-off date, 20 cases of pneumonitis were reported in subjects treated with trametinib, either as monotherapy or in combination with other anti-cancer agents, in six studies: five cases of grade 1, five cases of grade 2, nine cases of grade 3, and one case of grade 4.

#### 2.2.3.4 Rationale for proposed starting dose

The safe and efficacious dose of trametinib is 2 mg PO daily as reflected by the FDA approval of this dose. Further, this dose is safe and effective in combination with dabrafenib. Given the known survival advantage with this therapy taken at this dose and schedule, we believe it is a priority to administer this dose of trametinib as the backbone of the proposed combination therapy.

## 2.3 **Rationale**

It has been shown that inhibition of the mitogen-activated protein kinase (MAPK) pathway in patients with melanoma, through inhibition of BRAF and/or MEK, leads to increased melanoma antigen expression, decreased immunosuppressive cytokines, increased trafficking of CD8+ T-lymphocytes, and upregulation of immune exhaustion markers such as PD1 and PDL1. Also, in collaboration with Adaptive Biotechnologies, we have shown that patients with a higher proportion of preexisting tumor infiltrating lymphocyte clones, determined by T-Cell Receptor (TCR) rearrangement sequencing (so-called ImmunoSeq), have better outcomes with BRAF/MEKi therapy, although nearly all patients have an increase in TCR clonality in the setting of BRAF/MEKi therapy. The major conclusion from this data is that MAPK pathway targeting enhances anti-tumor immunity within the tumor microenvironment, which provides a strong rationale for combination MAPK targeted therapy with immune targeted therapy.

A number of trials of combination MAPK targeted therapy (MTT) with immune targeted therapy have been opened in recent years with mixed results. One of the major issues has been tolerability, particularly with combinations of MTT and ipilimumab. We believe that the value of combining MTT with immunotherapies, such as pembrolizumab, is that MTT may enhance immune responses in the tumor microenvironment and convert an immunologically non-responsive tumor microenvironment into an environment that is responsive to anti-PD1 immunotherapy. Thus, we are interested in testing trial designs that incorporate a lead-in phase of MTT and a brief period of concomitant MTT and immune targeted therapy.

This trial has two planned cohorts, BRAFV600 mutant (cohort 1) and BRAF-wild-type melanoma (cohort 2). Treatment commences with 2 weeks of MTT (dabrafenib plus trametinib in cohort 1, trametinib in cohort 2), then two 3 weeks cycles of concomitant MTT and pembrolizumab, followed by single-agent pembrolizumab thereafter. Serial biopsies will be performed prior to MTT, and (if feasible) following the 2-week lead-in of MTT, and (if feasible) following six weeks of combination immune therapy and MTT. An optional biopsy at time of disease progression will also be performed. This trial represents a unique opportunity to not only test the effectiveness of combined immune and MTT, but also to determine which patients are most likely to benefit from this approach and, more importantly, to begin to understand why patients do or do not benefit. This valuable information that may lead to the next wave of combinatorial therapy in melanoma that involves MTT, anti-PD1 therapy, and other agents that target critical mediators of adaptive or innate resistance to combined MTT and pembrolizumab. Due to a recent shortage of research grade dabrafenib and trametinb, we plan to use commercially availability dabrafenib and trametinib. Since we cannot guarantee supply of “off-label” trametinib for cohort 2, we will open cohort 1 initially and consider

opening cohort 2 in mid-2017, after more data is available for MEK inhibitors in patients with BRAF wild-type melanoma.

## 2.4 Correlative Studies Background

Recently, a number of predictive biomarkers of response to anti-PD1 antibodies in melanoma and other malignancies have been reported. These include tumor factors (such as mutational burden and tumor neoantigen expression) immunologic factors (including cytotoxic T-lymphocyte infiltration with functional capacity to recognize tumor antigens, so-called T-cell diversity), and tumor-immune microenvironmental factors like PD-L1 expression. With each new report, a clear picture is emerging that tumors with high mutational burden and neoantigen expression, increased T-cell diversity, and elevated PD-L1 expression are primed for immune destruction in the setting of single-agent anti-PD1 antibodies. Conversely, tumors with low mutational load and neoantigen expression, low T-cell diversity, and low PD-L1 expression are less susceptible to immune-mediated tumor cell destruction. One of the next great challenges in the field is to develop strategies that convert an immunologically non-responsive tumor microenvironment into one primed to respond to anti-PD1 therapy.

In this trial, we expect that lead-in MTT will trigger changes in the tumor immune microenvironment that will allow patients to have a more robust response to the anti-PD1 antibody, pembrolizumab. However, it will be critical to document these changes in tumor immune microenvironment. As such, tumor biopsies will be performed in all patients enrolled onto the first Stage of the Simon 2-stage design for both cohorts at four time points:

1. Prior to commencing MTT
2. Following 2-week MTT lead-in
3. Following 6-weeks of combined pembrolizumab with MTT (if feasible)
4. At the time of disease progression (optional)

Multiple cores will be obtained and/or sufficient tumor excised at each biopsy time-point, assuming that it is safe to do so (e.g. patients with rapid responses may have very little tumor to biopsy safely at the latter time points). Core biopsies or portions of the excised tumor will be processed with the following hierarchy in mind:

1. DNA/RNA/protein extraction (Sullivan)
2. Disaggregation and flow cytometry (Sharpe)
3. FFPE (Sullivan)
4. Single-cell RNA seq of T cells (Hacohen)
5. Single-cell RNA seq of tumor cells (Garraway)

Analysis will then be carried out to compare changes seen longitudinally and to determine if those changes are associated with improved outcomes to therapy with study treatment. Most of this work will exploratory in nature, as analysis such as the technology for single-cell sequencing of tumor and T-cells is emerging and no data is presently available to inform how these analyses might be used to help predict which patients are most likely to benefit from single-agent or combination therapy. However, based on previous work, there are two assays that will be incorporated into a predictive model, PD-L1 expression by immunohistochemistry (IHC) and immuno-sequencing (immunoSEQ) using the Adaptive Biotechnologies platform.

### *PD-L1 Immunohistochemistry*

Immunohistochemistry for PD-L1 and CD8 was performed using a monoclonal anti-PD-L1 antibody (Clone E1L3N, Cell Signaling Technologies; Danvers, MA) and a CD8 monoclonal antibody (4B11, RTU, Leica Biosystems, Buffalo Grove, IL), respectively, with an automated stainer (Bond Rx, Leica Microsystems, Buffalo Grove, IL). As there is no recognized standard PD-L1 scoring protocol, we will define PD-L1 positivity as membranous +/- cytoplasmic staining of tumor cells of any intensity using two different cut-offs (≥1% and ≥5 tumor cells) since both have been associated with clinical benefit to PD-1 pathway blockade in melanoma.

### *Adaptive Biotechnologies immunoSEQ*

FFPE specimens (or isolated tumor DNA) and frozen PBMC samples will be shipped to Adaptive. Samples sent to Adaptive will be handled in accordance with Adaptive SOPs. All arriving packages will be opened the same day they arrive and inspected thoroughly for correct labeling and packaging integrity. Adaptive will perform “survey level” sequencing on each tissue sample and “deep level” sequencing on each of the PBMC derived gDNA samples provided. As part of these analyses, Adaptive uses a proprietary assay for the amplification and massive parallel sequencing of millions of CDR3 regions in the T cell receptor beta gene (TCRB) using a multiplex PCR amplification across the VDJ junction of rearranged TCRB.

Using Adaptive’s unique capacity to perform next generation sequencing of T-cell receptors for this study, a large amount of data will be generated using tissue specimens and PBMC samples from all collection time points. In addition to the immunoSEQ Analyzer software, which is available to all of Adaptive clients to aid in data analysis and visualization, Adaptive can directly involve its computational biology and biostatistics personnel in the data analysis.

### *Tumor Microenvironment Assessment*

To determine how the tumor cells are modulated during MTT alone, anti-PD1

plus MTT and anti-PD1 monotherapy combination, we will use single-cell and bulk RNA-seq, whole exome, etc. to assess the evolution of tumor cells during therapy. Sequential biopsies will provide a means to analyze evolution of tumor cell responsiveness and resistance to therapy

To determine how immune cells in the tumor microenvironment are modulated during MTT alone, anti-PD1 plus MTT and anti-PD1 monotherapy combination, we will use single-cell and bulk RNA-seq, and assays to compare immune cell function (e.g., T cell activation vs. exhaustion using cellular immunologic assays, as well as analysis of phosphoproteins and ubiquitination signaling) in temporal biopsies. These studies should reveal how anti-tumor immunity is modulated during the lead-in phase of the clinical trial with MTT, during MTT and anti-PD1 combination therapy and during subsequent anti-PD1 monotherapy.

## 3. PARTICIPANT SELECTION

### 3.1 Inclusion Criteria

3.1.1 Participants must have histologically confirmed metastatic or unresectable melanoma

3.1.2 Participants must have BRAFV600-mutation status known (molecularly confirmed using validated, commercially available assay performed in a CLIA-approved laboratory).

- If test at CLIA-certified lab used a non-FDA approved method, information about the assay must be provided. (FDA approved tests for BRAF V600 mutations in melanoma include: THxID BRAF Detection Kit and Cobas 4800 BRAF V600 Mutation Test).

3.1.3 Participants must have measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) as $\geq$ 20 mm with conventional techniques or as $\geq$ 10 mm with spiral CT scan. See section 11 for the evaluation of measureable disease.

3.1.4 Age greater than or equal to 18 years. Because no dosing or adverse event data are currently available on the use of the combination of trametinib with or without dabrafenib, and pembrolizumab in participants less than 18 years of age, children are excluded from this study.

3.1.5 ECOG performance status ≤ 1 (see Appendix A).

3.1.6 Life expectancy of greater than three months in the opinion of the investigator.

3.1.7 Participants must have normal organ and marrow function as defined below:

- Leukocytes (WBC) ≥ 3,000/uL
- Absolute neutrophil count ≥ 1,500uL
- Platelets ≥ 100,000/uL
- total bilirubin ≤ 1.5 X institutional upper limits of normal; total bilirubin > 1.5X above institutional upper limits of normal will be allowed if direct bilirubin is within normal limits or if patients has a documented history of Gilbert's disease
- AST (SGOT)/ALT (SGPT) ≤ 2.5 X institutional upper limit of normal
- Creatinine within normal institutional limits or creatinine clearance (eGFR) ≥ 60 mL/min/1.73 $m^2$ for subjects with creatinine levels about institutional normal.

3.1.8 Participants must have disease amenable to and be willing to undergo serial core or excisional biopsies of a tumor lesion(s).

3.1.9 Because both dabrafenib and trametinib are class D agents with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk of adverse events in nursing infants secondary to treatment of the mother with either dabrafenib and trametinib, breastfeeding should be discontinued if the mother is treated with either dabrafenib, trametinib, or the combination of dabrafenib and trametinib. Female subjects of childbearing potential should be willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication or 14 days prior to the initiation of study medication for oral contraceptives (Reference Section 5.8. Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Male subjects with partners of childbearing potential should agree to use an adequate method of contraception starting with the first dose of study therapy through 120 days after the last dose of study therapy. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

3.1.10 Ability to understand and the willingness to sign a written informed consent document.

3.1.11 Ability to swallow and retain oral medication.

3.2 **Exclusion Criteria**

3.2.1 Participants with any clinically significant gastrointestinal abnormalities that may alter absorption.

3.2.2 Participants treated with prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to the prior chemotherapy, targeted small molecule therapy, and radiation therapy

- Note: Subjects with ≤ Grade 2 neuropathy and/or alopecia are an exception to this criterion and may qualify for the study.

- Note: If subject received major surgery, they must have had the surgery > 2 weeks prior to Study Day 1 and recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy

3.2.3 Participants previously treated with BRAF inhibitors (vemurafenib, dabrafenib, encorafenib), MEK inhibitors (selumetinib, trametinib, binimetinib, cobimetinib), and/or anti-PD1/PDL1 monoclonal antibodies for metastatic or unresectable disease. Any other prior therapy will be allowed (including ipilimumab, adjuvant anti-PD1 therapy, high-dose IL-2).

3.2.4 Participants with any prior ≥ Grade 3 immune-related adverse event (irAE) which began while receiving immunotherapy.

3.2.5 Participants with any unresolved immune-related adverse event (irAE) at time of study entry.

- Note: Subjects with ≤ Grade 2 thyroiditis and/or hypophysitis are an exception to this criterion and may qualify for the study.

3.2.6 Participants who have had a prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.

3.2.7 Patients may not be receiving any other anti-neoplastic agents.

3.2.8 Participants with symptomatic brain metastases will be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events. Subjects with asymptomatic, stable brain metastases and subjects who, if they have been previously treated for these conditions that are asymptomatic in the absence of corticosteroid therapy are allowed to enroll. Brain metastasis must be stable with verification by imaging (brain MRI completed at screening demonstrating no current evidence of progressive brain metastases). If asymptomatic brain metastasis are first identified on the required pre-study scans, another set of scans must be completed to confirm that they are stable.

3.2.9 Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment. Corticosteroids to prevent contrast reactions is allowable.

3.2.10 Pregnant women are excluded from this study because both dabrafenib and trametinib are class D agents with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk of adverse events in nursing infants secondary to treatment of the mother with either dabrafenib and trametinib, breastfeeding should be discontinued if the mother is treated with either dabrafenib, trametinib, or the combination of dabrafenib and trametinib.

3.2.11 Participants known to be HIV-positive and on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with either dabrafenib or trametinib. Appropriate studies will be undertaken in participants receiving combination antiretroviral therapy when indicated.

3.2.12 Has a known history of active TB (Bacillus Tuberculosis)

3.2.13 Known hypersensitivity to pembrolizumab or any of its excipients.

3.2.14 Symptomatic or untreated leptomeningeal disease.

3.2.15 Participants are not permitted to receive enzyme inducing anti-epileptic drugs.

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

3.2.17 Has a history of non-infectious pneumonitis that required steroids or has current/active pneumonitis.

3.2.18 History of or current evidence of retinal vein occlusion (RVO) or retinal pigment epithelial detachment (RPED):

- History of RVO or RPED
- Current evidence of visible retinal pathology as assessed by pre-study ophthalmic exam that is considered a risk factor for RVO or RPED such as evidence of new optic disc cupping, evidence of visual field defects, and/or intraocular pressure >21 mm Hg.

3.2.19 Uncontrolled intercurrent illness including, but not limited to:

a. Ongoing or active infection
b. LVEF <50% as determined by either MUGA scan or Echo
c. Edema > Grade 1
d. Documented myocardial infarction or unstable/uncontrolled cardiac disease (eg, unstable angina, severe arrhythmias, congestive heart failure [New York Heart Association (NYHA) > Class II]) within 6 months of study entry
e. Arterial thrombosis or vascular ischemic events, such as transient ischemic attack, cerebral infarction, within 6 months prior to study entry
f. Serious or non-healing wound
g. History of any medical condition including cardiovascular disease or chronic obstructive pulmonary disease (COPD), that in the opinion of the investigator, may increase the risks associated with study participation or study treatments or may interfere with the conduct of the study or interpretation of study results

h. Psychiatric illness/social situations that, in the opinion of the investigator, would limit compliance with study requirements

3.2.20 Individuals with a history of a different malignancy are ineligible except for the following circumstances:

- Individuals with a history of other malignancies are eligible if they have been disease-free for at least 3 years and are deemed by the investigator to be at low risk for recurrence of that malignancy.
- Individuals with the following cancers are eligible if diagnosed and treated within the past 3 years: cervical cancer *in situ* and basal cell or squamous cell carcinoma of the skin.

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

3.2.22 Has received a live vaccine within 30 days of planned start of study therapy.

### 3.3 Inclusion of Women and Minorities

Men and women and members of all races and ethnic groups are eligible for this trial. There are no inclusion/exclusion criteria that should explicitly lead to reduced enrollment of any specific minority population. Malignant melanoma is similarly prevalent in men and women; however its incidence varies greatly among ethnic groups.

## 4. REGISTRATION PROCEDURES

### 4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered 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 begin protocol therapy. Lab values do not need to re-meet eligibility criteria on Day 1 Week 1 prior to first. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy 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.

## 5. TREATMENT PLAN

### 5.1 Treatment Regimen

Treatment will be administered on an outpatient basis. Expected toxicities and potential risks as well as dose modifications for dabrafenib, trametinib, and pembrolizumab are described in Section 6 (Expected Toxicities and Dosing Delays/Dose Modification). No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Response and progression will be assessed at the end of the lead-in phase, and at the start of each 3-week visit thereafter by history and physical exam or in the interim if any new symptoms arise. In addition, radiological imaging (either CT or MRI) will be obtained within 4 weeks of lead-in therapy commencement, within 1 week prior to Week 9, and at 6-week intervals (with a window of -1 week), following the commencement of pembrolizumab for 24 weeks and then extended to 12-week intervals (+2 weeks) while patients remain on the study. Response and progression will be determined based on the Response Evaluation Criteria in Solid Tumors 1.1 (RECIST 1.1). Further, if a patient does not tolerate the combination phase of combination MTT plus pembrolizumab, then MTT will be discontinued and pembrolizumab single-agent phase will begin, presuming that it is safe to treat with pembrolizumab (as outlined in Section 5.3.1).

All patients will be evaluated for anti-tumor activity of the combination. Disease control rate (DCR) at the 24-week time point after commencement of pembrolizumab (in combination with MTT) is the primary anti-tumor end-point. DCR is defined as the percentage of patients per cohort who have stable disease (SD), complete or partial response (CR, PR) without requiring restart of MTT for any reasons. Progression free survival (PFS), CR and PR rates, and overall survival (OS) will also be summarized for patients in each cohort.

A Simon 2-stage design will be implemented for two disease cohorts, $BRAF^{V600}$ mutant and $BRAF^{V600}$ wild-type unresectable Stage III or Stage IV melanoma. Patients will be treated with a two-week lead-in of MTT, followed by a 6-week course of combined MTT and pembrolizumab (at dose of 200 mg IV every three weeks), followed by maintenance single-agent pembrolizumab in all patients with RECIST defined stable disease (SD), partial response (PR), and complete response (CR). *MTT only will be resumed* if patients develop symptomatic or radiographic evidence of progression. If patients do not develop evidence of progressive disease (PD), then they will continue maintenance single-agent

pembrolizumab for up to two years; at which time treatment will be discontinued. In each cohort, patients will be accrued to first stage of the Simon design. If a pre-specified number of patients maintain SD/PR/CR off MTT but on pembrolizumab at the 24-week time point, then the second stage of the two-stage design will be opened. (Please see details in statistical section). Biopsies will be performed on all patients enrolled to the first stage of the Simon two-stage for each cohort. These will be obtained at baseline, after the two-week lead-in, and after the completion of induction therapy if feasible. An optional biopsy at the time of disease progression will also be performed.

## 5.2 **Pre-Treatment Criteria**

Please note: TSH results are not required to be available prior to dosing.

### 5.2.1 Targeted Therapy Lead-in Phase, Day 1 Week 1

- Physical examination including vital signs and performance status
- Labs values do not need to re-meet eligibility criteria on Week 1, Day 1
- Comprehensive metabolic panel including liver function tests
- Complete blood count (CBC w/diff, plts)
- ECG (in $BRAF^{V600}$ mutant cohort only)

### 5.2.2 Combination Targeted Therapy Plus Phase, Day 1 Week 3 and Day 22 Week 6

- Physical exam including vital signs and performance status
- Comprehensive metabolic panel including liver function tests
- Complete blood count (CBC w/diff, plts)
- ECG (in $BRAF^{V600}$ mutant cohort)
- Serum pregnancy test (Day 1 Week 3 for women of childbearing potential only)

### 5.2.3 Single-agent pembrolizumab Phase, Week 9

- Physical examination including vital signs and performance status
- Dermatology Exam
- Ophthalmology Exam
- Echo/MUGA
- Comprehensive metabolic panel including liver function tests
- Complete blood count (CBC w/ diff, plts)
- Serum pregnant test (for women of childbearing potential only)

### 5.2.4 Single-agent pembrolizumab Phase, Week 12 onwards

- Physical examination including vital signs and performance status
- Comprehensive metabolic panel including liver function tests
- Complete blood count (CBC w/ diff, plts)
- Serum pregnant test (Week 5 and 21 only and for women of childbearing potential only)

## 5.3 **Agent Administration**

### 5.3.1 Pembrolizumab

- Intravenous
- 200 mg IV every three weeks, to commence after 2-week lead-in Phase of MTT
- Missed doses held for toxicity will not be made up.
- On days when this treatment is given, it should be done so after trametinib dosing, during the concurrent MTT phase

### 5.3.2 Dabrafenib *(*$BRAF^{V600}$ mutant cohort only)

- Oral
- 150 mg PO BID, to be taken every twelve hours; can be taken together with trametinib
- Dabrafenib should be taken on an empty stomach at least one hour before or two hours after a meal; water is permitted during this fasting period.
- Missed or vomited doses will not be made up.
- If a dose is missed, it can be taken up to 6 hours prior to the next dose to maintain the twice daily regimen.
- The drug cannot be crushed, chewed, or dissolved
- In the BRAF mutant cohort, this should be taken before trametinib
- The medication diary and pill bottles will be returned to clinic staff at the end of the lead in period and at the end of each three week cycle.

### 5.3.3 Trametinib ($BRAF^{V600}$ mutant or $BRAF^{V600}$ wild-type cohorts)

- Oral
- 2 mg PO daily; can be taken together with dabrafenib
- Trametinib should be taken on an empty stomach at least one hour before or two hours after a meal; water is permitted during this fasting period.
- Missed or vomited doses will not be made up.
- If a dose is missed, it can be taken up to 12 hours after scheduled dose time.
- The drug cannot be crushed, chewed, or dissolved
- In the $BRAF^{V600}$ mutant cohort, this should be taken after dabrafenib.
- On days when pembrolizumab is given to patients in either the $BRAF^{V600}$ mutant or $BRAF^{V600}$ wild-type cohorts, this should be taken before pembrolizumab therapy.
- The medication diary and pill bottles will be returned to clinic staff at the end of the lead in period and at the end of each three week cycle.

### 5.3.4 Other Modality(ies) or Procedures

Palliative sterotactic radiation or surgery may be performed during study participation. Dabrafenib and/or trametinib must be held for two days before and two days after surgery or the commencement and completion of stereotactic radiation. Pembrolizumab dosing must be held for two weeks before and after surgery or the commencement and completion of stereotactic radiation. If palliative, standard fractionated, external beam radiation is indicated, then a discussion with the investigator, the principal investigator, and Merck will be held to discuss whether the patient should be allowed to remain on trial, and if so, if and for how long dabrafenib, trametinib, and/or pembrolizumab should be held.

## 5.4 General Concomitant Medication and Supportive Care Guidelines

### 5.4.1 Acceptable Concomitant Medications

All treatments (outside of the medications or vaccinations specifically prohibited in the exclusion and in Section 5.4.2) that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date must also be included on the CRF.

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should only be recorded for SAEs and ECIs as defined in Section 7.

### 5.4.2 Prohibited Concomitant Medications

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

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab not specified in this protocol

- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.

- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the Overall PI.
- Strong inhibitors/inducers of selected CYP450 isoenzymes. Dabrafenib mesylate is a substrate for CYP 3A4, 2C8, p-glycoprotein (Pgp), and breast carcinoma resistance protein (Bcrp). These enzymes and transporters are prohibited for eligibility and during the study. Because the lists of these agents are constantly changing, it is important to regularly consult a frequently-updated list such as http://medicine.iupui.edu/clinpharm/ddis/table.aspx; medical reference texts such as the Physicians' Desk Reference may also provide this information.
- Concomitant medications should be reviewed for the potential risk of inducing nephrotoxicity and, if so, should be modified if clinically possible (See 6.2.6)

If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the Merck Clinical team. The final decision on any supportive therapy or vaccination rests with the subject's primary investigator. Subjects may receive other medications that the investigator deems to be medically necessary.

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

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

### 5.4.3 Concomitant Medications to be used with caution

The following therapies are to be used with caution during the Screening and Treatment Phase (including retreatment for post-complete response relapse) of this trial:

- Mild or moderate inhibitors/inducers of CYP 3A4, 2C8, Pgp, and Bcrp should be used with caution as dabrafenib serum concentrations may be altered. Because the lists of these agents are constantly changing, it is important to regularly consult a frequently-updated list such as http://medicine.iupui.edu/clinpharm/ddis/table.aspx; medical reference texts such as the Physicians' Desk Reference may also provide this information.
- Dabrafenib may induce CYP 3A4, 2B6, and possibly 2C8/9 and 2C19. Use concomitant medications that are substrates of these isoenzymes with caution as there may be loss of efficacy. Substitute with other medications that are not affected if possible.
- Because there is a low potential for interaction of trametinib with other concomitantly administered drugs through the cytochrome P450 system, the Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential to affect selected CYP450 isoenzymes.

### 5.4.4 Alerting the Principal Investigator about specific agents and capturing the concurrent use of all drugs, over-the-counter medications, or alternative therapies in the case report form

- Because there is a potential for interaction of dabrafenib with other concomitantly administered drugs through the cytochrome P450 system, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator must be alerted if the patient is taking any agent known to affect or with the potential to affect selected CYP450 isoenzymes.
- Because there is a low potential for interaction of trametinib with other concomitantly administered drugs through the cytochrome P450 system, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential to affect selected CYP450 isoenzymes.

### 5.4.5 Rescue Medications & Supportive Care

#### 5.4.5.1 Supportive Care Guidelines

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

Note: if after the evaluation the event is determined not to be related, the investigator is instructed to follow the ECI reporting guidance but does not need to follow the treatment guidance (as outlined in the ECI guidance document). Refer to Section 6 for dose modification.
It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event. Suggested conditional procedures, as appropriate, can be found in the ECI guidance document.

- **Suggestions for Pneumonitis**:
  - For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
  - For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
  - Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.
- **Suggestions for Diarrhea/Colitis**:
  Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).

- Advise all subjects who experience diarrhea/colitis to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, substitute fluid and electrolytes via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
- For **Grade 2 diarrhea/colitis** that persists greater than 3 days, administer oral corticosteroids.
- For **Grade 3 or 4 diarrhea/colitis** that persists > 1 week, treat with intravenous steroids followed by high dose oral steroids.
- When symptoms improve to Grade 1 or less, start steroid taper and continue over no less than 4 weeks.

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

  - For **T1DM** or **Grade 3-4** Hyperglycemia

    - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.

    - Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

- **Suggestions for Hypophysitis**:

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

- **Suggestions for Hyperthyroidism or Hypothyroidism**:
  Thyroid disorders can occur at any time during treatment. Monitor patients for changes in thyroid function (at the start of

treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.

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

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

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

- **Suggestions for Management of Infusion Reactions**: Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

Table 5.4 below shows treatment suggestions for subjects who experience an infusion reaction associated with administration of pembrolizumab (MK-3475).

Table 5.4 Infusion Reaction Treatment Suggestions

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

## 5.5 Criteria for Taking a Participant off Protocol Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse events, treatment may continue until one of the following criteria applies:

- Completion of two years of pembrolizumab therapy
- Disease progression, unless patient meets criteria for treatment beyond progression in Section 5.5.1
- Intercurrent illness that prevents further administration of treatment,

- Unacceptable adverse event(s),
- Participant demonstrates an inability or unwillingness to comply with the oral medication regimen and/or documentation requirements
- Participant decides to withdraw from the study, or
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the opinion of the treating investigator.

5.5.1 Treatment Beyond Progression

Following initial disease progression, if a participant is treated beyond progression per the criteria outlined below, tumor assessments must be repeated ≥ 4 weeks after initial disease progression in order to confirm disease progression. The tumor assessment must not be repeated any sooner than 4 weeks after initial disease progression.

Treatment beyond progression will be allowed. In general, patients who develop disease progression (per RECIST1.1) will remain on trial. Since this trial potentially involves four specific treatments "zones", namely 1) MTT induction; 2) concomitant MTT plus pembrolizumab; 3) pembrolizumab single-agent; and 4) follow up after pembrolizumab discontinuation in setting of CR, the specific treatment beyond progression will be dependent on which "zone" the patient is in when progression occurs as described below.

5.5.1.1 MTT Induction (Targeted Therapy Lead-In)

Patients who develop clinical progression or radiographic progression (e.g. if non-planned imaging is performed) during MTT induction, then patients will be allowed to move onto the concomitant MTT plus pembrolizumab phase of the therapy.

5.5.1.2 Concomitant MTT plus pembrolizumab (Combined Targeted Therapy Plus)

Patients who develop clinical progression during or radiographic during (e.g. if non-planned imaging is performed) or on routine imaging performed at the end of the concomitant MTT plus pembrolizumab phase will be allowed to be treated with single-agent pembrolizumab beyond progression as long as the following criteria are met:

A. Absence of signs and symptoms (including worsening of laboratory values) indicating disease progression.

B. No decline in ECOG performance status.

C. Absence of rapid progression of disease.

D. Absence of progressive tumor at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention.

5.5.1.3 Pembrolizumab single-agent

Patients who develop progression of disease on the pembrolizumab single-agent phase will be allowed to be treated beyond progression as long as the following criteria are met:

A. Absence of signs and symptoms (including worsening of laboratory values) indicating disease progression.

B. No decline in ECOG performance status.

C. Absence of rapid progression of disease.

D. Absence of progressive tumor at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention.

5.5.1.4 Follow up after discontinuation of pembrolizumab in setting of a CR

Discontinuation of treatment may be considered for subjects who have attained a confirmed CR that have been treated for at least 24 weeks with pembrolizumab and had at least two treatments with pembrolizumab beyond the date when the initial CR was declared. Subjects who discontinue treatment and then experience radiographic disease progression may be eligible for up to one year of additional treatment with single-agent pembrolizumab at the discretion of the investigator if no cancer treatment was administered since the last dose of pembrolizumab, the subject meets the safety parameters listed in the Inclusion/Exclusion criteria, and the trial is open. Subjects will resume therapy at the same dose and schedule at the time of initial discontinuation.

## 5.6 **Duration of Follow Up**

If treatment is discontinued for completion of two years of pembrolizumab, or for disease progression, or for withdrawal of consent, participants will be followed for adverse events. All adverse events encountered from time of the first dose of study treatment up until 90 days after the last dose of study treatment will be followed until one of the following occurs:

- The event resolves,
- The participating investigator assesses the event as stable,
- The participating investigator determines the event to be irreversible,
- The participant is lost to follow-up,
- The participant dies.

If the subject is not seen in clinic after the off-study visit, required Adverse Event details must be collected over the phone.

If treatment is discontinued for a reason other than disease progression, study discontinuation, or withdrawal of consent, participants will be seen every 12 weeks (+/- 2 weeks) for imaging until progressive disease or death, whichever comes first. If there are any adverse events that have not resolved, or been assessed by the participating investigator as stable or irreversible at the time of progressive disease, the participant will be followed until the adverse events resolve, or are assessed by the participating investigator as stable or irreversible.

## 5.7 Criteria for Taking a Participant Off Study

Participants will be removed from study when any of the criteria listed in Section 5.6 applies. The reason for study removal and the date the participant was removed must be documented in the study-specific case report form (CRF). Alternative care options will be discussed with the participant.

In the event of unusual or life-threatening complications, participating investigators must immediately notify the Principal Investigator, Ryan Sullivan at 617-724-4000.

## 5.8 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm. For this trial, male subjects will be considered to be of non-reproductive potential if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

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

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

OR

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

OR

(3) Has a congenital or acquired condition that prevents childbearing.
Female and male subjects of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving study drug and for 120 days after the last dose of study drug by complying with one of the following:

Female subjects of childbearing potential and male subjects with partners of childbearing potential must:

(1) Practice abstinence† from heterosexual activity;

OR

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

Acceptable methods of contraception are:

Single method (one of the following is acceptable):
• Intrauterine device (IUD)
• Vasectomy of a female subject's male partner
• Contraceptive rod implanted into the skin

Combination method (requires use of two of the following):
• Diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
• Cervical cap with spermicide (nulliparous women only)
• Contraceptive sponge (nulliparous women only)
• Male condom or female condom (cannot be used together)
• Hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

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

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

Subjects should be informed that taking the study medication may involve

unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study subjects of childbearing potential must adhere to the contraception requirement (described above) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 120 days after the last dose of trial therapy. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

## 6. DOSING DELAYS/DOSE MODIFICATION CRITERIA

Dose delays and modifications must be made using the following criteria. Toxicity assessments will be done using the CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) which is identified and located on the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

If possible, symptoms should be managed symptomatically. In the case of toxicity, appropriate medical treatment should be used (including anti-emetics, anti-diarrheals, etc.).

### 6.1 Pembrolizumab Dose Modifications

Pembrolizumab will be withheld for drug-related Grade 4 hematologic toxicities, non- hematological toxicity ≥ Grade 3 including clinically significant laboratory abnormalities despite appropriate replacement therapies when appropriate, and severe or life-threatening AEs as per Table 6.1.

**Table 6.1** Pembrolizumab dose modification requirements for drug-related adverse events

| Toxicity | Hold Treatment For Grade | Timing for Restarting Treatment | Discontinue Subject |
|---|---|---|---|
| Diarrhea/Colitis | 2-3 | Toxicity resolves to Grade 0-1. | Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks. |
| | 4 | Permanently discontinue | Permanently discontinue |
| AST, ALT, or Increased Bilirubin | 2 | Toxicity resolves to Grade 0-1 | Toxicity does not resolve within 12 weeks of last dose. |
| | 3-4 | Permanently discontinue (see exception below)[1] | Permanently discontinue |
| Type 1 Diabetes Mellitus (if new onset) or Hyperglycemia | T1DM or 3-4 | Hold pembrolizumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure. | Resume pembrolizumab when patients are clinically and metabolically stable. |
| Hypophysitis | 2-3 | Toxicity resolves to Grade 0-1 | Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of |

| Toxicity | Hold Treatment For Grade | Timing for Restarting Treatment | Discontinue Subject |
|---|---|---|---|
| | | | prednisone or equivalent per day within 12 weeks. |
| | 4 | Permanently discontinue | Permanently discontinue |
| Hyperthyroidism | 3 | Toxicity resolves to Grade 0-1 | Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks. |
| | 4 | Permanently discontinue | Permanently discontinue |
| Hypothyroidism | 2-4 | Therapy with pembrolizumab can be continued while treatment for the thyroid disorder is instituted | Therapy with pembrolizumab can be continued while treatment for the thyroid disorder is instituted. |
| Infusion Reaction | 3-4 | Permanently discontinue | Permanently discontinue |
| Pneumonitis | 2 | Toxicity resolves to Grade 0-1 | Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks. |
| | 3-4 | Permanently discontinue | Permanently discontinue |
| Renal Failure or Nephritis | 2 | Toxicity resolves to Grade 0-1 | Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks. |
| | 3-4 | Permanently discontinue | Permanently discontinue |
| Hematologic Toxicity | 4 | Toxicity resolves to Grade 0-1 or baseline | Toxicity does not resolve within 12 weeks of last infusion. *Permanent discontinuation should be considered for any severe or life-threatening event.* |
| All Other Drug-Related Toxicity[2,3] | 3 or Severe | Toxicity resolves to Grade 0-1 | Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks. |
| | 4 | Permanently discontinue | Permanently discontinue |

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

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

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

[3] Exception to be treated similar to grade 1 toxicity: Grade 2 alopecia, grade 2 fatigue

**6.1.1** Dosing interruptions are permitted in the case of medical / surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). In these cases only, subjects must be placed back on study therapy within 2 weeks of the scheduled interruption, unless otherwise discussed with the PI of the study. The reason for interruption must be documented in the patient's study record.

## 6.2 Dabrafenib + Trametinib Dose Modifications

The table below outlines the dose levels to be used for any necessary dabrafenib and trametinib dose modifications in studies which include the combination:

| Current Dose Dabrafenib | If Dose Reduction Required | Reduce To |
|---|---|---|
| 150mg BID | → | 150 mg QAM, 75 mg QPM |
| 150 mg QAM, 75 mg QPM | → | 75mg BID |
| 75mg BID | → | Discontinue dabrafenib |

| Current Dose Trametinib | If Dose Reduction Required | Reduce To |
|---|---|---|
| 2mg QD | → | 1.5mg QD |
| 1.5mg QD | → | 1.0mg QD |
| 1.0mg QD | → | Discontinue trametinib |

If an AE resolves to grade 1 or baseline at the reduced dose level, and no additional toxicities are seen after 4 weeks of study treatment at the reduced dose, the dose may be increased to the previous dose level. A dose reduction below 75 mg BID for dabrafenib and 1 mg once daily for trametinib is not allowed. If a dose reduction below 75 mg BID is required, dabrafenib will be permanently discontinued, but the patients will be allowed to continue trametinib. If a dose reduction below 1.0 mg once daily for trametinib is required, then trametinib will be permanently discontinued, but these patients will be allowed to continue pembrolizumab and/or dabrafenib.

The dose modifications may involve one or both agents, and should be based on the nature, severity and attributions of the AEs. General guidelines are provided below.

In the event that trametinib and pembrolizumab need to be discontinued due to toxicity reasons, treatment with dabrafenib may continue at the discretion of the investigator until time of disease progression.

### 6.2.1 Dabrafenib +/or Trametinib Dose Modification Requirements for drug-related adverse events **Not Specified** in Subsequent Sections

**Table 6-2: Dabrafenib +/or Trametinib Dose Modification Requirements for drug-related adverse events Not Specified in Subsequent Sections**

| CTCAE Grade | Action and Dose Modification |
|---|---|
| **Grade 1** | • Continue study treatment at same dose level (no dose modification).<br>• Monitor closely.<br>• Provide supportive care according to institutional standards. |
| **Grade 2 (Tolerable)** | • Interrupt study treatment if clinically indicated.<br>• Monitor closely.<br>• Provide supportive care according to institutional standards.<br>• When toxicity resolves to grade 1 or baseline, restart study treatment at current dose level. |
| **Grade 2 (Intolerable)**<br>**Grade 3** | • Interrupt study treatment.<br>• Monitor closely.<br>• Provide supportive care according to institutional standards.<br>• When toxicity resolves to grade 1 or baseline, restart study treatment **reduced by one dose level**.<br>• If the grade 3 toxicity recurs, interrupt study treatment.<br>• When toxicity resolves to grade 1 or baseline, **restart study treatment reduced by another dose level**. |
| **Grade 4** | • Interrupt study treatment.<br>• Monitor closely.<br>• Provide supportive care according to institutional standards.<br>• Restart with study treatment **reduced by one dose level** once toxicity resolves to grade 1 or baseline.<br>• If the grade 4 toxicity recurs, either **permanently discontinue study treatment** or, if the patient is clinically benefiting, discuss continuation of study treatment with the overall PI. |
| *If the AEs are thought to be due to one of the two agents, resumption of the other agents may be considered if the first agent is discontinued due to toxicities and treatment interruption is <21 days. Overall PI should be consulted for resumption of single agent.<br>** In the event of abdominal pain or suspected pancreatitis, amylase and lipase laboratory samples should be collected for confirmation of the diagnosis. | |

### 6.2.2 Dabrafenib + Trametinib Dose Modification Requirements for **Pyrexia**

- Pyrexia is defined as a body temperature equal to or above 38.5◦ Celsius or 101.3◦ Fahrenheit
- Pyrexia is an adverse event associated with dabrafenib. In a minority of cases, pyrexia was accompanied by symptoms such as severe chills/rigors, dehydration, hypotension, dizziness or weakness and required hospitalization.
- Subjects should be instructed on the importance of immediately reporting febrile episodes.
- Pyrexia accompanied by hypotension, dehydration, renal insufficiency and/or severe (grade ≥3) rigors/chills in the absence of an obvious infectious cause should be reported as an SAE.

## Table 6-2: Dabrafenib + Trametinib dose Modification and Management Requirements for Pyrexia

| Event | Management Guideline | Dose Modification |
|---|---|---|
| ***Work up:*** <br>• Clinical evaluation for infection and hypersensitivity, especially if pyrexia is complicated by rigors, severe chills, dehydration, *etc.* <br>• Laboratory work-up (recommend include full-blood-count, electrolytes, creatinine, BUN, CRP, liver-function tests, blood and urine culture). <br><br>***Management***: <br>• Anti-pyretic treatment should be started immediately at the first occurrence. Anti-pyretic treatment may include acetaminophen (paracetamol), ibuprofen, or suitable anti-pyretic medication per institutional standards. <br>• Oral hydration is encouraged in subjects without evidence of dehydration. Intravenous hydration is recommended if pyrexia is complicated by dehydration/hypotension. <br>• In subject experiencing pyrexia complicated by rigors, severe chills, *etc.*, which cannot be controlled with anti-pyretic medication, recommend oral corticosteroids be started. <br>• Prophylactic anti-pyretic treatment is recommended after the 2nd event. | | |
| 1st Event: | • Clinical evaluation for infection and hypersensitivity <br>• Laboratory work-up <br>• Hydration as required <br>• Administer anti-pyretic treatment if clinically indicated and continue prophylactic treatment | • Interrupt dabrafenib. <br>• Continue trametinib. <br>• Upon recovery to baseline, restart dabrafenib at the same dose level. If fever was associated with dehydration or hypotension, **reduce dabrafenib by one dose level**. |
| 2nd Event | • Clinical evaluation for infection and hypersensitivity <br>• Laboratory work-up <br>• Hydration as required <br>• Within 3 days of onset of pyrexia: <br>– Optimize anti-pyretic therapy. <br>– Consider oral corticosteroids (*i.e.*, prednisone 10 mg) for at least 5 days or as clinically indicated. | • **Interrupt dabrafenib.** <br>• Continue trametinib. <br>• Upon recovery to baseline, restart dabrafenib at the same dose level. If fever was associated with dehydration or hypotension, **reduce dabrafenib by one dose level**. |

**Table 6-2: Dabrafenib + Trametinib dose Modification and Management Requirements for Pyrexia**

| Event | Management Guideline | Dose Modification |
|---|---|---|
| Subsequent Events: | • Clinical evaluation for infection and hypersensitivity<br>• Laboratory work-up<br>• Hydration as required<br>• Blood sample for cytokine analysis<br>• Within 3 days of onset of pyrexia:<br>– Optimize oral corticosteroid dose as clinically indicated for recalcitrant pyrexia.<br>– If corticosteroids have been tapered and pyrexia recurs, restart steroids.<br>– If corticosteroids cannot be tapered, consult medical monitor. | • **Interrupt dabrafenib.**<br>• Continue trametinib.<br>• Once pyrexia resolves to baseline, restart dabrafenib **reduced by one dose level**.[h]<br>• If dabrafenib must be reduced to <75 mg BID, permanently **discontinue dabrafenib**. |
| [h] Dabrafenib should be reduced by one dose level after three episodes of pyrexia which cannot be managed by best supportive care and increasing doses of oral steroids. Escalation of dabrafenib is allowed if no episode of pyrexia is observed in the 4 weeks subsequent to dose reduction. | | |

### 6.2.3 Dabrafenib +/or Trametinib Dose Modification for Drug-Related **Rash**

Rash is a frequent AE observed in patients receiving trametinib, dabrafenib, or the combination of both therapies. Recommendations for supportive care and guidelines for dose modifications for rash are based on experience with other MEK inhibitors and EGFR inhibitors (Balagula *et al.*, 2010; Lacouture *et al.*, 2011).

The institutional standards for the management of skin-related AEs can differ from these guidelines. In this case, best clinical judgment should be applied and a consultation with the study overall PI may be required.

**Table 6-3: Dabrafenib +/or Trametinib Supportive care and Dose Modification Requirements for Drug-Related Rash**

| CTCAE Grade | Adverse Event Management | Action and Dose Modification |
|---|---|---|
| ***Recommended supportive care:***<br>• **Prevention:** Promote sunscreen use and avoid unnecessary sun exposure, use alcohol-free emollient creams, topical steroids and antibiotics as needed.<br>• Pruritic lesions: cool compresses and oral antihistamines.<br>• Fissuring lesions: Monsel's solution, silver nitrate or zinc oxide cream.<br>• Desquamation: thick emollients and mild soap.<br>• Paronychia: antiseptic bath, local potent corticosteroids, antibiotics, surgery as needed.<br>• Infected lesions: topical or systemic antibiotics. | | |
| **Grade 1**<br>• | • Initiate prophylactic and symptomatic treatment measures.[1]<br>• Use moderate strength topical steroid.[2]<br>• Reassess after 2 weeks. | • Continue study treatment.<br>• If rash does not recover to baseline within 2 weeks despite best supportive care, **reduce study treatment by one dose level**.[3] |
| **Grade 2**<br>• | • Initiate prophylactic and symptomatic treatment measures.[1]<br>• Use moderate strength topical steroid.[2]<br>• Reassess after 2 weeks. | • **Reduce study treatment by one dose level.**<br>• If rash recovers to ≤ grade 1 within 2 weeks, increase dose to previous dose level.<br>• If no recovery to ≤ grade 1 within 2 weeks, interrupt study treatment until recovery to ≤ grade 1.<br>• **Restart study treatment at reduced dose level.**[3] |
| **Grade ≥3**<br>• | • Use moderate strength topical steroids PLUS oral methyl-prednisolone dose pack.[2]<br>• Consult dermatologist. | • Interrupt study treatment until rash recovers to ≤ grade 1.<br>• **Restart with study treatment reduced by one dose level.**[3,4]<br>If no recovery to ≤ grade 2 within 21 days, **permanently discontinue study treatment**. |
| 1. Rash prophylaxis is recommended for the first 6 weeks of study treatment.<br>2. Moderate-strength topical steroids: Hydrocortisone 2.5% cream or fluticasone propionate 0.5% cream.<br>3. Approval of overall PI is required to restart study treatment after >21 days of interruption.<br>4. Study treatment may be escalated to previous dose level if no rash is evident 4 weeks after restarting study treatment. | | |

### 6.2.4 Dabrafenib +/or Trametinib Dose Modification for **Hand-Foot Skin Reaction (HFSR)**

**Table 6-4: Dabrafenib + /or Trametinib Dose Modification for HFSR**

| CTCAE Grade | Adverse Event Management | Action and Dose Modification |
|---|---|---|
| **Grade 1**[a] | • Lifestyle changes recommended.[b]<br>• Initiate symptomatic treatment if clinically appropriate[c] | Continue study treatment at current dose level. |
| **Grade 2** | • Lifestyle changes recommended.[b]<br>• Initiate symptomatic treatment.[c] | • Interrupt study treatment until recovery to ≤ grade 1.[d]<br>• Recovery to ≤ grade 1 within 7 days: restart study treatment at previous dose level.<br>• If no recovery to ≤ grade 1 within 7 days or ≥ 2nd occurrence: **restart with study treatment reduced by one dose level**. |
| **Grade ≥3** | • Lifestyle changes recommended.[b]<br>• Initiate symptomatic treatment.[c]<br>• Consult dermatologist. | • Interrupt study treatment until rash recovers to ≤ grade 1.[d]<br>• Restart with study treatment **reduced by one dose level.**[e]<br>• If 3rd occurrence, discontinue study treatment permanently. |

[a] A full-body skin examination and removal of pre-existing calluses and keratotic skin is recommended prior to initiation of study treatment.
[b] Life-style changes: (1) reduce exposure of hands and feet to hot water; (2) avoid traumatic activity including vigorous exercise especially in the first 4 weeks after start of study treatment; (3) avoid constrictive footwear; (4) avoid excessive friction on the skin when applying topical treatments; (5) wear thick cotton socks and gloves, and shoes with padded insoles.
[c] Symptomatic treatments: (1) use moisturizing creams frequently and especially on hands and feet; (2) consider topical keratolytics: urea 20-40% cream, or salicylic acid 6%, or tazarotene 0.1% cream, or fluorouracil 5% cream; (3) erythematous areas: clobetasol propionate 0.05% ointment; (4) pain: topical lidocaine 2%, and/or systemic pain medication such as nonsteroidal anti-inflammatory drugs, codeine, and pregabalin.
[d] Approval of overall PI is required to restart study treatment after ≥21 days of interruption.
[e] escalation of study treatment to the previous dose level is allowed if no HFSR is observed in the 4 weeks subsequent to dose reduction.

### 6.2.5 Dabrafenib + Trametinib Dose Modification for **Squamous Cell Carcinoma**

Cutaneous squamous cell carcinoma (SCC) has been observed in patients treated with dabrafenib and the combination of dabrafenib and trametinib. These treatment-related SCC should be surgically removed according to institutional practice; dose modifications or interruptions of the study treatment are not required. Occurrence of SCC must be reported as an SAE.

### 6.2.6 Dabrafenib +/or Trametinib Dose Modification for **Renal Insufficiency**

Cases of renal insufficiency have occurred in patients receiving the combination of dabrafenib and trametinib. Prior to start of study treatment, concomitant medications should be reviewed for the potential risk of inducing nephrotoxicity and, if so, should be modified if clinically possible.

**Table 6-5: Dabrafenib +/or Trametinib Treatment Modification Requirements for Renal Insufficiency**

| Serum Creatinine Level | Management Guideline | Action and Dose Modification |
|---|---|---|
| Serum creatinine increase >0.2 mg/dL (18 mcmol/L)<br>***BUT***<br>≤0.5 mg/dL (44 mcmol/L) above baseline | • Recheck serum creatinine within 1 week.<br>• Serum creatinine increase >1 week: contact Overall PI.<br>• If pyrexia is present, treat pyrexia as per guidelines.[a] | Continue study treatment at the same dose level. |
| Serum creatinine increase >0.5 mg/dL (44 mcmol/L)<br>***OR***<br>>2 mg/dL (>177 mcmol/L) | • Monitor serum creatinine ≥2-times per week.<br>• Hospitalization may be necessary if serum creatinine cannot be monitored frequently.<br>• If pyrexia is present, treat pyrexia per guidelines.<br>• Consult nephrologist if clinically indicated.<br>• Perform renal biopsy if clinically indicated, for example:<br>– Renal insufficiency persists despite volume repletion.<br>– Patient has new rash or signs of hypersensitivity (such as elevated eosinophil count). | • Interrupt study treatment until serum creatinine recovers to baseline.<br>• Restart study treatment.[b] |

[a] NSAIDs can induce renal insufficiency, especially in patients with dehydration; encourage oral fluids or consider IV fluids as clinically indicated.
[b] Investigator may restart at either the same or a reduced dose level. Escalation of study treatment to previous dose level is allowed if another episode of renal insufficiency does not occur after 4 weeks of dose reduction. Consultation with the overall PI is required before restarting study treatment if there is evidence of thrombotic microangiopathy.

### 6.2.7 Dabrafenib +/or Trametinib Dose Modification for **Reduced Left Ventricular Ejection Fraction**

Decreases of the left ventricular ejection fraction (LVEF) have been observed in patients receiving trametinib. Therefore, ECHOs must be performed at intervals outlined in the Study Calendar. The same procedure (either ECHO or MUGA, although ECHO is preferred) should be performed at baseline and at Week 9.

**Table 6-6: Dabrafenib +/or Trametinib Treatment Modification and Management Requirements for LVEF Decrease**

| Clinic | LVEF-drop (%) or CTCAE grade | Dose Modification |
|---|---|---|
| **Asymptomatic** | Absolute decrease of >10% in LVEF compared to baseline and ejection fraction below the institution's LLN. | • Interrupt study treatment and repeat ECHO within 2 weeks.[a]<br>• If the LVEF **recovers** within 4 weeks (defined as LVEF ≥LLN and absolute decrease ≤10% compared to baseline):<br>– Consult with the overall PI and request approval for restart.<br>– Restart trametinib and dabrafenib (if cohort 1) at reduced doses by one dose level.<br>– Repeat ECHO 2, 4, 8, and 12 weeks after re-start; continue in intervals of 12 weeks thereafter.<br>• If LVEF **does not recover** within 4 weeks:<br>– Consult with cardiologist.<br>– **Permanently discontinue trametinib**.<br>– Resumption of dabrafenib (if cohort 1) may be considered after consultation with the overall PI.<br>– Repeat ECHO after 2, 4, 8, 12, and 16 weeks or until resolution. |
| **Symptomatic[b]** | • Grade 3: resting LVEF 39-20% or >20% absolute reduction from baseline<br>• Grade 4: Resting LVEF ≤20%. | • **Permanently discontinue trametinib. Consult overall PI for continuation of dabrafenib (if cohort 1). Consult with cardiologist.**<br>• Repeat ECHO after 2, 4, 8, 12, and 16 weeks or until resolution. |
| [a] If ECHO does not show LVEF recovery after 2 weeks, repeat ECHO 2 weeks later.<br>[b] Symptoms may include: dyspnea, orthopenea, and other signs and symptoms of pulmonary congestion and edema. | | |

### 6.2.8 Dabrafenib +/or Trametinib Dose Modification for **Hypertension**

Increases in blood pressure (BP) have been observed in patients receiving trametinib. Recommendations for BP monitoring and

management are provided below.

*Monitoring:* All BP assessments are to be performed as follows:

- In subjects with an initial BP reading within the hypertensive range, a second reading should be taken at least 1 minute later, with the two readings averaged to obtain a final BP measurement. The averaged value should be recorded in the eCRF.
- Visits to monitor increased blood pressure can be scheduled independently from the per-protocol visits outlined in the study calendar. Ideally, subsequent blood pressure assessments should be performed within 1 week.
- Persistent hypertension is defined as an increase of systolic BP (SBP) >140 mmHg and/or diastolic BP (DBP) >90 mmHg in three consecutive visits with blood pressure assessments from two readings.
- Asymptomatic hypertension is defined as an increase of SBP >140 mmHg and/or diastolic BP (DBP) >90 mmHg in the absence of headache, light-headedness, vertigo, tinnitus, episodes of fainting, or other symptoms indicative of hypertension.

**Table 6-7: Dabrafenib +/or Trametinib Treatment Modification and Management Requirements for Hypertension**

| Event | Management Guideline | Dose Modification |
|---|---|---|
| **(Scenario A)**<br>• Asymptomatic and persistent[a] SBP of ≥140 and <160 mmHg, or DBP ≥90 and <100 mmHg<br>OR<br>• linically significant increase in DBP of 20 mmHg (but still below 100 mmHg) | • Adjust current or initiate new antihypertensive medication(s).<br>• Titrate antihypertensive medication(s) during the next 2 weeks to achieve well-controlled BP. If BP is not well-controlled within 2 weeks, consider referral to a specialist and go to scenario (B). | • Continue protocol therapy.<br>• If BP is not well-controlled within 2 weeks, consider referral to a specialist and go to scenario (B). |
| **(Scenario B)**<br>• Asymptomatic SBP ≥160 mmHg, or DBP ≥100 mmHg,<br>OR<br>• Failure to achieve well-controlled BP within 2 weeks in Scenario A. | • Adjust current or initiate new antihypertensive medication(s).<br>• Titrate antihypertensive medication(s) during the next 2 weeks to achieve well-controlled BP. | • Interrupt trametinib if clinically indicated.<br>• Once BP is well-controlled, restart trametinib **reduced by one dose level**.<br>• Dabrafenib (if cohort 1) may be continued |
| **(Scenario C)**<br>• Symptomatic hypertension<br>OR<br>• Persistent SBP ≥160 mmHg, or DBP ≥100 mmHg, despite antihypertensive medication and dose reduction of study treatment | • Adjust current or initiate new antihypertensive medication(s).<br>• Titrate antihypertensive medication(s) during the next 2 weeks to achieve well-controlled BP.<br>• Referral to a specialist for further evaluation and follow-up is recommended. | • Interrupt trametinib.<br>• Once BP is well controlled, restart trametinib **reduced by one dose level**.<br>• Dabrafenib (if cohort 1) may be continued. |
| **(Scenario D)**<br>Refractory hypertension unresponsive to above interventions or hypertensive crisis. | Continue follow-up per protocol. | • Discontinue trametinib.<br>• Hold dabrafenib until BP is controlled. If the treatment delay is >21 days, overall PI should be consulted for resumption of dabrafenib. |

### 6.2.9 Dabrafenib +/or Trametinib Dose Modification Requirements for **QTc Prolongation**

**Table 6-8: Dabrafenib +/or Trametinib Withholding and Stopping Criteria for QTc Prolongation**

| Prolongation | Action and Dose Modification |
|---|---|
| • QTcB ≥501 msec, or<br>• Uncorrected QT >600 msec, or<br>• QTcB >530 msec for subjects with bundle branch block | • Interrupt dabrafenib and trametinib (cohort 1) or trametinib (cohort 2) until QTcB prolongation resolves to grade 1 or baseline.<br>• If the event resolves, restart dabrafenib and trametinib (cohort 1) or trametinib (cohort 2) treatment at current dose level.<br>• **If the event does not resolve, permanently discontinue dabrafenib and trametinib treatment.**<br>• **If the event recurs, permanently discontinue study treatment.** |
| Abbreviations: msec = milliseconds; QTcB = QT interval on electrocardiogram corrected using the Bazett's formula | |

### 6.2.10 Dabrafenib +/or Trametinib Dose Modification for **Diarrhea**

Episodes of diarrhea have been observed in patients receiving dabrafenib, trametinib, or both therapies in combination. Other, frequent causes for diarrhea including concomitant medications (*e.g.*, stool softeners, laxatives, antacids, *etc.*), infections caused by *C. difficile* or other pathogens, partial bowel obstruction, *etc.*, should be clinically excluded.

**Table 6-9: Dabrafenib +/or Trametinib Treatment Modification and Management Requirements for Diarrhea**

| CTCAE Grade | Management | Action and Dose Modification |
|---|---|---|

**Table 6-9: Dabrafenib +/or Trametinib Treatment Modification and Management Requirements for Diarrhea**

| CTCAE Grade | Management | Action and Dose Modification |
|---|---|---|
| **Uncomplicated Diarrhea,[1] Grade 1 or 2** | • Diet: Stop all lactose containing products; eat small meals, BRAT-diet (banana, rice, apples, toast) recommended.<br>• Hydration: 8-10 large glasses of clear liquids per day (*e.g.*, Gatorade or broth).<br>• Loperamide[3]: Initially 4 mg, followed by 2 mg every 4 hours or after every unformed stool; maximum 16 mg/day. Continue until diarrhea-free for 12 hours.<br>• Diarrhea >24 hours: Loperamide 2 mg every 2 hours; maximum 16 mg/day. Consider adding oral antibiotics.<br>• Diarrhea >48 hous: Loperamide 2 mg every 2 hours; maximum 16 mg/day. Add budesonide or other second-line therapies (otreotide, or tincture of opium) and oral antibiotics. | • Continue study treatment.<br>• If diarrhea is grade 2 for > 48 hours, interrupt study treatment until diarrhea resolves to grade ≤1.<br>• Restart study treatment at the same dose level. |
| **Uncomplicated Diarrhea,[1] Grade 3 or 4**<br><br>**Any Complicated Diarrhea[2]** | • Clinical evaluation mandatory.<br>• Loperamide[3]: Initially 4 mg, followed by 2 mg every 4 hours or after every unformed stool; maximum 16 mg/day. Continue until diarrhea-free for 12 hours.<br>• Oral antibiotics and second-line therapies if clinically indicated<br>• Hydration: Intravenous fluids if clinically indicated.<br>• Antibiotics (oral or intravenous) if clinically indicated.<br>• Intervention should be continued until the subject is diarrhea-free for ≥24 hours.<br>• Intervention may require hospitalization for subjects at risk of life-threatening complications. | • Interrupt study treatment until diarrhea resolves to ≤ grade 1.<br>• Restart with study treatment reduced by one dose level.[4]<br>• If 3 dose reductions of study treatment are clinically indicated, **permanently discontinue study treatment**. |

1. **Uncomplicated diarrhea** defined by the absence of symptoms such as cramping, nausea/vomiting, ≥ grade 2, decreased performance status, pyrexia, sepsis, neutropenia ≥ grade 3, frank bleeding, and/or dehydration requiring intravenous fluid substitution.
2. **Complicated diarrhea** defined by the presence of symptoms such as cramping, nausea/vomiting, ≥ grade 2, decreased performance status, pyrexia, sepsis, neutropenia ≥ grade 3, frank bleeding, and/or dehydration requiring intravenous fluid substitution.
3. Loperamide should be made available prior to start of study treatment so loperamide administration can begin at the first signs of diarrhea.
4. Escalation of study treatment to previous dose level is allowed after consultation with the medical monitor and in the absence of another episode of complicated or severe diarrhea in the 4 weeks subsequent to dose reduction.

### 6.2.11 Dabrafenib +/or Trametinib Dose Modification for **Vision Changes**

Episodes of vision changes have been observed in patients

receiving dabrafenib, trametinib, or the combination of both therapies and can be caused by central serous retinopathy (CSR) with retinal pigment epithelial detachments (RPED), or Retinal Vein Occlusion (RVO). Patients are required to have a standard ophthalmic exam performed by an ophthalmologist at baseline, within 1 week prior to doing on week 9, and any time patients report visual disturbance. For Grade 2 visual changes or greater, this exam must occur within 24 hours of onset (or study team knowledge of the event). The exam will include indirect fundoscopic examination, visual acuity (corrected), visual field examination, tonometry, and direct fundoscopy. Special attention should be given to retinal (*e.g.*, CSR, RPED) or retinal vein abnormalities (*e.g.*, RVO).

Guidelines regarding event management and dose reduction for visual changes considered to be related to study treatment are provided in the table below.

**Table 6-10: Dabrafenib +/or Trametinib Treatment Modification Requirement for Vision Changes**

| CTCAE Grade | Management | Action and Dose Modification |
|---|---|---|
| **Grade 1**<br>Asymptomatic or symptomatic but not limiting ADL; intervention not indicated. | • Consult ophthalmologist any time when patient reports visual disturbance. **If there is visual loss or significant visual changes, consult ophthalmologist immediately (within 24 hours of study team awareness)**<br>• Workup to rule out CSR, RPED or RVO. Consult retinal specialist in case of CSR or RVO.<br>• **Report CSR and RVO as SAE.**<br>• Continue follow up examination(s) (by retinal specialist if available) for CSR, RPED and RVO. | • Continue study treatment at the same dose level until ophthalmologic examination can be conducted.*<br>• If ophthalmologic examination cannot be performed within 7 days of onset, interrupt study treatment until CSR and RVO can be excluded and symptoms resolve.<br>• If CSR and RVO excluded, restart study treatment at same dose level.<br>• If CSR: Interrupt study treatment until symptoms resolve and exam by retinal specialist shows resolution. May restart study treatment **with one dose level reduction of trametinib.**<br>• If RVO: **Permanently discontinue trametinib.** |

**Table 6-10: Dabrafenib +/or Trametinib Treatment Modification Requirement for Vision Changes**

| CTCAE Grade | Management | Action and Dose Modification |
|---|---|---|
| **Grade 2 and Grade 3**<br>Grade 2 defined as: Symptomatic with moderate decrease in visual acuity (20/40 or better); limiting instrumental ADL; local or non-invasive intervention indicated.<br><br>Grade 3 defined as: Symptomatic with marked decrease in visual acuity or marked visual field defect (worse than 20/40 but better than 20/200); severe pain or medically significant; operative intervention indicated. | • Consult ophthalmologist immediately (within 24 hours of study team awareness).<br>• Workup to rule out CSR, RPED or RVO. Consult retinal specialist in case of CSR or RVO.<br>• **Report CSR and RVO as SAE.**<br>• Continue follow up examination(s) (by retinal specialist if available) for CSR, RPED and RVO. | • Interrupt study treatment until signs and symptoms have resolved to baseline.<br>• If CSR and RVO excluded and symptoms resolved to baseline, restart study treatment reduced by one dose level.<br>• If CSR: Interrupt study treatment until symptoms resolve and exam by retinal specialist shows resolution. If CSR resolves restart study treatment **with trametinib dose reduced by one dose level**.<br>• If RVO: **Permanently discontinue** trametinib. |
| **Grade 4**<br>Sight-threatening consequences; urgent intervention indicated; blindness (20/200 or worse). | • Consult ophthalmologist immediately (within 24 hours of study team awareness).<br>• Workup to rule out CSR, RPED or RVO. Consult retinal specialist in case of CSR or RVO.<br>• **Report CSR and RVO as SAE.**<br>• Continue follow up examination(s) (by retinal specialist if available) for CSR, RPED and RVO. | **Permanently discontinue study treatment.** |
| Abbreviations: CSR = central serous retinopathy; RVO = retinal vein occlusion; SAE = serious adverse event<br>* If visual changes are clearly unrelated to study treatment (*e.g.*, allergic conjunctivitis), monitor closely but ophthalmic examination is not required.<br>**If ocular toxicities do not resolve within 21 days, permanently discontinue trametinib. | | |

### 6.2.12 Dabrafenib +/or Trametinib Dose Modification for **Pneumonitis**

Pneumonitis has been observed in patients receiving trametinib. To reduce the risk of pneumonitis, patients will be monitored closely for symptoms, evaluated with imaging and functional tests when appropriate.

**Table 6-11: Dabrafenib + Trametinib Treatment Modification Requirements for Pneumonitis**

| CTCAE Grade | Management | Action and Dose Modification |
|---|---|---|
| **Grade 1** | • CT scan (high-resolution with lung windows) recommended.<br>• Clinical evaluation and laboratory work-up for infection.<br>• Monitoring of oxygenation via pulse-oximetry recommended.<br>• Consultation with pulmonologist recommended. | Continue study treatment at current dose. |
| **Grade 2** | • CT scan (high-resolution with lung windows).<br>• Clinical evaluation and laboratory work-up for infection.<br>• Consult pulmonologist.<br>• Pulmonary function tests: If < normal, repeat every 8 weeks until ≥ normal.<br>• Symptomatic therapy including corticosteroids if clinically indicated. | • Interrupt study treatment until recovery to grade ≤1.<br>• Restart with study treatment **reduced by one dose level**.<br>• Escalation to previous dose level after 4 weeks and consultation with the overall PI may be considered.<br>• If no recovery to grade ≤1 within 4 weeks, **permanently discontinue study treatment**. |
| **Grade 3** | • CT scan (high-resolution with lung windows).<br>• Clinical evaluation and laboratory work-up for infection.<br>• Consult pulmonologist.<br>• Pulmonary function tests: If < normal, repeat every 8 weeks until ≥ normal.<br>• Bronchoscopy with biopsy and/or BAL if possible.<br>• Symptomatic therapy including corticosteroids as clinically indicated. | • Interrupt study treatment until recovery to grade ≤1.<br>• After consultation with the overall PI, study treatment may be restarted **reduced by one dose level**.<br>• If no recovery to grade ≤1 within 4 weeks, **permanently discontinue study treatment**. |
| **Grade 4** | Same as Grade 3. | **Permanently discontinue study treatment.** |

## 6.2.13 Dabrafenib +/or Trametinib Dose Modification for **Liver Chemistry Changes**

**Table 6-12: Dabrafenib +/or Trametinib Dose Modification Requirements for Liver Chemistry Changes**

| Stopping Criteria and Action |
|---|

## Table 6-12: Dabrafenib +/or Trametinib Dose Modification Requirements for Liver Chemistry Changes

| Stopping Criteria and Action |
|---|
| **Liver chemistry stopping criteria are defined as follows.** When any of the liver chemistry stopping criteria are met, **immediately discontinue study treatment**, perform liver event follow-up assessments, and monitor the patient until liver chemistries resolve, stabilize, or return to baseline values.<br>• ALT ≥3x ULN **and** bilirubin ≥2x ULN (>35% direct bilirubin) (or ALT ≥3x ULN and international normalized ratio [INR] >1.5, if INR measured). **NOTE:** If serum bilirubin fractionation is not immediately available, study treatment should be discontinued if ALT ≥3x ULN **and** bilirubin ≥2x ULN. Serum bilirubin fractionation should be performed if testing is available. If testing is unavailable, **record presence of detectable urinary bilirubin on dipstick**, indicating direct bilirubin elevations and suggesting liver injury.<br>• ALT ≥8x ULN.<br>• ALT ≥5x ULN but <8x ULN persists for 2 weeks.<br>• ALT ≥3x ULN if associated with the appearance or worsening of symptoms of hepatitis or hypersensitivity such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, or eosinophilia.<br>• ALT ≥5x ULN but <8x ULN and cannot be monitored weekly for >2 weeks. |

# 7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

An adverse event is any untoward medical occurrence associated with the use of the study drug(s), whether or not considered drug related. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the study drug(s).

## 7.1 Expedited Adverse Event Reporting to the Overall PI and Merck

Investigators **must** report to the DF/HCC Overall Principal Investigator (PI) any serious adverse event (SAE) that occurs from the time of the first dose of study treatment up until 90 days after the last dose of study treatment. The report should be submitted on the MedWatch 3500A form.

A serious adverse event is any adverse event that is any of the following:

- Fatal (i.e., the adverse event actually causes or leads to death)
- Life threatening (i.e., the adverse event, in the view of the investigator, places the patient at immediate risk of death)
- Requires or prolongs inpatient hospitalization
- Results in persistent or significant disability/incapacity (i.e., the adverse event results in substantial disruption of the patient's ability to conduct normal life functions)
- A congenital anomaly/birth defect in a neonate/infant born to a mother exposed to the investigational product
- Considered a significant medical event by the investigator (e.g., may jeopardize the patient or may require medical/surgical intervention to prevent one of the outcomes listed above)
- Pyrexia accompanied by hypotension, dehydration, renal insufficiency and/or severe (grade $\geq$3) rigors/chills in the absence of an obvious infectious cause should be reported as an SAE.
- Occurrence of Cutaneous squamous cell carcinoma (SCC) must be reported as an SAE.
- Central serous retinopathy (CSR) and Retinal Vein Occlusion (RVO) must be reported as an SAE.

All adverse events that do not meet any of the criteria for serious should be regarded as **nonserious adverse events**

Investigators must report each serious adverse event to the Overall PI within 24 hours of learning of the occurrence. In the event that the participating investigator does not become aware of the serious adverse event immediately (e.g.,

participant sought treatment elsewhere), the participating investigator is to report the event within 24 hours after learning of it and document the time of his or her first awareness of the adverse event. Report serious adverse events by telephone, email or facsimile using the MedWatch 3500A form to:

Ryan J. Sullivan, MD
Phone: 617-724-4000
Email: rsullivan7@mgh.harvard.edu
Fax: 617-726-1949

The participating investigator must provide follow-up information on the serious adverse event. Follow-up information should describe whether the event has resolved or continues, if and how the event was treated, and whether the participant will continue or discontinue study participation.

In addition, the participating investigator will be responsible for reporting these serious adverse events to Merck via the MedWatch 3500A form.

The contact for Merck is listed below:

Alicia M. Schneider
Scientific Leadership & Research Manager - Oncology
Merck Investigator Studies Program/Scientific Engagements
Global Center for Scientific Affairs| Merck Research Laboratories
Phone: 267-305-0191
Email: Alicia.Schneider@merck.com
Fax: 267-305-6534

## 7.2 Expedited Reporting to the Food and Drug Administration (FDA)

The Overall PI, as study sponsor, will be responsible for all communications with the FDA. The Overall PI will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

The Overall PI must report to the FDA any suspected adverse reaction to study treatment (i.e., including active comparators) that is both serious and unexpected.

## 7.3 **Expedited Reporting to Hospital Risk Management**

Participating investigators will report to their local Risk Management office any participant safety reports or sentinel events that require reporting according to institutional policy.

### 7.4 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must also be reported in routine study data submissions.**

### 7.5 Monitoring of Adverse Events and Period of Observation

All adverse events, both serious and non-serious, and deaths that are experienced by participants will be collected from the time of the first dose of study treatment up until 90 days after the last dose study treatment, or until death if death occurs first. The presence and resolution of AEs and SAEs (with dates) should be documented on the appropriate case report form and recorded in the participant's medical record to facilitate source data verification.

For some SAEs, the Overall PI or designee may follow-up by telephone, fax, and/or monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

## 8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the agents administered in this study can be found in the investigative drug brochure.

### 8.1 Pembrolizumab

#### 8.1.1 Description

Pembrolizumab (MK-3475) is a humanized anti-PD-1 mAb of the IgG4/kappa isotype with a stabilizing S228P sequence alteration in the fragment crystallizable (Fc) region. MK-3475 binds to human PD-1 and blocks the interaction between PD-1 and its ligands. The theoretical molecular weight of the polypeptide is 146,288 Da and its theoretical pI is 7.5. The parental murine anti-human PD-1 antibody (hPD-1.09A) was produced by immunizing mice with hPD-1 DNA. The MK-3475 antibody was generated by humanization of the parental antibody by the Medical Research Council (Cambridge, UK) using complementarily-determining region (CDR) grafting technology (U.S. Patent No. 5,225,539). The gene segments encoding the variable heavy and light chains of MK-3475, as well as human IgG4, were codon-optimized, synthesized, and ligated into a vector.

A single expression plasmid, pAPD11V1-GA was constructed for the expression of both the heavy and light antibody chains of MK-3475. The nucleotide sequences encoding the heavy and light chains, along with their respective

promoters and poly A signal sequences have been confirmed by DNA sequence analysis. The pAPD11V1-GA expression vector was subsequently used to transfect CHO-DXB-11 cells for the development of the MK-3475-producing cell line.

The theoretical molecular weight of the polypeptide is 146,288 Da and its theoretical pI is 7.5. Pembrolizumab exhibits linear pharmacokinetics at dose levels of clinical relevance (1-10 mg/kg). It exhibits low clearance and limited volume of distribution that is typical for therapeutic antibodies. Mean estimated t1/2 values are 14.1-21.6 days.

The nomenclature of pembrolizumab is provided in the table below:

The nomenclature of pembrolizumab is provided in the table below:

| | |
|---|---|
| Code Name | MK 3475 (Anti-PD-1) |
| Other Code Name | SCH 900475 (Anti-PD-1) |
| Chemical Name | Humanized X PD-1_mAb (H409A11) IgG4 |
| CAS Number | 1374853-91-4 |
| CAS Name | Anti-(human protein PDCD1 (programmed cell death 1)) immunoglobulin G4 (human-Mus musculus monoclonal heavy chain) disulfide with human-Mus musculus monoclonal light chain, dimer |
| Generic Name | Not available |
| Commercial Name | Pembrolizumab |

### 8.1.2 **Form**

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

Pembrolizumab is supplied as a clear to opalescent solution that is essentially free of extraneous particles and may contain proteinaceous particulates. One dosage form of pembrolizumab will be provided by Merck in Type I glass vials intended for single use only as summarized in the following table:

| **Product Name & Potency** | **Dosage Form** |
|---|---|
| Pembrolizumab 100 mg/ 4mL | Solution for Injection |

Pembrolizumab solution for infusion is a sterile, non-pyrogenic, aqueous, preservative-free solution. Pembrolizumab solution for infusion contains an excess fill of 6.25 mg (equivalent to 0.25 mL solution) to ensure the recovery of label claim of 100 mg pembrolizumab per vial (equivalent to

4.0 mL of solution).

### 8.1.3 **Storage and Stability**

As specified in the Pharmacy Manual for pembrolizumab as provided by the Overall PI.

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label.

### 8.1.4 **Compatibility**

The following infusion set materials are compatible with Pembrolizumab

- PVC infusion set that is plasticized using Di-2-ethylhexyl Terephthalate DEHP
- PVC and tri-(2-ethylhexyl) trimellitate (TOTM) infusion set
- Polyethylene lined PVC infusion set
- Polyrethane
- Plybutadiene A sterile, non-pyrogenic, low-protein binding 0.2 to 5 µm in-line filter made of polyethersulfone (PES) or polysulfone must be used during administration to remove any adventitious particles. If the infusion set does not contain a 0.2 to 5 µm in-line filter, it is recommended to use a 0.2 to 5 µm add-on filter which may contain an extension line (the materials of the extension line and filter should be as mentioned above).

A sterile, non-pyrogenic, low-protein binding 0.2 to 5 µm in-line filter made of polyethersulfone (PES) or polysulfone must be used during administration to remove any adventitious particles. If the infusion set does not contain a 0.2 to 5 µm in-line filter, it is recommended to use a 0.2 to 5 µm add-on filter which may contain an extension line (the materials of the extension line and filter should be as mentioned above).

### 8.1.5 **Handling**

Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site.

Clinical supplies may not be used for any purpose other than that stated in the protocol.

### 8.1.6 **Availability**

Pembrolizumab will be supplied free-of-charge from Merck.

### 8.1.7 **Administration**

Pembrolizumab will be administered as a 30 minute IV infusion using an infusion pump (treatment cycle intervals may be increased due to toxicity as described in Section 6). Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min). Attach the infusion line to the pump and prime the line, either with normal saline (at least 25 mL) or with infusion solution as per local SOP, before starting the infusion. Maximum infusion rate should not exceed 6.7 ml/min through a peripheral or indwelling catheter. Use 30 mL normal saline to flush the infusion line at the end of the infusion if institutional guidelines allow.

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

DO NOT administer the product as an intravenous push or bolus.

DO NOT combine, dilute or administer it as an infusion with other medicinal products.

A central line is not required for Pembrolizumab administration, but may be used if available.

### 8.1.8 **Ordering**

Participating Institutions will order their own investigational agent (Pembrolizumab) directly from Merck using the Drug Supply Request Form. Please allow for 3 weeks for drug to arrive after the order is submitted. The Participating Institution will ensure that the pharmacy will be able to receive and store the agent according to state and federal guidelines. The local IRB should be kept informed of who will supply the agent (i.e., Merck pharmaceuticals Inc.) so that any regulatory responsibilities can be met in a timely fashion.

### 8.1.9 **Accountability**

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

8.1.10 **Destruction and Return**

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

8.2 **Dabrafenib mesylate (GSK2118436B) (NSC 763760)**

8.2.1 **Chemical Name:** N-{3-[5-(2-Amino-4-pyrimidinyl)-2-(1,1-dimethylethyl)-1,3-thiazol-4-yl]-2-fluorophenyl}-2,6-difluorobenzene sulfonamide, methanesulfonate salt

8.2.2 **Other Names:** GSK2118436, GSK2118436A (free base), Tafinlar

8.2.3 **Classification:** BRAF inhibitor

8.2.4 CAS Registry Number: 1195768-06-9

8.2.5 **Molecular Formula:** $C_{23}H_{20}F_3N_5O_2S_2 \bullet CH_4O_3S$

8.2.6 **M.W.:** 615.68 (mesylate salt)

8.2.7 **Mode of Action:** Dabrafenib mesylate (GSK2118436B) is a potent and selective BRAF kinase inhibitor. This inhibition suppresses downstream activity of pERK, a biomarker, and has antiproliferative activity against BRAF mutant tumors. The mode of action is consistent with ATP-competitive inhibition.

8.2.8 **How Supplied:** Dabrafenib mesylate (GSK2118436B) capsules will be supplied commercially.

- 50 mg capsule is Swedish orange, size 2 with markings of four black bars.
- 75 mg capsule is pink, size 1 with markings of four black bars.

Capsule excipients include microcrystalline cellulose, magnesium stearate (vegetable source), colloidal silicon dioxide. Shell composition consists of an opaque hypromellose capsule, composed of red iron oxide (E172), titanium dioxide (E171), and hypromellose (E464). Four black bars are printed on the hypromellose capsules using black ink. The black ink contains black iron oxide (E172), shellac, propylene glycol, and ammonium hydroxide.

8.2.9 **Storage:** Store between 15°C to 30°C (59°F to 86°F).

8.2.10 **Stability:** Shelf-life studies of dabrafenib mesylate (GSK2118436B) are ongoing.

8.2.11 **Route of Administration:** Oral administration. Take dabrafenib mesylate (GSK2118436B ) 1 hour prior or 2 hours after a meal. If a dose is missed, it should not be taken if it is less than 6 hours until the next dose.

8.2.12 **Potential Drug Interactions:** *In vitro* studies show that dabrafenib mesylate (GSK2118436B) induces CYP3A4, 2B6, 2C9,8, 2C9, and 2C19 enzymes. Use caution in patients who are taking substrates that are metabolized in these enzyme pathways.

Dabrafenib mesylate (GSK2118436B) metabolism appears to be mediated by CYP3A4 and CYP2C8. Use caution if strong inducers or inhibitors of CYP2C8 or 3A4 are co-administered with dabrafenib.

Patients should avoid ingesting the fruit or juice of Seville oranges, grapefruit, pummelos, exotic citrus fruits, or grapefruit hybrids for at least 24 hours prior to the start of dosing because of inhibition of intestinal CYP3A4.

Dabrafenib solubility is pH-dependent and experiences decreased solubility at higher pH. Use caution in patients who are taking drugs that elevate gastric pH due to the theoretical risk of decreasing oral bioavailability of dabrafenib.

8.2.13 **Availability**

Dabrafenib mesylate (GSK2118436B) is FDA-approved for the treatment of unresectable or metastatic BRAF-mutant melanoma. It will be used at its standard dose and schedule for its indicated purpose. Commercial dabrafenib will be used throughout the study and obtained from specialty pharmacies and with coverage provided by participant's individual health insurance.

## 8.3 Trametinib dimethyl sulfoxide (GSK1120212B) (NSC 763093)

8.3.1 **Chemical Name (IUPAC):** equimolecular combination of N-(3-{3-cyclopropyl-5-[(2-fluoro-4-iodophenyl)amino]-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydropyrido[4,3-d]pyrimidin-1(2H)-yl}phenyl)acetamide with (methylsulfinyl)methane

8.3.2 **Other Names:** trametinib, GSK1120212, JTP-74057, JTP-78296, JTP-75303, Mekinist

8.3.3 **CAS Registry Number:** 1187431-43-1

8.3.4 **Classification:** MEK inhibitor

8.3.5 **Molecular Formula:** $C_{26}H_{23}FIN_5O_4 \cdot C_2H_6OS$

8.3.6 **M.W.:** 693.54 (dimethyl sulfoxide solvate), 615.41 (anhydrous parent)

8.3.7 **Approximate Solubility:** Trametinib dimethyl sulfoxide is almost insoluble in water (<0.0001 mg/mL at 25° C)

8.3.8 **Mode of Action:** Trametinib dimethyl sulfoxide is a reversible, highly selective, allosteric inhibitor of mitogen-activated extracellular signal regulated kinase 1 (MEK1) and MEK2. Tumor cells commonly have hyperactivated extracellular signal-related kinase (ERK) pathways in which MEK is a critical component. Trametinib dimethyl sulfoxide inhibits activation of MEK by RAF kinases and MEK kinases.

8.3.9 **Description:** Trametinib dimethyl sulfoxide is a white to almost white powder.

8.3.10 **How Supplied:** Tablets will be supplied commercially.

The tablet core contains mannitol, microcrystalline cellulose, hypromellose, croscarmellose sodium, magnesium stearate (non-animal), colloidal silicon dioxide, and sodium lauryl sulfate.

- 0.5 mg tablets are white or yellow, modified oval, biconvex and film-coated. Aqueous film coating consists of Opadry Yellow 03B120006 (hypromellose, titanium dioxide, polyethylene glycol, iron oxide yellow).
- 2 mg tablets are pink, round, biconvex and film-coated. Aqueous film coating consists of Opadry Pink YS-1-14762-A, hypromellose (titanium dioxide, polyethylene glycol, polysorbate 80, iron oxide red).

8.3.11 **Storage:** Store tablets at 2°C - 8°C (36°F - 46°F) in the original bottle. Bottles should be stored in the manufacturer's package carton for light protection. Protect from moisture. For additional storage instructions, please refer to package insert.

8.3.12 **Stability:** Shelf life studies of trametinib dimethyl sulfoxide are ongoing.

8.3.13 **Route of Administration:** Oral. Take by mouth on an empty stomach, either 1 hour before or 2 hours after a meal. If a dose is missed, it should not be taken if it is less than 12 hours until the next dose.

8.3.14 **Potential Drug Interactions**

*In vitro* studies suggest that trametinib dimethyl sulfoxide is not a substrate of CYP enzymes or of human Pgp, BCRP, OATP1B1 or OATP1B3 transporters.

Trametinib dimethyl sulfoxide is a weak CYP2C8 inhibitor and weak CYP3A4 inducer. Drug-drug interactions with sensitive substrates of 2C8 and 3A4 are not anticipated.

8.3.15 **Availability**

Trametinib dimethyl sulfoxide (GSK1120212B) is FDA-approved for the treatment of unresectable or metastatic BRAF-mutant melanoma. It will be used at its standard dose and schedule for its indicated purpose. Commercial trametinib will be used throughout the study and obtained from specialty pharmacies and with coverage provided by participant's individual health insurance.

## 9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

### 9.1 Collection of Specimen(s)

Tumor biopsies are required be performed in all patients for both cohorts at four time points (as all patients are enrolled onto the first Stage of the Simon 2-stage design):

1. Prior to commencing MTT
2. Following 2-week MTT lead-in
3. Following 6-weeks of combined pembrolizumab with MTT (if feasible)
4. A biopsy at the time of disease progression will also be performed (this is optional)

Multiple cores will be obtained and/or sufficient tumor excised at each biopsy time-point, assuming that it is safe to do so (e.g. patients with rapid responses may have very little tumor to biopsy safely at the latter time points). Core biopsies or portions of the excised tumor will be processed with the following hierarchy in mind:

1. DNA/RNA/protein extraction (Sullivan)
2. Disaggregation and flow cytometry (Sharpe)
3. FFPE (Sullivan)
4. Single-cell RNA seq of T cells (Hacohen)
5. Single-cell RNA seq of tumor cells (Sharpe)

Analysis will then be carried out to compare changes seen longitudinally and to determine if those changes are associated with improved outcomes to therapy with study treatment. Most of this work will exploratory in nature, as analysis

such as the technology for single-cell sequencing of tumor and T-cells is emerging and no data is presently available to inform how these analyses might be used to help predict which patients are most likely to benefit from single-agent or combination therapy. However, based on previous work, there are two assays that will be incorporated into a predictive model, PD-L1 expression by immunohistochemistry (IHC) and immuno-sequencing (immunoSEQ) using the Adaptive Biotechnologies platform.

9.1.1 Handling of Specimens(s)

As described above, four cores will be obtained and fixed. The SOP for collection, handling, and processing is attached (Appendix C). Samples will either be stored at the individual sites and sent in batch at the time of planned correlative study analysis or sent to and stored at the Sullivan Laboratory at MGH. FFPE samples will be stored at room temperature. Frozen samples will be stored in a -80 degree freezer.

9.1.2 Shipping of Specimen(s)

Samples may be sent at the time of collection or in batches via overnight shipping. Frozen samples should be sent on dry ice; paraffin blocks may be sent at room temperature conditions. Please email laboratory notification of shipment prior to sending. The address:

Sullivan/Flaherty Laboratory
c/o Michal Barzily, Ph.D.
55 Fruit Street; Jackson 9th Floor
Boston, MA 02114
Phone: 617-643-3614
Email: RSULLIVAN7@mgh.harvard.edu

Site(s) Performing Correlative Study
All sites are expected to participate in these correlative studies.

## 9.2 PD-L1 Immunohistochemistry

Immunohistochemistry for PD-L1 and CD8 will be performed in Dr. Jeffrey Engelman's laboratory using a monoclonal anti-PD-L1 antibody (Clone E1L3N, Cell Signaling Technologies; Danvers, MA) and a CD8 monoclonal antibody (4B11, RTU, Leica Biosystems, Buffalo Grove, IL), respectively, with an automated stainer (Bond Rx, Leica Microsystems, Buffalo Grove, IL). As there is no recognized standard PD-L1 scoring protocol, we will define PD-L1 positivity as membranous +/- cytoplasmic staining of tumor cells of any intensity using two different cut-offs (≥1% and ≥5 tumor cells) since both have been associated with clinical benefit to PD-1 pathway blockade in melanoma.

### 9.3 Adaptive Biotechnologies immunoSEQ

FFPE specimens (or isolated tumor DNA) and frozen PBMC samples will be shipped to Adaptive Biotechnologies.

Samples sent to Adaptive will be handled in accordance with Adaptive SOPs. All arriving packages will be opened the same day they arrive and inspected thoroughly for correct labeling and packaging integrity. Adaptive will perform "survey level" sequencing on each tissue sample and "deep level" sequencing on each of the PBMC derived gDNA samples provided. As part of these analyses, Adaptive uses a proprietary assay for the amplification and massive parallel sequencing of millions of CDR3 regions in the T cell receptor beta gene (TCRB) using a multiplex PCR amplification across the VDJ junction of rearranged TCRB.

Using Adaptive's unique capacity to perform next generation sequencing of T-cell receptors for this study, a large amount of data will be generated using tissue specimens and PBMC samples from all collection time points. In addition to the immunoSEQ Analyzer software, which is available to all of Adaptive clients to aid in data analysis and visualization, Adaptive can directly involve its computational biology and biostatistics personnel in the data analysis.

### 9.4 Tumor Microenvironment Assessment

Tumor microenvironment assessment will be performed in collaboration with Dr. Arlene Sharpe. At least two biopsies will be earmarked for this work and will be sent to Dr. Sharpe's laboratory.

To determine how the tumor cells are modulated during MTT alone, anti-PD1 plus MTT and anti-PD1 monotherapy combination, we will use single-cell and bulk RNA-seq, whole exome, etc. to assess the evolution of tumor cells during therapy. Sequential biopsies will provide a means to analyze evolution of tumor cell responsiveness and resistance to therapy

To determine how immune cells in the tumor microenvironment are modulated during MTT alone, anti-PD1 plus MTT and anti-PD1 monotherapy combination, we will use single-cell and bulk RNA-seq, and assays to compare immune cell function (e.g., T cell activation vs. exhaustion using cellular immunologic assays, as well as analysis of phosphoproteins and ubiquitination signaling) in temporal biopsies. These studies should reveal how anti-tumor immunity is modulated during the lead-in phase of the clinical trial with MTT, during MTT and anti-PD1 combination therapy and during subsequent anti-PD1 monotherapy.

### 9.5 Pharmacodynamic Studies

9.5.1.1 MITF

Biopsies will be obtained in patients

9.5.1.2 Blood-based BRAF assay

To explore the value of pretreatment BRAF level in predicting response to dabrafenib, trametinib, and Pembrolizumab, we will test isolated PBL and serum samples obtained prior to commencement of therapy. RNA will then be isolated from PBL and serum and $BRAF^{V600}$ analysis will be performed as described in Figure 2.4.1. Quantitative analysis will be performed and levels will be reported in ng/uL. Two categories of levels will be analyzed, based on our previous data, those with values >900 ng/uL and $\leq$ 900 ng/uL.

To explore the pharmacodynamic effects of dabrafenib, trametinib, and Pembrolizumab therapy on peripheral blood $BRAF^{V600}$ mutation detection, we will serial test isolated PBL and serum samples. RNA will then be isolated from PBL and serum and $BRAF^{V600}$ analysis will be performed as described in Figure 2.4.1. Patients will have quantitative analysis before and baseline values will be in ng/uL. Peripheral blood will be drawn pre-treatment, on Day 15 of the first cycle of treatment, and then on Day 1 of every subsequent cycle of therapy. 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 (>50% improvement vs. $\leq$ 50% improvement; or 100% increase vs. < 100% increase) with clinical response (CR/PR by RECIST1.1) and clinical progression (PD) will then be performed to assess concordance between changes over time with our assay and with imaging.

## 10. STUDY CALENDAR

Baseline evaluations are to be conducted within 2-weeks prior to start of protocol therapy. Scans must be done $\leq$4 weeks prior to the start of therapy. All assessments must be performed prior to administration of any study medication. On days when TSH is required, TSH is not required to be resulted prior to administration of study medication. All study assessments and medications should be administered within $\pm$ 3 days of the protocol-specified date, unless otherwise noted. Laboratory values do not need to re-meet eligibility criteria on Week 1, Day 1.

| | | Targeted Therapy Lead-In | | Combined Targeted Therapy plus Immune Therapy | | Single-agent pembrolizumab | | | | | | | Off-Study[m] | Follow Up |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre- Study<br>-14 to -1 days | Wk 1 | Wk 2 | Wk 3 | Wk 6 | Wk 9 | Wk 12 | Wk 15 | Wk 18 | Wk 21 | Wk 24 | Wk 27-107 | | |
| Dabrafenib[a] | | X---------- | | | | ----------X | | - - - - - - - - - - - - - - - - - - - - - - | | | | - - - - - - - - | | |
| Trametinib[a] | | X---------- | | | | ----------X | | - - - - - - - - - - - - - - - - - - - - - - | | | | - - - - - - - - | | |
| Pembro | | | | X | X | X | X | X | X | X | X | X[l] | | |
| Informed consent | X | | | | | | | | | | | | | |
| History[b] | X | X | | X | X | X | X | X | X | X | X | X | | |
| Dermatology Exam[c] | X | | | | | X | | | | | | | | |
| Ophthalmology Exam[d] | X | | | | | X | | | | | | | | |
| Concurrent meds | X | X---------- | | | | | | | | | ----------X | X | | |
| Physical exam (Wt, BSA[n], VS[o]) | X | X | | X | X | X | X | X | X | X | X | X | X | |
| Height | X | | | | | | | | | | | | | |
| ECG[e] | X | | | X | X | | | | | | | | | |
| Performance Status | X | X | | X | X | X | X | X | X | X | X | X | X | |
| CBC w/diff, plts | X | X | | X | X | X | X | X | X | X | X | X | X | |
| Serum chemistry[f] | X | X | | X | X | X | X | X | X | X | X | X | X | |
| TSH | X | | | X | | X | | X | | X | | X[f] | | |
| Adverse event evaluation[P] | | X---------- | | | | | | | | | ----------X | X | X[P] | X[P] |
| Radiologic evaluation[g] | X | | | | | X | | X | | X | | X | | X[r] |
| B-HCG[h] | X | | | X | | X | | X | | X | | X | X | |
| Echo/MUGA[i] | X | | | | | X | | | | | | | | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BRAF[j] | X | | | X | X | X | X | X | X | X | X | X[j] | | |
| PBMC[k] | X | | | X | | X | (X) | | | | | | | |
| Tumor Biopsies[k] | X | | | X | | X | (X) | | | | | | | |
| Rash Prophylaxis[q] | | X[q] | X[q] | X[q] | X[q] | | | | | | | | | |

a. MAPK targeted therapy (MTT): In cohort 1, participants will be treated with the combination of dabrafenib and trametinib. In cohort 2, participants will be treated with trametinib. MTT will commence week 1, day 1 and be given continuously (except in the setting of toxicity) for two weeks in the lead-in phase and then 6-weeks in combination with pembrolizumab. Only if patients develop symptomatic or radiographic progression at anytime AFTER initial MTT-phase completion, then MTT may be resumed at a previously tolerated dose in combination with ongoing pembrolizumab.
b. Participants will be seen on day 1 (+/-3 days) of week 1, week 3, and then every three weeks while remaining on therapy. If treatment is discontinued for a reason other than disease progression, study discontinuation, or withdrawal of consent, patients will be seen every 12 weeks (+/- 2 weeks).
c. Dermatological examination done at screening, and within 1 week prior to dosing on week 9. A full-body skin examination and removal of pre-existing calluses and keratotic skin is recommended prior to initiation of study treatment (See Table 6-4).
d. Ophthalmological examination done at screening and within 1 week prior to treatment on week 9. For Grade 2 visual changes or greater, an exam must occur within 24 hours of onset (or study team knowledge of the event). Consult ophthalmologist any time when patient reports visual disturbance. If there is visual loss or significant visual changes, consult ophthalmologist immediately (within 24 hours)
e. Single EKG will be collected at screening and repeated on day 1 of weeks 3 and 6 in cohort 1.
f. Albumin, alkaline phosphatase, total bilirubin, BUN, creatinine, calcium, chloride, glucose, HCO3, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium. TSH will be performed at baseline, week 3 and then every 6 weeks.
g. Radiographic imaging will be performed within 4 weeks of lead-in therapy commencement, within 1 week prior to Week 9, every six weeks thereafter (within 1 week prior to W15, W21, W27) until week 27, and then every 12 weeks (+/- 2 weeks) through 48 weeks of pembrolizumab therapy. Additionally, following initial disease progression, if a participant is treated beyond progression, tumor assessments must be repeated $\geq$ 4 weeks after initial disease progression in order to confirm disease progression. The tumor assessment must not be repeated any sooner than 4 weeks after initial disease progression.
h. Serum pregnancy test (women of childbearing potential).
i. Ejection fraction measurement done at screening and within 1 week prior to dosing on week 9 . The same modality (ECHO or MUGA) should be used at baseline and at follow-up; should be performed with increased frequency if clinically indicated.

j. Correlative blood analysis will be performed prior to MTT, prior to pembrolizumab therapy initiation, prior to every pembrolizumab dose through week 27, prior to every other pembrolizumab dose after week 27 (week 33, week 39, etc.), and at time of disease progression. BRAF samples will be collected in 1-10 mL green sodium heparin and 1-10 mL red top serum tubes
k. Tumor biopsies and PBMC blood analysiswill be performed pretreatment, prior to pembrolizumab therapy initiation (up to 5 days prior), at the conclusion of combined targeted therapy plus pembrolizumab therapy (up to 5 days prior), and then at the time of disease progression. PBMC samples will be collected in 2-8 mL blue tiger top CPT tubes.
l. Pembrolizumab will be administered at a dose of 200 mg IV every three weeks.
m. Off-study evaluation visit is completed within 30 days after the last dose of study medication. If treatment is discontinued for a reason other than completion of two years of pembrolizumab, disease progression or withdrawal of consent, patients will be seen every 12 weeks (+/- 2 weeks) with imaging performed prior to these evaluations. If patients have an ongoing response or stable disease 18 months (+/- 2 months) after their last dose of pembrolizumab, patients will be followed every three-twelve months, based on investigator discretion, with imaging performed every 3-12 months.
n. BSA should be calculated using the baseline height.
o. Vitals signs include pulse, body temperature, blood pressure (BP), and respiratory rate. In subjects with an initial BP reading within the hypertensive range, a second reading should be taken at least 1 minute later per Section 6.2.8BRAD
p. Adverse Event Reporting: All adverse events, both serious and non-serious, will be collected from the time of the first dose of study treatment up until 90 days after the last dose of study treatment, or until death if death occurs first. The adverse events are followed to their resolution, or until the participating investigator assesses them as stable, or the participating investigator determines the event to be irreversible, or the participant is lost to follow-up. If the subject is not seen in clinic after the off-study visit, required Adverse Event details must be collected over the phone.
q. Rash prophylaxis is recommended for the first 6 weeks of study treatment. See Table 6-3.
r. If treatment is discontinued for a reason other than disease progression, study discontinuation, or withdrawal of consent, participants will be seen every 12 weeks (+/- 2 weeks) for imaging until progressive disease or death, whichever comes first.

# 11. MEASUREMENT OF EFFECT

Although response is not the primary endpoint of this trial, participants with measurable and/or non-measurable disease will be assessed by RECIST 1.1.

## 11.1 Antitumor Effect – Solid Tumors

Radiographic imaging will be performed within 4 weeks of lead-in therapy commencement, within 1 week prior to Week 9, every six weeks thereafter (within 1 week prior to W15, W21, W27) until week 27, and then every 12 weeks (+/- 2 weeks) through 48 weeks of pembrolizumab therapy.

In addition to a baseline scan, confirmatory scans should also be obtained not less than 4 weeks following initial documentation of objective response.

If treatment is discontinued for completion of two years of pembrolizumab, or for a reason other than disease progression or withdrawal of consent, patients will be seen every 12 weeks (+/- 2 weeks) with imaging performed within 2 weeks prior to these evaluations.

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 guideline. *(Eisenhauer et al., 2009).* Changes in the diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria.

### 11.1.1 Definitions

Evaluable for Target Disease response. Only those participants who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for target disease response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Participants who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

### 11.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be

accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm by chest x-ray or ≥10 mm with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable.

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

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all considered non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same participant, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as

**non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow up.

11.1.3 Methods for Evaluation of Disease

All measurements should be taken and recorded in metric notation using a ruler, calipers, or a digital measurement tool. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm in diameter as assessed using calipers (*e.g.*, skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung; however, CT is preferable.

Conventional CT and MRI. This guideline has defined measurability of lesions on CT scan based on the assumption that CT thickness is 5mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size of a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if

possible.

FDG-PET. While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

(a) Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.

(b) No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

(c) FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A ‘positive’ FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

PET-CT. At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound. Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later data and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI

is advised. If there is concern about radiation exposure from CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy. The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a participant to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

Cytology, Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (*e.g.*, residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

11.1.4 Response Criteria

11.1.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

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. (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 diameters while on study.

11.1.4.2 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.

Non-CR/Non-PD: 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. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of “non-target” lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

11.1.4.3 Evaluation of New Lesions

The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (for example, some ‘new’ bone lesions may be simply healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

11.1.4.4 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 patient's best response assignment will depend on the

achievement of both measurement and confirmation criteria.

**For Participants with Measurable Disease (*i.e.*, Target 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.

**For Participants with Non-Measurable Disease (*i.e.*, Non-Target 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

11.1.5 Duration of Response

Duration of overall 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, or death due to any cause. Participants without events reported are censored at the last disease evaluation).

Duration of overall complete response: The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented, or death due to any cause. Participants without events reported are censored at the last disease evaluation.

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, including the baseline measurements.

11.1.6 Progression-Free Survival

Overall Survival: Overall Survival (OS) is defined as the time from randomization (or registration) to death due to any cause, or censored at date last known alive.

Progression-Free Survival: Progression-Free Survival (PFS) is defined as the time from randomization (or registration) to the earlier of progression or death due to any cause. Participants alive without disease progression are censored at date of last disease evaluation.

Time to Progression: Time to Progression (TTP) is defined as the time from randomization (or registration) to progression, or censored at date of last disease evaluation for those without progression reported.

11.1.7 Response Review

Independent, central review of the radiology assessment will be performed by through the Dana Farber Harvard Cancer Center Tumor Imaging Metrics Core (TIMC).

## 12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7 (Adverse Events: List and Reporting Requirements).

### 12.1 Data Reporting

#### 12.1.1 Method

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

**Note**: If your study has been assigned to CDUS-Complete reporting, **all** adverse events (both routine and expedited) that have occurred on the study and meet the mandatory CDUS reporting guidelines must be reported via the monitoring method identified above. If your study has been assigned to CDUS-Abbreviated reporting, no adverse event reporting (routine or expedited) is required to be reported via CDUS.

#### 12.1.2 Responsibility for Data Submission

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

*For CDUS and CMTS submissions:*

Participant institutions are responsible for submitting CDUS data and/or data forms to either the Coordinating Center or to the Lead Organization on the study quarterly. The date for submission to the Coordinating Center or to the Lead Organization will be set by them. CDUS does not accept data submissions from the participant institutions on the study. When setting the dates, allow time for Coordinating Center compilation, Overall PI review, and timely submission to CTEP by the quarterly deadlines. For trials monitored by CTMS, a quarterly report of data will be provided by Theradex to the Coordinating Center.

Either the Coordinating Center or the Lead Organization is responsible for compiling and submitting CDUS data to CTEP for all participant institutions and for providing the data to the Overall PI for review.

### 12.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the

study. Information that raises any questions about participant safety will be addressed with the Overall PI and study team.

The DSMC will review each protocol up to four times a year or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring with 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

## 12.3 **Regulatory Considerations**

### 12.3.1 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 agreed upon by the DFHCC Overall Principal Investigator, shall 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.

### 12.3.2 Informed Consent

All participants 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. 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.

### 12.3.3 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.

## 12.4 **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.

### 12.5 Records Retention

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

## 13. STATISTICAL CONSIDERATIONS

This is a phase II trial to assess the effect of adding an abbreviated MAPK-targeted therapy to pembrolizumab in patients with unresectable or metastatic melanoma. Two cohorts of patients will be enrolled and analyzed separately: Patients with $BRAF^{V600}$ mutant melanoma, and patients with $BRAF^{V600}$ wild-type melanoma. The trial will be based on parallel Simon two-stage designs according to disease cohort and will provide preliminary estimates of toxicity and efficacy.

### 13.1 Study Design/Endpoints

**Primary Endpoint**: The rate of clinical benefit (CBR), defined as the proportion of patients who have CR/PR/SD at the week-24 scan while remaining off MAPK-targeted therapy (MTT) after induction. To be evaluable for the primary endpoint, patients must receive at least one dose of pembrolizumab, any patient who is unevaluable for response will be classified as a non-responder.

**Secondary Endpoints:**

13.1.1 Overall Survival (OS): The time between the first dose of targeted therapy and death from any cause. For patients who are lost to follow-up or who have no documentation of death at the time of final analysis, follow-up will be censored at the date of last assessment of vital status.

13.1.2 Safety and tolerability: Safety and toxicity will be monitored throughout the study. An early, comprehensive safety review will be conducted at the end of the first-stage of the Simon two-stage design to review the data for unexpected severe toxicities due to the drug combination.

13.1.3 Describe temporal changes in the tumor microenvironment as a function of therapy.

**Design**: For each disease cohort, the Simon two-stage design is based on a type-I error rate of 0.1, at least 85% power, and a total sample size of 25 subjects. A complete safety assessment is planned to coincide with the review of clinical benefit at the end of the first stage.

Within cohort, the null hypothesis of a true CBR of 0.50 will be tested against a one-

sided alternative CBR of 0.75. The benchmark CBR rate of 0.50 is based on the results of the Keynote 001 trial in patients with refractory or first-line malignant melanoma treated with pembrolizumab (Robert, ASCO 2016) that reported a CBR of 0.51 regardless of BRAF mutation status. A short course of MAPK-targeted therapy in addition to pembrolizumab would be considered worthy of further investigation if there is evidence of an improvement in CBR to 0.75. Fourteen (14) subjects that are evaluable for the primary endpoint (defined as receiving at least 1 dose of dabrafenib/trametinib/pembrolizumab combination therapy prior to treatment discontinuation for any reason.) will be enrolled in the first stage. If 8 or fewer subjects with clinical benefit are observed, enrollment will stop. If there are 9 or more subjects with clinical benefit, then an additional 11 subjects will be enrolled for a total of 25. The null hypothesis will be rejected if 16 or more subjects with clinical benefit are observed in 25 subjects. This design yields power of 86% (target type II error of 0.15) when the CBR rate is 0.75. If the null hypothesis is true, the probability is 0.79 that enrollment to a cohort will stop at the end of the first stage. This high probability of stopping under the null was chosen to require strong evidence of benefit before opening the second stage.

## 13.2 **Sample Size, Accrual Rate and Study Duration**

The total sample size for this study is 50 patients (25 patients per cohort). At an anticipated accrual rate of 2 patients per month, enrollment is expected to be completed in approximately two years.

## 13.3 **Analysis of Primary Endpoints**

Clinical benefit rates will be presented for each cohort with 90% confidence intervals based on the method of Atkinson and Brown, which allows for the two-stage design. For samples of size 25, each confidence interval will be no wider than 0.35.

## 13.4 **Analysis of Secondary Endpoints**

PFS and OS: The distributions of secondary endpoints of PFS and OS will be based on the method of Kaplan-Meier. Medians of the distributions of PFS and OS will be estimated and presented with 90% confidence intervals based on log(-log(endpoint)) methodology. One-year estimates of OS will also be presented using the same methods.

Early Safety Review: An early, comprehensive safety review will occur at the end of the first stage of the Simon two-stage design and will take place when one cohort has reached the end of its respective first stage according to the Simon design. Since we do not anticipate that adverse events will differ according to BRAF mutation status, all available safety data will be combined from the two cohorts, resulting in an early safety review for up to 28 patients. All reported AEs will be summarized.

For a sample of 28 patients, there is high probability of observing at least one event if a toxicity has an incidence of at least 5%. If the true incidence of an unexpected or severe

toxicity is 5% or greater, the probability is at least 0.75 that one or more patients out of 28 will experience the toxicity during the early safety monitoring period. If the true incidence of unexpected or severe toxicity is 1% or less, the probability is 0.25 or less that at least one patient of 28 will experience the toxicity during the early safety monitoring period. 0 summarizes the probabilities of observing at least one patient with a severe or unexpected toxicity during the initial safety review for a range of true incidence rates.

**Table 13.1. Operating Characteristics of Early Safety Monitoring**

| True Incidence of Unexpected or Severe Toxicity | Probability of Observing One or More Patients with Toxicity among First 28 |
|---|---|
| 1% | 0.25 |
| 3% | 0.57 |
| 5% | 0.76 |
| 8% | 0.90 |
| 9% | 0.93 |
| 10% | 0.95 |
| 15% | 0.99 |

## 13.5 Analysis of Correlative Measures

Analysis of MTT induced changes on T-cell clonality / PDL1 expression and correlation with clinical benefit. To investigate whether MTT-associated changes following a brief course of MTT can predict who will benefit from the addition of anti-PD1 therapy, assessments will be based on: (a) clonality of tumor-infiltration lymphocytes (TILs), and (b) PD-L1 IHC. TIL clonality is a term used to quantify the diversity of clones and frequency of any given clone. It is calculated by ImmunoSeq Adaptive Biotechnologies software using a clonality score, defined as [1-(entropy)/log2(# of productive unique sequences)], where the entropy term takes into account the varying clone frequencies of TILs. A maximally diverse population, where every sequence is represented one time, is associated with a clonality score of zero, and a perfectly monoclonal population with a clonality score of one. Assessment times will be pre-treatment and after the 2-week MTT lead-in. Each cohort will be investigated separately.

To investigate the hypothesis that there will be an increase in TIL clonality after MTT lead-in, the overall changes in clonality of TILs after MTT will be expressed as a fold-change (2-week/pre) and compared with unity using a Wilcoxon signed-rank test with 80% power, and a 0.1, two-sided significance level. For a range of standard deviations of the fold-change between 0.8 and 1.2, we will be able to detect effect sizes between 1.4 and 1.65 for this comparison. We will further refine this question to address whether increases in clonality are related to response. Clones that comprise the largest percentage of the total T-cell population after MTT lead-in will be identified and sorted by frequency. The percentage of the overall T-cell population included in the top 5% of clones will

be compared according to response (achieving/not achieving clinical benefit) using Wilcoxon rank-sum tests. If the observed CBR is 0.75, resulting in 19 patients with clinical benefit and 6 without, a Wilcoxon rank-sum test with 0.1, two-sided significance level will have 80% power to detect a difference in percentage T-cells that is approximately 1.3 times the common standard deviation. Similar analyses will be performed for the top 2.5% or 1% of clones.

To assess if changes in clonality after MTT lead-in are associated with infiltration by new TIL clones, we will compare pre-treatment and post-MTT biopsies to identify clones that were absent from the pre-treatment biopsies, and summarize the percentage of individual clones that were identified to be new. This will be assessed for all patients in a cohort using Wilcoxon signed-rank tests, and according to clinical response using Wilcoxon rank-sum tests. In additional analyses, the percentage of pre-existing clones, rather than new clones, will be compared according to response to see if patients with high proportions of pre-existing clones after MTT lead-in exhibit a better response than patients with low proportions.

For PD-L1 expression, patients will be classified, retrospectively, into two subgroups defined by clinical benefit at 24 weeks. Fold-changes in PD-L1 IHC (2-week/pre) will be compared for patients achieving/not achieving clinical benefit using Wilcoxon rank-sum tests. If the observed CBR is 0.75, resulting in group sizes of 19 and 6, a Wilcoxon rank-sum test with 0.1, two-sided significance level will have 80% power to detect a difference in fold-changes that is approximately 1.3 times the common standard deviation.

## 13.6 Analysis of Safety and Toxicity Data

**Analysis set and grouping for the analyses:** All patients who receive one or more doses of the drug combination will be included in the safety analysis. All listings and tables will be presented by treatment.

**Adverse events (AEs):** All adverse events recorded during the study will be summarized. The incidence of treatment-emergent adverse events will be summarized by treatment according to primary system organ class, severity based on the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) version 4.0, type of adverse event, and relationship to treatment. Deaths reportable as SAEs and non-fatal serious adverse events will be listed by patient and tabulated by primary system organ class, type of adverse event, and dose level.

**Laboratory abnormalities:** All laboratory values will be converted into SI units, as appropriate, and the severity grade calculated using CTCAE, version 4.0. Parameters for which a grading does not exist will be classified into low/normal/high group by means of laboratory normal ranges. For each laboratory test (e.g., hematology, biochemistry) a listing of laboratory values will

be provided by laboratory parameter, patient and treatment group. The frequency of notable lab abnormalities (i.e., newly occurring CTCAE grade-3 or -4 laboratory toxicities), will be displayed by parameter, cycle, and dose level. Similarly, the frequency of all laboratory abnormalities will be displayed by parameter, worst CTCAE version 4.0 grade experienced and dose level. A separate listing will display notable laboratory abnormalities (i.e., newly occurring CTCAE grade-3 or -4 laboratory toxicities). Laboratory data will be summarized by presenting grade shift tables for those parameters for which CTCAE version 4.0 allows classification. All remaining data will be summarized by presenting shift tables based on normal ranges.

Laboratory data will be also be displayed by presenting summary statistics of raw data and change from baseline values (means, medians, standard deviations, ranges).

## 13.7 Reporting and Exclusions

### 13.7.1 Evaluation of Toxicity

**Evaluation of toxicity.** *Define "evaluable."* For example: All participants will be evaluable for toxicity from the time of their first treatment.

### 13.7.2 Evaluation of the Primary Efficacy Endpoint

**Evaluation of response.** All participants included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each participant should be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.

All of the participants who met the eligibility criteria (with the possible exception of those who did not receive triplet therapy) should be included in the main analysis of the response rate. Participants in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9, and sometimes category 3, will be protocol specific.

All conclusions should be based on all eligible participants. Subanalyses may then be performed on the basis of a subset of participants, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these subanalyses may not serve as the basis for drawing conclusions

concerning treatment efficacy, and the reasons for excluding participants from the analysis should be clearly reported. The 95% confidence intervals should also be provided.

## 14. 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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## APPENDIX A: PERFORMANCE STATUS CRITERIA

| ECOG Performance Status Scale | | Karnofsky Performance Scale | |
|---|---|---|---|
| Grade | Descriptions | Percent | Description |
| 0 | Normal activity. Fully active, able to carry on all pre-disease performance without restriction. | 100 | Normal, no complaints, no evidence of disease. |
| | | 90 | Able to carry on normal activity; minor signs or symptoms of disease. |
| 1 | Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work). | 80 | Normal activity with effort; some signs or symptoms of disease. |
| | | 70 | Cares for self, unable to carry on normal activity or to do active work. |
| 2 | In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours. | 60 | Requires occasional assistance, but is able to care for most of his/her needs. |
| | | 50 | Requires considerable assistance and frequent medical care. |
| 3 | In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours. | 40 | Disabled, requires special care and assistance. |
| | | 30 | Severely disabled, hospitalization indicated. Death not imminent. |
| 4 | 100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair. | 20 | Very sick, hospitalization indicated. Death not imminent. |
| | | 10 | Moribund, fatal processes progressing rapidly. |
| 5 | Dead. | 0 | Dead. |

## APPENDIX B: INFORMATION ON POSSIBLE DRUG INTERACTIONS

### Information on Possible Interactions with Other Agents for Patients and Their Caregivers and Non-Study Healthcare Team

The patient _____ is enrolled on a clinical trial using the experimental agents, Dabrafenib plus Trametinib dimethyl sulfoxide or trametinib dimethyl sulfoxide. This clinical trial is sponsored by the Dana Farber Harvard Cancer Center. This form is addressed to the patient, but includes important information for others who care for this patient.

Dabrafenib mesylate and trametinib dimethyl sulfoxide interact with many drugs that are processed by your liver. Because of this, it is very important to tell your study doctors about all of your medicine before you start this study. It is also very important to tell them if you stop taking any regular medicine, or if you start taking a new medicine while you take part in this study. When you talk about your medicine with your study doctor, include medicine you buy without a prescription at the drug store (over-the-counter remedy), or herbal supplements such as St. John's wort.

Many health care prescribers can write prescriptions. You must also tell your other prescribers (doctors, physicians' assistants or nurse practitioners) that you are taking part in a clinical trial. **Bring this paper with you and keep the attached information card in your wallet**. These are the things that you and they need to know:

Dabrafenib mesylate interacts with certain specific enzymes in your liver.

- The enzymes in question are ***CYP450 3A4, 2C8, 2C9, 2C19, 2B6.*** Dabrafenib mesylate levels are affected by some of these enzymes and can lower the levels of other medicines you take.
- Dabrafenib mesylate must be used very carefully with other medicines that need these liver enzymes to be effective or to be cleared from your system.
- Other medicines may also affect the activity of the enzyme.
    - Substances that increase the enzyme's activity ("inducers") could reduce the effectiveness of the drug, while substances that decrease the enzyme's activity ("inhibitors") could result in high levels of the active drug, increasing the chance of harmful side effects. Dabrafenib mesylate should not be taken with any other drugs that are strong inducers or inhibitors of CYP 3A4 or 2C8. Prohibited medications include azole antifungals, some antiepileptic drugs, some antibiotics and some immunosuppressants. Please check with the study investigator before prescribing or dispensing strong inhibitors/inducers of CYP 3A4 or 2C8. Mild/moderate inhibitors/inducers should be used with caution.
    - Dabrafenib mesylate is considered an inducer of CYP 3A4, CYP2C8/9, 2B6 and possibly 2C19 , meaning that it can decrease the levels of other drugs that are processed by these enzymes. This can lead to harmful

side effects and/or reduce the effectiveness of those medications.

- You and healthcare providers who prescribe drugs for you must be careful about adding or removing any drug in this category.
- Before you start the study, your study doctor will work with your regular prescriber to switch any prohibited medicines that are considered "strong inducers/inhibitors or substrates of ***CYP 3A4 and 2C8***."
- Your prescribers should look at this web site http://medicine.iupui.edu/clinpharm/ddis/table.aspx
- or consult a medical reference to see if any medicine they want to prescribe is on a list of drugs to avoid.
- Please be very careful! Over-the-counter drugs have a brand name on the label—it's usually big and catches your eye. They also have a generic name—it's usually small and located above or below the brand name, and printed in the ingredient list. Find the generic name and determine, with the pharmacist's help, whether there could be an adverse interaction.
- Be careful:
    - If you take acetaminophen regularly: You should not take more than 3 grams a day if you are an adult or 2.4 grams a day if you are older than 65 years of age. Read labels carefully! Acetaminophen is an ingredient in many medicines for pain, flu, and cold.
    - If you take herbal medicine regularly: You should not take St. John's wort while you are taking dabrafenib mesylate

Other medicines can be a problem with your study drugs.

- You should check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.

- Your regular prescriber should check a medical reference or call your study doctor before prescribing any new medicine for you.

**Your study doctor's name is**

_____

**and he or she can be contacted at**

_____

**INFORMATION ON POSSIBLE DRUG INTERACTIONS**

You are enrolled on a clinical trial using the experimental agent **_____.** This clinical trial is sponsored by the NCI. _____ interacts with drugs that are processed by your liver. Because of this, it is very important to:

- Tell your doctors if you stop taking regular medicine or if you start taking a new medicine.
- Tell all of your prescribers (doctor, physicians' assistant, nurse practitioner, and pharmacist) that you are taking part in a clinical trial.
- Check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.

_____ interacts with a specific liver enzyme called **CYP_____**, and must be used very carefully with other medicines that interact with this enzyme.

- Before you start the study, your study doctor will work with your regular prescriber to switch any medicines that are considered "strong inducers/inhibitors or substrates of **CYP_____**."
- Before prescribing new medicines, your regular prescribers should go to http://medicine.iupui.edu/clinpharm/ddis/table.aspx for a list of drugs to avoid, or contact your study doctor.
- Your study doctor's name is _____

  and can be contacted at _____**.**

## APPENDIX C: BIOASSAY

PROCESSING OF TISSUE SPECIMENS

Adapted from:

1. Leyland-Jones, B.R. et al. Recommendations for collection and handling of specimens from group breast cancer clinical trials. J Clinical Oncology, 26 (34): 5638-5644, 2008.

-Full guidelines from that manuscript are referenced on the World Wide Web at http://ctep.cancer.gov/guidelines/spec_bc_grptrials.html

2. University of Texas MD Anderson Cancer Center Institutional Tissue Bank Standard Operating Procedures, Version 7.0

## MATERIALS/EQUIPMENT

| | |
|---|---|
| Liquid Nitrogen in approved LN2 transport carrier | Surgical mask/eye protection |
| Safety glasses or face shield | Clean Laboratory coat |
| Freezer gloves | Clean protective shoes |
| Disposable latex gloves | Cryovials |
| Disposable scalpels or scalpel blades (or single edge razor blade) | Racks for cryovials |
| Forceps | Petri dish |
| Histoprep Marker | OCT cyro-compound |
| TissueTek cryomold | Kimwipes |
| 10% Neutral Buffered formalin | 100% Isopropanol |
| 95% Ethanol (alcohol) | |

## HAZARDOUS MATERIALS

1. 10% Neutral Buffered Formalin
Formaldehyde : severe eye and skin irritant. Sensitizes by skin and respiratory contact. Toxic by ingestion and inhalation. Target organs effects on respiratory system. Corrosive. Carcinogen.

| | |
|---|---|
| Emergency First Aid Procedures: | Eye: Irrigate immediately with large quantity of water for a least 15 minutes. Get medical attention immediately<br>Skin: Flush with water for at least 15 minutes<br>Ingestion: Dilute immediately with water or milk. Induce vomiting. Call physician. |

| | |
|---|---|
| | Inhalation: Remove to fresh air. Give artificial respiration if necessary. |
| If released or spilled: | Use formaldehyde spill kit for small spills of 1000ml; For larger spills contact Environmental Health & Safety |
| Waste Disposal Method | Whatever cannot be saved for recovery or recycled should be disposed of according to local, state, and federal regulations. |
| Respiratory Protection | If the exposure level is exceeded, wear a full facepiece respirator equipped with a formaldehyde cartridge. |
| Ventilation | Work in well ventilated areas |
| Precautionary Labeling | Label with formaldehyde label |
| Handling and Storage Considerations | Wash hands thoroughly after handling. Avoid eye contact. Protect from freezing and physical damage. Store at controlled room temperature, 15-30° C. |

2. Liquid Nitrogen

| | |
|---|---|
| Emergency First Aid Procedures: | For Cold Liquid Frostbite:<br>If any liquefied atmosphere gas contacts the skin or eyes, remove any clothing that may constrict blood circulation to the frozen area. Immediately flood or submerge the affected body area with large quantities of clean, unheated water and then apply cold compresses. A source of water should be nearby and easily accessible wherever liquid nitrogen activities are being conducted. If the skin is blistered, or there is any chance that the eyes have been affected, get the patient to a physician immediately for treatment. |
| | For Grogginess or Unconsciousness While Handling Liquid Nitrogen:<br>If a person seems to become groggy or loses consciousness while working with liquid nitrogen, get him to a well-ventilated area immediately. Use a self-contained breathing apparatus if necessary. If breathing has stopped, apply artificial respiration. Whenever a person loses consciousness, call 911 immediately. |
| If released or spilled: | |
| Waste Disposal Method | |
| Respiratory Protection | |
| Ventilation | |
| Precautionary Labeling | |
| Handling and Storage | Use only containers specifically designed for holding |

| | |
|---|---|
| Considerations | liquefied gases |

## FRESH/FROZEN TISSUE PROCESSING

### Processing Options for Fresh/Frozen Tissue:

- OCT is especially useful for preserving histology, and may improve RNA recovery, and thus is recommended. A frozen section resulting from an OCT-embedded specimen will provide important information on the presence and quantity of tumor. Moreover, OCT prevents the tissue from desiccation and crumbling and also acts as an insulator from thermal change and limits ice crystal formation. If tissue samples are obtained by core (punch or needle) biopsies, each core should be separately embedded in the OCT.
- If OCT processing is not possible, controlled snap-freezing in cooled isopentane or with a heat extractor is recommended over simple dry-ice freezing. Snap-frozen specimens should be placed in an appropriate container (e.g., cryovial or cassette) and transferred to -80°C or colder for storage

### Some guiding principles for fresh/frozen tissue collection include the following

- Do not slow-freeze. Samples should be snap-frozen. Slow freezing promotes the formation of ice crystals, which damage the nucleic acids (e.g., RNA) in the specimen. The slower a sample freezes, the larger the ice crystals. Older models of cryostats (bath/dewar or vacuum type) that require > one minute to freeze a specimen should be avoided.
- Do not place the specimen directly in liquid nitrogen. Instead, place the OCT-filled cryomold onto a stable structure (i.e. Petri dish) that is on the surface of the liquid nitrogen.
- If only dry ice is available, adding alcohol (e.g., isopropanol or ethanol) to the dry ice can make a slurry that will help freeze the specimen more effectively (the alcohol will increase the thermal conductivity of the dry ice). This, however, is not a preferred over liquid nitrogen for snap-freezing.
- Do not add serum to the specimen
- Do not touch the biopsy without sterile gloves
- Sterile or disposable equipment should be used, including for dissection and for snap-freezing.
- Instruments should be changed or cleaned between dissecting normal and tumor tissue.
- If a pen is used to label cryovials or other receptacles that will be stored in freezing conditions, ensure that the pen is waterproof/solvent-proof and can withstand long-term freezing conditions.
- Copies of any relevant pathology reports and material submission forms should be sent along with the specimens to the central bank. Reports should be coded

in a way they can be matched to the specimen(s) while also protecting patient confidentiality requirements.

- If possible, representative adjacent normal tissue should be provided in addition to the tumor tissue. Normal tissue should be maximally distant from the tumor (minimum 2 cm). Collection of germline DNA (i.e. from PBMCs) should be considered in all protocols that include the collection of tumor tissue for DNA isolation.

**Size/Number of fresh/frozen biopsies**

Punch biopsies (4-6 mm preferred, 2 mm minimum) of cutaneous and subcutaneous lesions are preferable to core needle biopsies. A single biopsy is generally sufficient for research purposes.

Core needle biopsies: It should be stressed that core biopsy – whether collected as fresh/frozen or FFPE – is superior to fine needle aspiration (FNA) (Singh, 2007). Cores are more representative than FNA, providing more accurate diagnostic information – including invasive versus in situ, grade, percent tumor, and other important histopathologic information. The minimum size for fresh/frozen tissue collection is generally 0.25 cubic cm, which is generally achieved with approximately four passes of a 14-gauge needle. In the diagnostic setting, the collection of 2-4 fresh/frozen cores for research is recommended, in addition to 2 cores for diagnosis. The fraction of cores that will contain viable tumor will decrease if/as the tumor shrinks in response to therapy. Therefore, fewer cores may be obtainable as lesions grow smaller.

If a previous biopsy site is noted in the specimen to be sampled, biopsies should not be taken from near that site. Core biopsies alter the biology of tissue – e.g., they introduce inflammatory material from wound reaction and biomolecules involved in wound healing – which can be problematic if a subsequent core taken from that tissue is used for assay development. Notably, genes involved in wound healing are very similar to those involved in cancer progression (Riss et al., 2006).

**Fresh/Frozen Tissue Collection in the Surgical Setting**

During surgery, fresh/frozen tissue may be acquired by the transfer of the surgical specimen from the operating room to the pathology department in a tumor container without fixatives. Once it arrives in the pathology department, the sample may be sectioned, and then cores taken with a punch biopsy instrument and snap-frozen. This method is preferred if ischemia time can be limited to 30 minutes or less. ***Note:*** use a sterile RNAse free container if RNA work will be done with any of the tissue.

- *Alternative*: Taking cores and immediately freezing them in the operating room – i.e., a core biopsy is taken from the specimen immediately after the specimen is removed from the patient. Operating room acquisition of cores from the specimen right after excision helps keep ischemia time under 10 minutes, especially in situations where pathologists are some distance from the operating room.

**Time to Freezing and Storage Temperature for Fresh/Frozen Samples**

- Fresh tissue samples should be frozen as soon as possible. If they cannot be frozen immediately, they should be frozen within 30 minutes.
- Once snap-frozen, samples should be immediately transferred either to liquid nitrogen (preferred) or to a -80°C freezer. Specimens should be carried to such freezers on dry ice.
- Frozen samples should be stored long-term either in liquid nitrogen or in a locked freezer with a temperature of -80°C or colder. The freezer should be on electrical emergency power line and alarmed. If future uses of the tissue are unknown, storing the tissue in the vapor phase of liquid nitrogen will help to ensure long-term viability.
- Storage equipment may include small (2x3 inch) plastic, zip-top bags; mega-cassettes (for example, with each tissue or cryomold wrapped in aluminum foil); and cryoboxes and plastic racks (cryovial storage).

**Quality Assurance for Fresh/Frozen Samples**

A frozen section should be cut from the OCT block and stained with H&E to confirm tumor presence, percent of tumor cells, preservation of morphology, and the presence of any undesirable material, such as necrotic or inflammatory material. The H&E slide may be used as a guide to isolate specific portions of the sample for molecular analyses (i.e. viable tumor; adjacent normal tissue; etc).

**Database Annotation for Fresh/Frozen Tissue Collection**

When possible, the following should be recorded on the specimen submission form with respect to fresh/frozen tissue collection:

- Time before freezing: if >30 minutes, note time in 15-minute increments beyond 30 minutes.
- Freezing temperature
- If previous punch biopsy(ies) were performed on the patient or noted in the specimen
- Time point of sample – e.g., after which cycle of therapy

**LABELING**

Vials, including cryovials, should be labeled with the study name or number, a specimen ID number that is linked to the subject's study ID, contents of the vial, and date of collection. The subject's study ID should not be on the vial unless patient confidentiality is determined to be secure according to the clinical trial protocol. Specific procedures for labeling specimens should clearly be defined in the protocol. The central bank itself should have standardized labeling (printed or written) for archiving samples, such as unique sample IDs and or barcodes. The information included on a sample label must not include patient-identifying information, and should be compliant with the Health Insurance Portability and Accountability Act (HIPAA). The information should be sufficiently specific such that the encoded information (e.g., tracking number) can be linked to the sample in the database.

## Recommended Procedures for OCT Embedding and Snap-Freezing

### Specimen Preparation Prior to Fixation

- Using universal precautions, remove any excess blood from the tissue using a paper towel or KimWipe.
- Tissue should be cut on a dissection board using a razor blade or scissors with at least one dimension at a maximum thickness of 0.5 cm.
- Discard any unused tissue according to procedures for disposing of biological waste material.
- Discard the blade according to procedures for disposing of contaminating sharps.
- Weigh each section of the specimen prior to fixation
- For labeling, Markers specifically made for cryo temperatures are recommended (alcohol-based permanent markers will smudge).
- ***Note:*** if any of the tissue will be used for RNA work later use RNAse free technique.

### 1. OCT Embedding

a. Pre-Procedure Preparation: label cryomolds with permanent marker or printed cryolabel, and prepare (preferably print) sticker labels for foil wrappers. Labels should include sample ID number, tissue type, and date. Place cryomolds on a flat surface in a manner that will allow for easy differentiation of those intended for normal tissue and those for tumor tissue.
b. When sample is available, fill the bottom of each cryomold with a thin layer (less then 1 mm) of OCT by slowly and carefully filling the mold. It is important to avoid formation of bubbles and avoid uneven surfaces.
c. Weigh each section of the specimen prior to placing it in a cryoomold. Record the weight in appropriate log and database
d. If possible, pre-chill the prepared cryomold (step b). This can be done by: (1) setting on ice for 2-5 minutes, or (2) just prior to transferring specimen (step e) partially freezing by holding over liquid nitrogen until OCT starts to become cloudy.
e. Transfer the specimen to the OCT-filled cyromold using forceps.
f. Cover the tissue with OCT; ensure the top surface of the OCT compound completely covers the tissue and is level.
g. The OCT can be hardened by placing a Petri dish on the surface of liquid nitrogen and, using forceps, place the filled cryomold in the Petri dish. Avoid allowing the cryomold to come in direct contact with the liquid nitrogen. Specimen(s) are sufficiently frozen when the OCT has become completely white and hard.
h. After the OCT has hardened, place the mold in pre-labeled aluminum foil (heavy duty is best) with the appropriate label with the sample identification information.
i. Place samples on dry ice until transferred to -80° freezer for storage.

### 2. Snap Freezing

a. Pre-Procedure Preparation: Label 1.8 ml cryovials using a permanent cryo-marker (EtOH- and freezer-resistant).

b. To determine specimen weight: record weight of the empty vial, add tissue, then re-weigh. Subtract weights to determine the weight of the specimen, and record.
c. Place specimen in a 1.8ml cryovials using forceps. Use separate forceps for each type (tumor, normal) of specimen to avoid cross contamination.
d. Tightly secure the cap and submerged in liquid nitrogen for “snap freezing”. Use freezer gloves and a face shield during this procedure. Take precautions to avoid accidental spillage or spattering of liquid nitrogen.
e. Place samples on dry ice until transferred to -80° freezer for storage.

**FFPE TISSUE PROCESSING**

Fixation and paraffin infiltration

One fixative and one buffer type should be used across participating centers. All tissue samples should be fixed in 10% neutral phosphate-buffered formalin (i.e., 3.7% formaldehyde), pH 7. Resection specimens should be grossly dissected (macrosectioned) prior to fixation, to ensure adequate penetration of the fixative; ideally, the sections should be approximately 3 to 5 mm thick prior to placement in tissue cassettes for fixation. It is essential that surgical margins are appropriately marked and that these steps are carried out by a pathologist or their designate. Different blades should be used when dissecting normal tissue vs. tumor tissue. The time that elapses from resection to dissection and formalin fixation (the warm ischemia time) should be minimized, and typically should be no longer than 4 hours. Placing the specimen in fixative without dissection for overnight or longer is NOT adequate.

The following are recommended acceptable ranges of duration of fixation for FFPE specimens:

- Biopsies (core, needle, and skin biopsies): 8-24 hours
- Excision specimens: 12-24 hours in formalin (36 hours - absolute maximum)
- Tissue sections (0.25-1.0 grams): Overnight-24 hours in formalin (36 hours - absolute maximum)
- Weekend specimens: Although fixing and shipping of specimens over the weekend (i.e., Friday-Monday) is discouraged, if specimens must be left in formalin over the weekend, they should be oriented, “blocked”, bread-loaf sectioned and placed in a large amount of formalin in closed containers.

If the collecting center is not associated with a pathology group and does not have access to a tissue processor, then the specimen should be either 1) shipped in formalin on the day of collection for next-day delivery to the central bank, or 2) fixed in formalin for no more than 24 hours and then transferred to 70% ethanol, then shipped to the central bank within a few days. Samples should be protected from excessive heat when appropriate by packaging them in a styrofoam container (without ice packs). See Shipping section for more detailed information about appropriate packaging of samples.

The following are recommended ranges of time from formalin to paraffin on the processor (variable according to the specific manufacturer):

- 5-8 hrs for biopsies
- 6-14 hrs for other specimens (time depending on the instrumentation and tissue size)

Completion of the processes of dehydration with alcohols, clearing with xylene, and impregnation with paraffin is important. Some findings have suggested that extended processor times may result in higher-quality analytes, although which step should be prolonged has not yet been determined (Stephen Hewitt, personal communication, 2006). Use of low-melt paraffins has been recommended. Contamination with beeswax should be avoided (Hewitt et al., 2008).

**Storage of FFPE tissue**

FFPE blocks should be stored at temperatures below 80ºF (below 26ºC) in an "office-like" environment – i.e., a controlled-temperature environment with room temperature typical for an office, and protected from excessive heat (>28°C), humidity (>70%), and dryness (<30% humidity). FFPE tissue should not be stored in basements (danger of water) or warehouses (danger of insects). Light exposure for FFPE tissue is a key problem and should be minimized. Storage of unstained FFPE slides (whether from a single block or from a TMA block) should be discouraged due to antigen loss (DiVito et al., 2004; Fergenbaum et al., 2004). Biomarker analyses may best be carried out on freshly cut FFPE sections.

**Annotation of Laboratory Methods for FFPE samples**

We recommend that fixative type, buffer type, and time from resection to dissection and formalin fixation should be reported and recorded in the central bank's database.

**Recommended Procedures for Formalin-Fixation of Tissues**

1. Preserving the tissue in formalin enables the embedding of specimens into paraffin blocks. Neutral buffered formalin is used to stabilize protein in fresh tissue, and prevent autolysis and putrefaction.
    a. Specimens intended for formalin fixation should be processed after the completion of other fresh tissue procedures such as snap freezing, embedding in OCT compound, and submersion in RNA stabilizing reagent.
    b. Minimize time interval
        i. The time interval from of removal of tissues to fixation is very important in this procedure. The faster the tissue is placed in fixative, the better. Artifact will be introduced by drying, so if tissue is left out, please keep it moist with saline. The longer the interval between excision and fixation, the more cellular organelles will be lost and the more nuclear shrinkage and artifactual clumping will occur.

c. The volume of formalin should be a minimum of 15-20 times the volume of the tissue sample – e.g., 20ml of formalin per 1$cm^3$ of tissue.
d. Sectioning tissue for better penetration
    i. Penetration of tissues depends upon the diffusibility of each individual fixative, which is a constant. One way to get around this problem is sectioning the tissues thinly (3 to 5 mm). To preserve tissue and process for paraffin embedding, cut fresh tissue into appropriate size pieces.
    ii. **NOTE:** Tissues to be fixed and processed should be cut to a size no larger than 3-5 mm in thickness. Larger sized tissues will not permeate well with formaldehyde and will result in poor fixation and poor cellular morphology.
e. Record weight of each section
    i. Weigh the specimen prior to placing it in a tissue cassette. Record the weight in appropriate log and database
f. Labeling
    i. Place specimen in tissue cassette using proper orientation. Be sure to label the cassette using the cassette labeler. Include the tissue ID number and the tissue type on the cassette.
    ii. ***Note***: If the tissue is for a primary investigator, include their last name and protocol number on the side of the cassette.
g. Fixation
    i. Place cassette in a specimen cup containing a 10:1 ratio of 10% Neutral buffered formalin to tissue. Let tissue fix in the 10% formalin at room temperature from a minimum of 16 hours up to 24 hr.
    ii. ***Note:*** If unable to embed after 24 hours of 10% formalin fixation, transfer specimen in specimen cassette to 70% alcohol and embed within 72 hrs.

**SHIPPING**

Shipping personnel must receive training and be current in certification for biological specimen shipping. International Air Transport Authority (IATA) requires recertification every 2 years. For international studies, each country should consider identifying a tissue bank where tissue can be held before final shipping to a central bank across borders. A site should consult the central bank to determine the best times to ship samples that are frozen. This will help to avoid inadvertent thawing due to the evaporation of dry ice.

Batch shipping of samples will help to reduce the time required for organizing shipments and, in the case of frozen samples, dry-ice shipping costs (see “Note on Nucleic Acid Extraction”, below.) A good guideline for the interval of time between procurement and shipment is one month.

## Packaging for All Specimens

Packaging should comply with International Air Transport Association (IATA) criteria (please see http://www.iata.org). If ground overnight is used for FFPE samples, then shipment should conform to ground transportation standards (e.g., Department of Transportation packaging standards if in the US). The shipping box should be secured and appropriate stickers should be placed, such as "Biological Substance, Category B UN 3373", and the type of shipment, e.g., next-day. The IATA shipping category appropriate to the specimens collected should be used, both in labeling and in the training required for packaging. In addition to "Biological Substance, Category B, other IATA categories include "Exempt Human Specimens" and "Infectious Substance, Category A".

## Packaging for Shipping FFPE Specimens

Shipment of FFPE blocks requires that the blocks be protected from excessive heat. High outdoor temperatures are only one hazard: placement of an unprotected paraffin block on a warm surface can result in significant damage that could require re-embedding. Blocks should be individually wrapped or placed in small, jewelry-size, labeled plastic zip-top bags (not 2-10 blocks in a single sandwich bag). Blocks should then be placed in a Styrofoam shipping container, without dry ice or cold packs. Additional space should be filled with packing peanuts and other filler. Use of sealed bags with a desiccant can be used, if deemed necessary, to help control humidity.

Slides should be placed in appropriate slide carriers after the Permount has dried. At a minimum, the slide container should be wrapped in bubble wrap or placed in a padded envelope.

If alternative tissue block punchers are sent to a site for FFPE tissue collection, they should be shipped packed into a sleeve and in secured Styrofoam.

## Packaging for Shipping Frozen Specimens

Multi-level, watertight packaging with the appropriate biohazard and dry ice labels should be used to ship frozen solid tissue and aliquoted serum or plasma. These specimens should be contained in non-breakable – i.e., non-glass – cryovials or tube containers.

For example:

- Cryovial is placed into bubble-wrap, then into a plastic zip-top bag containing a sheet of absorbent material, for biohazard protection, then the bag and documentation into a watertight Styrofoam container packed with dry ice, the content list placed on top of Styrofoam container, then the Styrofoam container into a cardboard box with a biohazard label and a dry ice label.

Absorbent material, such as cotton balls, paper towels, or bubble wrap, should be used for additional cushioning as needed. Fragile containers should be wrapped with cushioning material. Again, though, plastic (not glass) vials should be used.

Shipping containers should not be sealed airtight so that $CO_2$ gas created from the evaporation of dry ice can escape the container. Pack dry ice and samples with paper, cardboard, or Styrofoam so that as the dry ice sublimates the samples will not move freely inside of the insulated box. The volume of air to which the dry ice is exposed should be minimized in order to slow the rate of sublimation. If there is any air space after filling the package with dry ice, it should be filled with packing peanuts or other material to reduce the volume of air space.

Temperature control for blood and fresh/frozen samples

- Samples should be shipped overnight, and shipped only Monday through Thursday to ensure delivery on a workday. If shipment cannot be made immediately, the samples can be stored at the appropriate temperature (e.g., -80°C for frozen tissue) until shipment can be made.
- Notification of shipment to the central repository is encouraged to ensure that specimens are properly received and processed. Communication can avoid mishaps due to absence or closure of the repository or variations from region to region. Tracking numbers and carrier information should be included in the communications.
- Dry ice should be used for shipping fresh/frozen tissue and aliquoted serum or plasma. The amount of dry ice needed will depend on the length of the trip and surrounding outside temperature, and should allow for a 24-hour delay in delivery. Discuss the amount required with the shipper in order to ensure that enough dry ice is added in order to maintain frozen specimens sufficiently to the destination.
- EDTA tubes containing blood for germline DNA extraction should be shipped on the same day as the blood draw (if possible), unfrozen, on a cold pack conditioned to maintain refrigerated temperatures during shipment. Blood should NOT be transported frozen, and particularly not at -20ºC.
- The amount of refrigerant or dry ice (depending on which type of specimen is being shipped) should allow for a 24-hour delay in transport.
- A consideration for larger sites is the inclusion of temperature monitors within the shipping containers of frozen specimens to validate that temperature has been maintained and indicate if significant warming has taken place.

The “NCI Best Practices for Biospecimens Resources” (June 2007) provides further information on specimen storage, under Section B.1.4, “Biospecimen Storage”, pages 4-5 of that document (Research, 2007).