For Protocol Amendment #18 submission to:

NCI Protocol #: 9557
Local Protocol #: 14-186

NCI Version Date: February 15, 2018
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Summary of Changes

#	Section	Change
1.	**Front Page**	Editortial updates to protocol amendment number and version date
2.	2.2.2.4	Revised CTCAE V.4 to V.5
3.	**3.1.4**	Eligibility criteria revised to change the CTCAE v4.0 to v5.0.
4.	7.2	Edited to state CTCAE V5.0 will be utilized starting April 1, 2018. A link to the new version was also provided.

NCI Protocol #: *CTEP#9557*

Local Protocol #: **14-186**

TITLE: Phase I study of AT13387 in combination with dabrafenib and trametinib in patients with BRAF-mutant melanoma and other solid tumors.

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Trametinib (GSK1120212), NSC# - 763093
Dabrafenib (GSK2118436), NSC# - 763760
AT13387, NSC# - 749712

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SCHEMA

Dose Escalation

Dose Level	Dabrafenib [BID/PO]	Trametinib [QD/PO]	AT13387 [D1,8,15/IV]
-1	75 mg	1 mg	180 mg/m^2
1	**150 mg**	**1 mg**	**180 mg/m^2**
2	150 mg	2 mg	180 mg/m^2
3	150 mg	2 mg	220 mg/m^2
4	150 mg	2 mg	260 mg/m^2

The preceding table summarizes the planned dose levels. The primary objective of the dose escalation component of the study is to define the maximum tolerated dose (MTD) of the triple combination of dabrafenib, trametinib, and AT13387. Dose escalation will follow a standard 3+3 design beginning in dose level 1. The doses of dabrafenib and trametinib for Dose Level 1 were selected based upon the results of the phase I study showing excellent tolerability and safety, and that this combination appears to be associated with a reduced incidence and severity of *some* of the toxic effects of monotherapy with either a BRAF or MEK inhibitor., as well as excellent clinical activity. (*Flaherty et al. 2012*) Of note, the first dose cohort (dose level 1) incorporates a trametinib dose of 1 mg daily as opposed to the FDA-approved dose of 2 mg daily. This dose was chosen due to the theoretical overlapping ocular toxicity of trametinib and AT13387.

It is conceivable that additional cohorts may be necessary to pick an optimal treatment regimen with the three agents. As such, if toxicity is seen at a dose level where dose modification is necessary and the prior, lower dose level was treated without DLT, then additional dose cohorts between the two dose levels will be considered (see section 5 for details of this process). If the toxicity is determined to be related to dabrafenib (i.e. palmar-plantar erythrodysesthesia syndrome, arthralgia) then the dose of dabrafenib will be reduced first to 75 mg BID and then increased to 100 mg BID if that first dose level is tolerated. If the toxicity is determined to be related to trametinib, then the dose of trametinib will be reduced to 1 mg QD (if a dose higher than 1 mg QD is being evaluated in the dose cohort) and then increased to 1.5 mg QD if that first dose level is tolerated. If the toxicity is determined to be related to AT13387 then the dose will be reduced by 1 dose cohort. At the end of the planned enrollment to the phase 1 portion of the study, a complete review of the PK data and safety will occur before cohort expansion at the MTD.

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1. OBJECTIVES

1.1 Phase I Objectives

1.1.1 Primary Objective

1.1.1.1 To determine the maximum tolerated dose (MTD), toxicity, and safety profile of AT13387 given weekly in combination with dabrafenib and trametinib in patients with BRAF-mutant metastatic or unresectable solid tumors.

1.1.2 Secondary Objectives

1.1.2.1 To obtain preliminary estimates of the objective response rate (ORR) and progression-free survival (PFS) and document the 6-month PFS and 1-year overall survival (OS) of patients with BRAF-mutant metastatic or unresectable melanoma treated with AT13387 given weekly in combination with dabrafenib and trametinib.

1.1.2.2 To describe the pharmacokinetics of treatment with dabrafenib, trametinib, and AT13387.

2. BACKGROUND

2.1 BRAF mutant melanoma and other solid tumors

The incidence of melanoma has dramatically risen over the past several decades and in 2013, an estimated 76,690 new cases and 9,480 deaths are expected in the United States. (*Seigel et al.,* 2013) As a result, melanoma is now the 5th and 7th most common malignancy in the US among men and women, respectively. The prognosis for patients with metastatic melanoma is 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, the 1-year survival rates of M1a, M1b, and M1c are 68%, 58%, and 38% respectively. (*Balch et al.,* 2009) Still, melanoma typically disseminates widely, and frequently involves sites that are uncommon in other cancers, such as the GI tract and the skin. Treatment options for stage IV melanoma have traditionally been extremely limited, with only dacarbazine (DTIC) and high-dose interleukin 2 (HD IL-2) receiving FDA-approval from 1976-2011. In 2011, however, two new agents were FDA-approved for use in metastatic melanoma; ipilimumab, an anti-cytotoxic T-lymphocyte 4 monoclonal antibody, and vemurafenib, a BRAF inhibitor.

Melanoma harbour oncogenic BRAF mutations in 40-50% of cases. (*Davies et al.,* 2002) These mutations lead to constitutive activation of the BRAF kinase that then hyperactivates mediators of the mitogen activated protein kinase (MAPK) pathway including MEK and ERK. The ramifications of constitutive activation of the MAPK pathway include cell cycle dysregulation,

apoptosis resistance, immune evasion, and increased invasion and metastases. Further, patients with BRAF mutant melanoma have a worse prognosis than those without BRAF mutations. (*Long et al.,* 2012) Since the discovery that BRAF mutations are present in nearly half of all melanomas, strategies to develop therapeutic inhibitors have been underway. The first such agent to be associated with substantial clinical activity was vemurafenib. In the phase I study, it became clear early that treatment of patients with BRAF-mutant melanoma was associated with remarkable responses. (*Flaherty et al.,* 2010) In subsequent phase II and III studies, the activity of vemurafenib has been clearly established, objective response rates of approximately 50% and progression free survival of 6-7 months, and shown to be superior to treatment with chemotherapy in terms of response rate, PFS, and overall survival. (*Chapman et al.,* 2011; *Sosman et al.,* 2012) In May 2013, two additional agents, the BRAF inhibitor dabrafenib and the MEK inhibitor trametinib, were approved by the FDA for the treatment of melanoma based on Phase III studies showing superiority of single-agent treatment of each agent compared with chemotherapy. (*Hauschild et al.,* 2012; *Flaherty et al.,* 2012 #1) In January 2014, the combination of dabrafenib plus trametinib was approved based on data suggesting that combination therapy is better than single-agent BRAF inhibitor therapy. (*Flaherty et al., 2012 #2)*

Oncogenic BRAF mutations are also seen across a number of other malignancies including 40-50% of papillary thyroid cancer, 10% of colorectal cancer, less than 5% of non-small cell lung cancer, and in lower percentages of other malignancies. BRAF inhibitor therapy has been tested widely in these diseases with variable results, and now dual BRAF/MEK inhibitor targeting is being tested. Specifically, responses have been seen with vemurafenib (*Dadu et al. 2014*) in nearly half of patients with BRAF-mutant papillary thyroid cancer, and there is an ongoing study evaluating the effectiveness of dabrafenib with or without trametinib in recurrent, BRAF-mutant recurrent thyroid cancer (NCT01723202). Additionally, there is a 12% response rate in BRAF-mutant colon cancer with the combination of dabrafenib and trametinib (*Corcoran et al. 2014*), and a number of clinical trials are looking to improve the efficacy of BRAF-targeted therapy in this subset of colon cancer patients (NCT01719380, NCT01750918, NCT01791309). Lastly, a number of reports are emerging detailing the responsiveness of BRAF-mutant lung cancer to single-agent BRAF inhibition. (*Peters et al. 2013*, *Robinson et al. 2014*)

2.2 CTEP Agents

2.2.1 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 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).

2.2.1.1 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.

2.2.1.2 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), $AUC_{0\text{-}24}$ 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 $AUC_{0\text{-}24}$ 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 $AUC_{0\text{-}24}$ 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%).

2.2.1.3 Trametinib Safety Profile

A **Comprehensive Adverse Events and Potential Risks (CAEPR)** list using NCI Common Terminology Criteria for Adverse Events (CTCAE) terms is included in Section 7.1 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.

- *Retinal pigment epithelial detachment (RPED):* RPED is a class side effect of MEK inhibitors. As of 22 May 2012, 13 cases of RPED 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.

2.2.1.4 Clinical Experience with the Combination of Trametinib + Dabrafenib

Preliminary data on 45 patients participating in the phase 1/2 study of dabrafenib and trametinib, BRF113220, have been reported (Infante *et al.*, 2011).

PK:
The plasma levels of dabrafenib were higher in combination with trametinib as compared to that with monotherapy. Geometric mean Day 15 AUC of dabrafenib in combination ranged from 3539 to 5187 ng•hr/mL, and the AUC observed in the monotherapy study was 2619 ng•hr/mL. Further data are required to understand this difference.

PK of trametinib did not appear to be affected by the addition of dabrafenib. Preliminary results showed that the geometric mean dose-normalized $AUC_{0-\tau}$ (CV%) for trametinib (dose normalized for the 2.0 mg QD dose) in combination with dabrafenib at 150 mg BID was 302 ng•hr/mL (n=17; 35%) on Day 15. Historical PK data from the trametinib FTIH study (MEK111054) indicated a mean Day 15 $AUC_{0-\tau}$ (CV%) of 360 ng•hr/mL (31%).

Safety and the RP2D for the combination of trametinib and dabrafenib
One DLT of a recurrent grade 2 neutrophilic panniculitis occurred, and pyrexia was common (51%). The RP2D was 150 mg BID dabrafenib plus 2 mg QD trametinib (both agents at the RP2D for single agent). Of the 137 patients enrolled, 32 patients were treated at the RP2D. SAEs experienced by more than one patient include: pyrexia (5%), hypotension (4%), nausea (3%), and 2% of patients had a constellation of AEs including vomiting, dehydration, or renal failure. The only grade 4 AE was a sepsis-like syndrome with fever/hypotension. Grade 3 AEs included generalized rash (n=2, 4%) and neutropenia (n=2, 4%). Skin toxicity (rash) occurred in 9 (20%) patients. Of note, the rate of SCC was 2% in this study. A single case of grade 5 hyponatremia was reported.

Activity
Among 77 evaluable patients with melanoma who had not received prior BRAF inhibitors, there were 43 responses (56%), including 4 CRs (5%) and 39 PRs (51%) (Weber *et al.*, 2012). Twenty-nine patients experienced SD, and three patients experienced PD. Patients were treated on four escalating dose levels of dabrafenib/trametinib (mg BID/mg QD): 75/1, 150/1, 150/1.5, 150/2. The confirmed RR for each dose level, respectively, was 67% (n=6), 64% (n=22), 48% (n=25), and 54% (n=24). Median PFS (months) for each of the first three dose levels, respectively, was 8.7, 8.3, and 5.5; PFS data are not mature for the fourth (150/2) dose level. Overall PFS was 7.4 months.

Currently, the randomized phase 2 portion (Part C) of the study of dabrafenib with or without trametinib has enrolled 162 patients as of September 1, 2011 (Investigator's Brochure, 2012b).

2.2.1.5 Clinical Experience with the Combination of Trametinib + GSK2141795 (AKT inhibitor) (TAC113886)

Twenty-three patients with advanced solid tumors received the combination using a zone-based escalation procedure enabling evaluation of multiple combination doses in parallel cohorts (Kurzrock *et al.,* 2011). While the RP2D for single agent trametinib and GSK2141795 are 2 mg/d and 75 mg/d, dose reductions were required for the combination. DLTs include grade 2 AST and ALT elevation, and grade 3 chest pain with sustained ventricular tachycardia; all DLTs were reversible with drug interruption. The most common AEs (≥10%) included nausea (26%), AST elevation (22%; grade 3/4, 9%), fatigue (22%) and rash (22%). Three MTDs were defined for variable dose ratios: 2 mg trametinib + 25 mg GSK2141795; 0.5 mg trametinib + 75 mg GSK2141795; and 1.5 mg trametinib + 50 mg GSK2141795. Three of 13 evaluable patients (unselected) had tumor shrinkage of 8% (ovarian), 16% (endometrial), and 17% (ovarian) after 8 weeks on study.

The dose regime of 1.5 mg trametinib + 50 mg GSK2141795 will be considered for further development. Additional trials to explore alternate schedules (*e.g.*, intermittent) and pharmacodynamic markers are ongoing.

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.* dacabazine (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).

Dabrafenib mesylate (GSK2118436B, Tafinlar®; referred to as dabrafenib hereafter), a 4-(3-aminosulfonylphenyl)-5-(pyrimidin-3-yl) thiazole, is an ATP-competitive, selective inhibitor of RAF kinase currently in clinical development. On May 29, 2013, the U.S. FDA approved dabrafenib for the treatment of patients with unresectable or metastatic melanoma with $BRAF^{V600E}$ mutation as detected by an FDA-approved test (FDA, 2013). On January 10, 2014, the FDA granted accelerated approval to dabrafenib and MEK inhibitor trametinib for use in combination to treat patients with unresectable or metastatic melanoma with either $BRAF^{V600E}$ or $BRAF^{V600K}$ mutation as detected by an FDA-approved test (FDA, 2014).

2.2.2.1 Mechanisms of Action and Preclinical Data with Dabrafenib

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 more than 270 kinases tested outside RAF isoforms, only 10 kinases were inhibited at a 50% inhibitory concentration (IC_{50}) <100 nM: LIM domain kinase 1 (LIMK1), activin receptor-like kinase 5 (ALK5)/ transforming growth factor (TGF)-beta receptor type-1 (TGFβ1R), Never In Mitosis Gene A (NIMA)-related kinase 11 (NEK11), salt-inducible kinase 1 (SIK1), salt-inducible kinase 2 (SIK2), polycystin-2 (PKD2), protein tyrosine kinase 6/breast tumor kinase (BRK), pancreatic eukaryotic initiation factor-2 alpha (eIF2α) kinase (PEK)/eIF2α kinase (PERK), endothelium-specific receptor tyrosine kinase 2 (TIE2) (R849W), and yeast casein kinase 1 (CK1) (IB, 2013a).

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 (IB, 2013a). 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 Pharmacokinetics of Dabrafenib

Following single-dose oral administration of dabrafenib HPMC capsules, plasma concentrations peaked approximately 2.0 hours post-dose. Oral bioavailability is near complete (94.5%) relative to an intravenous (IV) microdose.

Dabrafenib is highly bound to plasma proteins (99.6%). Its volume of distribution after IV dosing is 45.5 L. Intravenous plasma clearance (12.0 L/hr) is low relative to liver blood flow, suggesting a low hepatic extraction ratio drug. Median terminal half-life is approximately 8 hours after a single oral dose.

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 $t_{1/2}$ (21-22 hours) and accumulated following repeat dosing. M7 is the most abundant, accounting for 54% of the three metabolites. 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 for dabrafenib:
Dabrafenib induces CYP3A4 and CYP2C9. Dabrafenib decreased the systemic exposures of midazolam (a CYP3A4 substrate), S-warfarin (a CYP2C9 substrate), and R-warfarin (a CYP3A4/CYP1A2 substrate). Co-administration of dabrafenib 150 mg twice daily for 15 days and a single dose of midazolam 3 mg (a CYP3A4 substrate) decreased midazolam AUC by 74%. Co-administration of dabrafenib 150 mg twice daily for 15 days and a single dose of warfarin 15 mg decreased the AUC of S-warfarin (a CYP2C9 substrate) by 37% and the AUC of R-warfarin (a CYP3A4/CYP1A2 substrate) by 33%.

In vitro studies show that dabrafenib is a substrate of CYP3A4 and CYP2C8 while hydroxy-dabrafenib and desmethyl-dabrafenib are CYP3A4 substrates. Co-administration of dabrafenib 75 mg twice daily and ketoconazole 400 mg once daily (a strong CYP3A4 inhibitor) for 4 days increased dabrafenib AUC by 71%, hydroxy-dabrafenib AUC by 82%, and desmethyl-dabrafenib AUC by 68%. Co-administration of dabrafenib 75 mg twice daily and gemfibrozil 600 mg twice daily (a strong CYP2C8 inhibitor) for 4 days increased dabrafenib AUC by 47%, with no change in the AUC of dabrafenib metabolites. Dabrafenib is a substrate of human P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) in vitro.

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 for dabrafenib monotherapy:
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 in the FTIH monotherapy study (BRF112680). The study enrolled 114 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 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, 187 received dabrafenib and 63 received DTIC from February to September 2011. 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:
In phase 1 trial, 18 patients had cancers other than melanoma: CRC (7), papillary thyroid

cancer (PTC) (13), NSCLC (1) and ovarian cancer (1). Confirmed PRs were seen in one patient with CRC, and in 5 patients with PTC; the patient with NSCLC had an unconfirmed PR at 6 weeks. Eleven patients (6 with PTC and 5 with CRC) had stable disease (SD) as their best response; the ovarian cancer patient had SD for approximately 36 weeks.

2.2.2.3 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).

PK
Coadministration of dabrafenib 150 mg twice daily and trametinib 2 mg once daily resulted in no clinically relevant pharmacokinetic drug interactions.

RP2D for the combination of trametinib and dabrafenib
In the dose escalation portion (Part B) of study BRF113220, the MTD of the combination was not reached, and the RP2D was therefore 150/2. (Flaherty et al., 2012). Pyrexia, chills, and nausea were the most common reasons cited for dose reductions; pyrexia, chills, and decreased ejection fraction were the most common reasons cited for dose interruptions. Comprehensive safety data for the combination of dabrafenib and trametinib are presented in Section 2.2.2.4.

Activity of dabrafenib + trametinib
In the phase 2 portion of study BRF113220, among 162 patients with $BRAF^{V600E}$ or $BRAF^{V600K}$ mutation-positive melanoma, were randomized to 3 arms: dabrafenib 150 mg BID + trametinib 2 mg QD, dabrafenib 150 mg BID + trametinib 1 mg QD, and single-agent dabrafenib 150 mg BID, efficacy analyses were performed in the intention-to-treat population, 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 (see table below).

End Point (as assessed by the investigators)	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)

2.2.2.4 Safety profile of **Dabrafenib** or **Dabrafenib-Trametinib** Combination

Comprehensive Adverse Events and Potential Risks (CAEPR) lists using NCI

Common Terminology Criteria for Adverse Events (CTCAE) terms for dabrafenib and for trametinib are included in Section 7.1 of the protocol.

Based on available AE data from clinical studies involving **dabrafenib** to date, the most common drug-related AE was hyperkeratosis (29%). Other commonly reported (>15%) drug-related AEs included alopecia, arthralgia, fatigue, skin papilloma, pyrexia, and rash (IB, 2013).

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.

Common Adverse Events of Dabrafenib Monotherapy Based on Phase III Trial of Dabrafenib vs. Dacarbazine in Patients with Advanced Melanoma (adapted from Dabrafenib Package Insert)

Adverse Reaction or Laboratory Abnormality	Dabrafenib (n=187)		Dacarbazine (n=59)	
	All Grades[a]	Grades 3 and 4[b]	All Grades[a]	Grades 3 and 4
Skin and subcutaneous tissue disorders				
Hyperkeratosis	37	1	0	0
Alopecia	22	NA[f]	2	NA
Palmar-plantar erythrodysesthesia syndrome	20	2	2	0
Rash	17	0	0	0
Nervous system disorders				
Headache	32	0	8	0
General disorders and administration site conditions				
Pyrexia	28	3	10	0
Musculoskeletal and connective tissue disorders				
Arthralgia	27	1	2	0
Back pain	12	3	7	0
Myalgia	11	0	0	0
Neoplasm benign, malignant, and unspecified (including cysts and polyps)				
Papilloma[c]	27	0	2	0
cuSCC[d, e]	7	4	0	0
Respiratory, thoracic, and mediastinal				
Cough	12	0	5	0
Gastrointestinal disorders				
Constipation	11	2	14	0
Infections and infestations				

Common Adverse Events of Dabrafenib Monotherapy Based on Phase III Trial of Dabrafenib vs. Dacarbazine in Patients with Advanced Melanoma (adapted from Dabrafenib Package Insert)

Adverse Reaction or Laboratory Abnormality	Dabrafenib (n=187)		Dacarbazine (n=59)	
	All Grades[a]	Grades 3 and 4[b]	All Grades[a]	Grades 3 and 4
Nasopharyngitis	10	0	3	0

[a] NCI CTCAE v5.
[b] Gade 4 adverse reactions limited to hyperkeratosis (n=1) and constipation (n=1).
[c] Includes skin pappiloma and papilloma.
[d] Includes squamous cell carcinoma of the skin and keratoacanthoma.
[e] Cases of cutaneous squamous cell carcinoma were required to be reported as Grade 3 per protocol.
[f] NA = not applicable..

Common Adverse Events of Dabrafenib-Trametinib Combination vs. Dabrafenib Monotherapy. The phase 2 portion of study BRF113220 (referred to as Part C) included 3 arms: dabrafenib 150 mg BID + trametinib 2 mg QD, dabrafenib 150 mg BID + trametinib 1 mg QD, and single-agent dabrafenib 150 mg BID. The most common AE resulting in permanent discontinuation was pyrexia (4%). AEs led to dose reductions in 49% and dose interruptions in 67% of patients treated with dabrafenib in combination with trametinib. The table below presents selected adverse reactions and treatment-emergent laboratory abnormalities in this study.

Selected AEs and Laboratory Abnormalities Occurring in ≥10% at (All Grades) or ≥5% (Grades 3 or 4) of Patients Treated With Dabrafenib in Combination With Trametinib

Adverse Reaction or Laboratory Abnormality	Dabrafenib + Trametinib 2mg (n=55)		Dabrafenib + Trametinib 1mg (n=54)		Dabrafenib (n=53)	
	All Grades[a]	Grades 3 and 4	All Grades[a]	Grades 3 and 4	All Grades[a]	Grades 3 and 4
General disorders and administrative site conditions						
Pyrexia	71	5	69	9	26	0
Chills	58	2	50	2	17	0
Fatigue	53	4	57	2	40	6
Edema peripheral[b]	31	0	28	0	17	0
Skin and subcutaneous tissue disorders						
Rash[c]	45	0	43	2	53	0
Night sweats	24	0	15	0	6	0
Dry skin	18	0	9	0	6	0
Dermatitis acneiform	16	0	11	0	4	0
Actinic keratosis	15	0	7	0	9	0
Erythema	15	0	6	0	2	0
Pruritis	11	0	11	0	13	0
Gastrointestinal disorders						

Selected AEs and Laboratory Abnormalities Occurring in ≥10% at (All Grades) or ≥5% (Grades 3 or 4) of Patients Treated With Dabrafenib in Combination With Trametinib

Adverse Reaction or Laboratory Abnormality	Dabrafenib + Trametinib 2mg (n=55)		Dabrafenib + Trametinib 1mg (n=54)		Dabrafenib (n=53)	
	All Grades[a]	Grades 3 and 4	All Grades[a]	Grades 3 and 4	All Grades[a]	Grades 3 and 4
Nausea	44	2	46	6	21	0
Vomiting	40	2	43	4	15	0
Diarrhea	36	2	26	0	28	0
Metabolism and nutritional disorders						
Decreased appetite	22	0	30	0	19	0
Dehydration	11	0	6	2	2	0
Vascular disorders						
Hemorrhage[d]	16	5	11	0	8	2
Renal and urinary disorders						
Renal failure[e]	7	7	2	0	0	0
Hematology						
Leukopenia	62	5	46	4	21	0
Neutropenia	55	13	37	2	9	2
Liver function tests						
Increased AST	60	5	54	0	15	0
Increased alkaline phosphatase	60	2	67	6	26	2
Increased ALT	42	4	35	4	11	0
Hyperbilirubinemia	15	0	7	4	0	0
Chemistry						
Hyperglycemia	58	5	67	6	49	2
Hyponatremia	55	11	48	15	36	2
Hypophosphatemia	47	5	41	11	40	0
Increased creatinine	24	5	20	2	9	0

[a] NCI CTCAE v5.
[b] Includes the following terms: peripheral edema, edema, and lymphedema.
[c] Includes the following terms: rash, rash generalized, rash pruritic, rash erythematous, rash popular, rash vesicular, rash macular, rash maculo-papular.
[d] Includes the following terms: brain stem hemorrhage, cerebral hemorrhage, gastric hemorrhage, epistaxis, gingival hemorrhage, hematuria, vaginal hemorrhage, hemorrhage intracranial, eye hemorrhage, and vitreous hemorrhage.
[e] Includes the following terms: renal failure and renal failure acute.

AEs of special interest:

The following events observed with dabrafenib monotherapy and for dabrafenib plus trametinib are discussed in further detail because they may represent a class effect of BRAF and/or MEK inhibitor compounds, and/or are potentially life-threatening. AEs of special interest associated with dabrafenib or trametinib individually are listed in the table below:

AEs of special interest that are	AEs of special interest that are associated with trametinib

associated with dabrafenib (BRAF category AEs) are: • Skin-related toxicities • Pyrexia • Malignancies • Renal failure (renal failure, renal failure acute) • Uveitis • Hyperglycemia • Pancreatitis	(MEK category AEs) • Skin-related toxicities (e.g., rash – generalized, macular, maculopapular, pruritic, erythematous, etc; dermatitis acneiform; erythema; skin exfoliation • Diarrhea • Ocular events (e.g., RVO, RPED (previously termed CSR)) • Hepatic events (e.g., aspartate aminotransferase [AST], ALT, and blood bilirubin increased) • Cardiac-related events (e.g., LVEF decreased and left ventricular dysfunction) • Hypertension • Pneumonitis (pneumonitis, interstitial lung disease) • Hemorrhages

In general, the overall profile of "AEs of special interest" observed with the combination of trametinib-dabrafenib is consistent with the known profiles of each separate drug, the most notable differences being the increase in pyrexia and the decrease in skin-related toxicities with combination therapy relative to monotherapy.

For "MEK-related AEs of special interest," the overall incidence in the dabrafenib-trametinib combination arm in trial BRF113220 was 91%, which was similar to the incidence reported with trametinib ISS monotherapy (94%), but higher than the incidence in the dabrafenib alone arm. However, MEK-related skin toxicities, diarrhea and hypertension appeared to be lower in the combination arm, as compared with the trametinib-only treated population. The incidence rate of ocular events was higher relative to the trametinib ISS population.

For "BRAF-related AEs of special interest," the incidence of any event in combination arm was higher (84%) than either the Dabrafenib ISS population (49%) or the trametinib ISS population (19%). This increase is predominantly due to the increased incidence of pyrexia observed with combination treatment. Also noted were an increase in renal failure and a decrease in cuSCC and PPES when comparing the combination to dabrafenib ISS population.

The following sections provide more detailed description of the AEs of Special Interest.

Dermatologic toxicities (dabrafenib or dabrafenib-trametinib):

Dabrafenib monotherapy has been associated with skin-related toxicities including hyperkeratosis, skin papilloma, rash, seborrheic keratosis, acrochordon as well as rash and pruritis and cutaneous squamous cell carcinoma.

With the combination of dabrafenib-trametinib at 150/2 (Part C of the phase II trial), skin-related toxicity occurred in 65% of subjects (IB, 2013). This incidence was lower than observed in the trametinib ISS population (88%, 288 out of 329 subjects). The most frequent skin-related toxicities (affecting >10% treated with combination) were rash,

dermatitis acneiform, erythema, and rash generalized. The incidence and severity of the majority of skin-related toxicities and especially those most often seen with either trametinib- or dabrafenib therapy alone appear to be reduced when both compounds are combined.

Malignancies (dabrafenib or dabrafenib-trametinib):

Cutaneous SCC and keratoacanthomas*:* 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.

Across clinical trials of dabrafenib monotherapy (n=586), the incidence of cutaneous SCC was 11%. Of those patients who developed new SCC, approximately 33% developed one or more SCC with continued administration of dabrafenib.

In randomized trial with dabrafenib vs. dabrafenib-trametinib combination (BRF113220), the incidence of cutaneous SCC/keratocanthoma was statistically lower with150/2 combination therapy relative to dabrafenib alone (7 vs. 19%). The median time to the first occurrence of keratoacanthoma/cuSCC was 152 days in the combination treatment group as compared to 30.5 days in the dabrafenib alone arm.

New primary malignant melanoma: In the randomized trial for dabrafenib-trametinib combination (BRF113220), new primary melanoma occurred in 2% (1/53) on dabrafenib monotherapy [similar to the dabrafenib ISS population (1%)] and in none of 55 patients receiving dabrafenib + trametinib (IB, 2013). The overall frequency of new primary melanomas observed with dabrafenib treatment approximates that expected for untreated subjects with antecedent melanoma.

Other treatment-emergent malignancies: Non-cutaneous secondary malignancies have also been reported in patients receiving dabrafenib or dabrafenib-trametinib combination. In patients receiving dabrafenib-trametinib combination, five cases out of 365 subjects (1%) were identified as having non-cutaneous malignancies: KRAS mutation-positive pancreatic adenocarcinoma (n=1), recurrent NRAS mutation-positive CRC (n=1), head and neck carcinoma (n=1), glioblastoma (n=1), and pre-existing renal cell carcinoma (n=1) (FDA label). No increase was detected in the overall frequency of treatment emergent malignancies in melanoma subjects receiving dabrafenib and trametinib treatment in Study BRF113220 as compared to the dabrafenib safety population. Dabrafenib should be permanently discontinued for RAS mutation-positive non-cutaneous malignancies.

Pyrexia (dabrafenib or dabrafenib-trametinib): Pyrexia and pyrexia-related events, including influenza-like illness, cytokine release syndrome, and systemic inflammatory response syndrome are common side effects associated with dabrafenib. In dabrafenib-trametinib combination study BRAF113220 Part C, pyrexia and related events in the

combination arm (150/2) were increased in frequency and severity (76%; 5% grade 3, no grade 4), as compared with dabrafenib monotherapy ISS population (33%; 2% grade 3, no grade 4). Eleven percent of subjects in the combination group required hospitalization for episodes of serious pyrexia (IB, 2013). Approximately 50% of the pyrexia-related events in the Part C 150/2 arm resulted in dose interruption and/or dose reduction, a higher proportion than in the dabrafenib ISS population (15% to 30%). The majority of subjects (>80%) who dose-reduced dabrafenib due to AEs were able to be dose re-escalated.

All SAEs of pyrexia-related events (pyrexia, influenza-like illness, cytokine release syndrome, and systemic inflammatory response syndrome) were manually reviewed to identify cases described as having experienced **serious non-infectious febrile events** with complications of hypotension, dehydration, severe rigors/chills, or renal failure in the absence of another identifiable etiology (*e.g.*, infection). Ten such subjects were identified among 404 subjects (2.5%) in the entire combination therapy population as compared to 1% in the dabrafenib ISS population; 9 of these subjects were hospitalized. All of these subjects required dose interruption(s) and/or dose modification(s); one subject permanently discontinued study drug after experiencing fever, muscle weakness, dehydration, and hyponatremia. All subjects responded to symptomatic therapy with either NSAIDs, paracetamol, or corticosteroids and best supportive care including IV fluids.

Renal failure (dabrafenib or dabrafenib-trametinib): Renal failure was observed in the dabrafenib ISS population (<1%; <1% grade 3/4) and trametinib ISS population (2%; no grade 3, <1% grade 4), and was increased in incidence and severity in the combination arm in study BRF113220 (7%, all grade 3) (IB, 2013). Most cases of acute renal failure presented as a secondary event in the setting of pyrexia where dehydration appeared to be a contributing factor and/or in concert with other risk factors such as hemolytic uremic syndrome (HUS), antibiotic toxicity, or hypercalcemia. There was one case of advanced renal failure which may have been drug-induced but whose precise etiology was not clear. The renal events led to permanent discontinuation of study drugs in one subject, and to dose interruptions in three subjects.

Hypertension (dabrafenib-trametinib): Hypertension has been associated with trametinib therapy. In the combination study of dabrafenib-trametinib, the combination arm had a higher rate of hypertension compared to the dabrafenib ISS population (9% vs. 2%); however, this rate was lower than that in the trametinib ISS population (15%) (IB, 2013).

In either the combination or the dabrafenib monotherapy population, there were no SAEs related to hypertension, and hypertension did not lead to treatment discontinuation, dose reduction or dose interruption in any of the patients.

Cardiac valvular abnormalities (dabrafenib or dabrafenib-trametinib)*:* 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.

Cardiomyopathy (dabrafenib-trametinib): Cardiomyopathy has been associated with trametinib use and therefore the incidence was increased in the dabrafenib-trametinib combination compared to dabrafenib alone. Cardiac-related AEs occurred in 9% of subjects in the Part C 150/2 group, which is the same incidence as in the Trametinib ISS population (9%), but a higher incidence compared with the Dabrafenib ISS population (2%) (IB, 2013). Decreased ejection fraction was the only AE reported in the Part C 150/2 group, and all reports were either grade 1 or 2.

Ocular adverse events: Ocular events occurred at a higher frequency in study BRF113220 Part C 150/2 combination group (25%) compared to trametinib (13%) and dabrafenib (8%) monotherapy ISS populations (IB, 2013). Blurred vision, dry eye, and visual impairment were the most commonly reported ocular events in the Part C 150/2 group. All ocular events in Part C 150/2 were grade 1 to 2 with the exception of one case of grade 3 retinal pigment epithelial detachment (RPED).

RPED and RVO (dabrafenib-trametinib): These two events are associated with trametinib therapy and therefore were observed in the combination of dabrafenib and trametinib. Of 365 subjects in Study BRF113220, the incidence of RPED remained at 1% and is thus similar to the frequency observed in the overall trametinib development program so far. Thus, the addition of dabrafenib appears to have no impact on the frequency or severity of RPED previously reported for trametinib.

RVO has not been reported as an AE in the dabrafenib ISS population of 586 subjects. Addition of dabrafenib to trametinib in the combination treatment regimen in Study BRF113220 did not increase the frequency of RVO observed thus far with trametinib monotherapy.

Uveitis, iritis, and iridocyclitis (dabrafenib or dabrafenib-trametinib): Uveitis and iritis can occur when dabrafenib is administered as a single agent or in combination with trametinib. In the 365 subjects with melanoma treated on the dabrafenib-trametinib combination arm in Study BRF113220, the incidence of ocular events including uveitis, iritis, or iridocyclitis was 2%, and responded to symptomatic therapy, which included primarily the use of topical corticosteroids. This rate is slightly higher than in the dabrafenib ISS population (1%). In addition, the severity of the inflammatory ocular events also appeared to be slightly increased, with 2 cases of uveitis Grade 3 and 1 case of Grade 4.

Hyperglycemia (dabrafenib or dabrafenib-trametinib): Hyperglycemia can occur when dabrafenib is used as a monotherapy or in combination with trametinib. In study BRF112680 (dabrafenib monotherapy), 5/12 patients with a history of diabetes required more intensive hypoglycemic therapy while taking dabrafenib; the incidence of grade 3 hyperglycemia was 6% (12/187) in patients treated with dabrafenib compared with none

of the dacarbazine-treated patients. In study BRF113220 (combination with trametinib), the incidence of hyperglycemia was 5% (3/55) in patients treated with dabrafenib-trametinib compared with 2% (1/53) in patients treated with dabrafenib (FDA label).

Pancreatitis (dabrafenib or dabrafenib-trametinib): Pancreatitis (<1%) and/or increased lipase/amylase (2%) have been reported at low frequency with dabrafenib. In the phase 2 combination study BRAF113229, AEs of acute pancreatitis or pancreatitis occurred in six (1%) subjects on the dabrafenib-trametinib arm (IB, 2013), and none with dabrafenib monotherapy. The time to onset of pancreatitis ranged from Study Day 21 to 292 (median: 138 days). At the data cut-off, 4 subjects had recovered from the event of pancreatitis. Discontinuation of study drugs due to pancreatitis was not deemed necessary by the investigators in any of the 6 cases. The incidence of pancreatitis was <1% in the dabrafenib ISS population (2 subjects) and in the trametinib ISS population (1 subject).

Hepatic events (dabrafenib-trametinib combination): In the Part C 150/2 group, 15% of subjects experienced hepatic AEs as compared to 13% of subjects in the trametinib ISS population and 6% of subjects in the dabrafenib ISS population (IB, 2013). Of the hepatic AEs, increased ALT and AST were the most common events in all groups, and most were either grade 1 or 2. No cases of Hy's law were observed among any of the subjects in the BRF113220 study.

Diarrhea (dabrafenib-trametinib combination): The proportion of subjects in the Part C 150/2 group who experienced diarrhea was 36% compared with 49% in the trametinib ISS population and 16% in the dabrafenib ISS population (IB, 2013). Most subjects across the monotherapy and combination therapy dabrafenib and trametinib clinical programs reported grade 1 or grade 2 diarrhea.

Pneumonitis (dabrafenib-trametinib combination): Pneumonitis was not reported as an AE in the 365 subjects enrolled in Study BRF113220 (Investigators Brochure, 2013). However, pneumonitis was the most common drug-related SAE (1% of subjects) Trametinib ISS population. Overall, the addition of dabrafenib to trametinib does not appear to increase the frequency or severity of pneumonitis previously observed with trametinib monotherapy.

Hypersensitivity: There has been a single report of hypersensitivity (blisters) to dabrafenib, occurring on the same day as the 1st dose of study drug as well as upon rechallenge (IB, 2013). The subject recovered after interruption and then discontinuation of dabrafenib. Grade 1 AEs of blisters on limbs (4 subjects) and drug hypersensitivity (rash, 1 subject) have been reported in previous studies with dabrafenib. However, the precise etiology of these events is unclear.

Hypersensitivity to trametinib was reported by one subject 7 days after starting trametinib who experienced fever, asthenia, visual disturbance, and symptoms suggestive of a hypersensitivity reaction described by the investigator as "vascularity." This subject also developed LFT elevations, lower limb nodules that by biopsy showed "dermo-

hypodermatitis with plasmocyte and lymphocyte infiltrate." The subject recovered after discontinuation of trametinib.

Hemorrhages (dabrafenib-trametinib combination): Hemorrahge is an AE identified with the dabrafenib-trametinib combination therapy. Hemorrhages, including major hemorrhages defined as symptomatic bleeding in a critical area or organ, can occur with dabrafenib plus trametinib combination therapy (FDA label). In study BRF113220, treatment with dabrafenib in combination with trametinib resulted in an increased incidence and severity of any hemorrhagic event: 16% (9/55) of patients treated with trametinib in combination with dabrafenib compared with 2% (1/53) of patients treated with dabrafenib as a single agent. The major hemorrhagic events of intracranial or gastric hemorrhage occurred in 5% (3/55) of patients treated with trametinib in combination with dabrafenib compared with none of the 53 patients treated with dabrafenib as a single agent. Intracranial hemorrhage was fatal in two (4%) patients receiving the combination of trametinib and dabrafenib.

Glucose-6-phosphate dehydrogenase (G6PD) deficiency (dabrafenib or dabrafenib-trametinib combination): Dabrafenib, which contains a sulfonamide moiety, confers a potential risk of hemolytic anemia in patients with G6PD deficiency; these patients should be closely observed for signs of hemolytic anemia.

Embryofetal toxicity: Based on the mechanisms of action, dabrafenib and/or trametinib can cause fetal harm when administered to a pregnant woman. Dabrafenib was teratogenic and embryotoxic in rats at doses three times greater than the human exposure at the recommended clinical dose. Trametinib was embryotoxic and abortifacient in rabbits at doses greater than or equal to those resulting in exposures approximately 0.3 times the human exposure at the recommended clinical dose.

2.2.3 **AT13387**

AT13387AU (2,4-dihydroxy-5-isopropyl-phenyl)-[5-(4-methyl-piperazin-1-ylmethyl)-1,3-dihydro-isoindol-2-yl]-methanone) is the *L*-lactic acid salt of AT13387 and is the active pharmaceutical ingredient in AT13387.

2.2.3.1 Mechanisms of Action and Preclinical Data with AT13387

HSP90 is a ubiquitously expressed and highly abundant molecular chaperone, accounting for as much as 1-2% of total cell protein. Both HSP90α and heat shock protein 70 (HSP70) are induced under conditions of cellular stress to ensure the cell has an increased capacity to maintain proper protein folding. Heat shock factor-1 (HSF-1) is bound by HSP90 under non-stressed conditions. In response to a variety of cellular stresses, or direct inhibition of HSP90, HSF-1 dissociates from HSP90 and as a consequence upregulates HSP90α, HSP70, and HSP40 gene transcription. In contrast to other chaperones, which generally act to aid the folding and refolding of proteins, HSP90 is

only involved in the final maturation of a distinct set of "client" proteins. The precise manner in which HSP90 influences the folding of these proteins is not fully understood, but the process is known to be adenosine triphosphate (ATP)-dependent. HSP90 is required for the functional stabilization of numerous client proteins. Inhibition of the binding of ATP to HSP90 induces the degradation of these client proteins, growth arrest, and apoptosis. HSP90 client degradation profoundly inhibits multiple cell signaling pathways known to be critical for cell growth and survival.

In vitro studies with AT13387 have shown that the compound binds to the N-terminal ATP site on HSP90 with sub-nanomolar affinity.

AT13387 inhibits the growth of a variety of tumor cell lines including melanoma cell lines. AT13387 has single agent activity in BRAF-mutant cell lines, including those that are sensitive (A375, SkMel-28) and those that are resistant (A2058, RPMI-7951) to BRAF inhibitors. AT13387 depleted BRAF and CRAF levels as well as AKT. In the RPMI-7951 cell line, levels of the client protein COT, which is overexpressed and leads to resistance in this cell line, were also depleted. In addition, levels of pERK and phospho-S6 Ribosomal protein (pS6) were ablated in all cell lines, indicating an inhibition of AKT and ERK signaling, which are the key pathways in the majority of vemurafenib resistance mechanisms.

Activity of AT13387 in a Panel of Melanoma Cell Lines

Melanoma Cell line	**Vemurafenib IC_{50} (nM)**	**AT13387 IC_{50} (nM)**	**Mutation status**
A375	87	22	$BRAF^{V600E}$
SK-MEL-28	340	73	$BRAF^{V600E}$
SK-Mel-5	370	190	$BRAF^{V600E}$
A2058	1700	34	$BRAF^{V600E}/PTEN^{-null}$
RPMI-7951	>10000	30	$BRAF^{V600E}/COT\uparrow$
SK-Mel-2	> 10000	45	$BRAF^{WT}/N\text{-}Ras^{Q61R}$

IC_{50} values are the mean of at least 2 individual experiments

AT13387 has also been evaluated in both vemurafenib-sensitive (A375, SK-MEL-28) and vemurafenib-resistant (A2058) xenograft mouse models. AT13387 significantly inhibited the growth of all three xenografts. As expected, oral administration of vemurafenib at 50 mg/kg twice a day caused significant regression of the A375 and SK-MEL-28 tumors over the period of dosing but not in A2058. Combination of vemurafenib and AT13387 enhanced tumor growth inhibition over either of the monotherapies in the A2058 xenograft. The combination of AT13387 and vemurafenib was well tolerated (no major adverse effects on body weight) and not antagonistic.

The effects of AT13387 treatment on modulation of HSP90 clients and signaling pathways were also evaluated in the tumor xenografts models. Mice bearing A375 tumors were treated with a single dose of AT13387 and tumors were removed at various timepoints up to 72 hrs. Levels of the clients, BRAF, AKT, CRAF and cyclin dependent kinase 4 (CDK4) were depleted over this timecourse, whilst levels of HSP70, an indicator of HSP90 inhibition, were induced. Depletion of HSP90 clients and induction of HSP70 was also observed in vemurafenib resistant RPMI-7951 xenograft tumors 24 hrs after treatment. In addition an ablation of pAKT, pS6 and pERK indicated that signaling through the ERK and AKT pathways was inhibited at this time point.

2.2.3.2 Nonclinical safety of AT13387

Further information on the nonclinical program of AT13387 is provided in the AT13387 investigator's brochure (March 2013).

A CNS safety pharmacology study in rats demonstrated no adverse CNS effects. A cardiovascular safety pharmacology study was conducted in Beagle dogs. A dose-related increase in heart rate was observed at 4 mg/kg and above from 10 minutes after the start of infusion which peaked at the end of infusion and returned to control levels by approximately 5 hrs after the end of infusion. The increased heart rate observed at 15 mg/kg was associated with a concomitant decrease in blood pressure (systolic, diastolic and mean arterial). AT13387 did not have any significant effect on QT interval or QTc at any dose level tested.

Toxicity studies were conducted by 1-hr IV infusion in rodent (rat) and non-rodent (dog) species. Both species exhibited decreased body weight and food consumption, and decreases in white blood cell counts. While clinical pathology changes suggestive of adverse effects in the bone marrow, kidney and liver were observed for both species, histopathologic changes were only seen in dogs and included testes, gallbladder, bone marrow (sternum), kidneys and thymus. The overall effects observed in surviving rats and dogs were transient and reversible, with the exception of the testicular lesions observed in dogs at high doses, for which the recovery period of 14 days was not sufficient. No microscopic ophthalmic changes were seen in either species. The histological changes in the testes seen in dogs indicate that treatment with AT13387 may be associated with impaired fertility, which may be only slowly reversible or permanent.

2.2.3.3 Clinical Pharmacokinetics (PK) and Activity of AT13387

As detailed in the AT13387 March 2013 Investigator Brochure five studies have been initiated with AT13387; two Phase 1 clinical studies of AT13387 monotherapy and 3 Phase 2 combination studies;

- AT13387-01; a phase 1 monotherapy study in adults with metastatic solid tumors
- AT13387-03, a phase 1 monotherapy study sponsored by the National Cancer Institute (NCI) in adults with refractory solid tumors

- AT13387-02 a phase 2 study in adults with gastrointestinal stromal tumor (GIST) who receive AT13387 in combination with imatinib
- AT13387-04; a Phase 2 study of AT13387 alone or in combination with abiraterone acetate in the treatment of castration-resistant prostate cancer (CRPC) no longer responding to abiraterone
- AT13387-05; AT13387 alone and in combination with crizotinib in the treatment of non-small cell lung cancer (NSCLC)

AT13387-01 Study (data from AT13387/01 Clinical Study Report Version 2 signed January 2014)

AT13387-01 was the FTIH study designed to determine the safety, tolerability, pharmacokinetics, and preliminary efficacy of AT13387, administered as a 1-hour infusion either twice-weekly or once-weekly for 3 weeks in a 4-week cycle, in subjects with advanced malignancies. Sixty-two subjects were treated with AT13387 in 10 cohorts; 5 with the twice-weekly regimen (ranging from 10 mg/m^2 to 120 mg/m^2) and 5 with the once-weekly regimen (ranging from 150 mg/m^2 to 310 mg/m^2).
PK and metabolism of AT13387

In study AT13387/01 blood samples for PK analysis were collected either on Day 1 and Day 18 of Cycle 1, for the twice-weekly regimen or Cycle 1, Day 1 and Cycle 2, Day 15 for the once-weekly regimen. The PK evaluable population was 61.

The PK of AT13387 showed a dose-proportional increase in $AUC_{0\text{-}t}$ and C_{max} from 10 to 120 mg/m^2 (twice-weekly) and then from 150 to 310 mg/m^2 (once-weekly) with relatively low inter-individual variability. The elimination half-life ($t_{1/2}$) was dose-independent and ranged from 6.6 to 11.5 hrs between cohorts. There was no notable accumulation or reduction in exposures between Cycle 1, Day 1 and Day 18 in the twice-weekly regimen or Cycle 1, Day 1 and Cycle 2, Day 15 in the once-weekly regimen. From urine data available, the urinary excretion of AT13387 appeared to be low. The maximum rate of excretion was observed within 3.50 to 11.9 hours (mean T_{max}) following the IV infusion for the 10 to 120 mg/m^2 cohorts. The percent of the dose recovered in urine ranged from 0.92 to 2.98%.

AT13387 clearance or volume of distribution was independent of sex, race (albeit with significant variability) or previous treatment (radiotherapy, chemotherapy or surgery). However, AT13387 exposures (AUC) were higher and clearance was lower in two subjects with high baseline bilirubin levels, which may be indicative of reduced UGT enzyme activity in these subjects as glucuronidation constitutes the main pathway for metabolic clearance of AT13387.

As detailed in the March 2013 AT13387 Investigator Brochure in Study AT13387-02, subjects received once weekly IV infusion doses of AT13387 in combination with daily oral doses of imatinib. The plasma PK of AT13387 was assessed on Day 8 of the study (second dose of AT13387); as it was considered that the imatinib plasma concentrations would be close to steady state. Subjects in Cohort 1 of Study AT13387-02 received doses

of 180 mg/m^2: the principal mean PK parameters derived from 6 subjects are summarized in the Table below. The mean $AUC_{0\text{-}t}$ value obtained in Study AT13387-02 was greater, and showed wider inter-subject variability, than the mean AUC0-t for the same dose level in Study AT13387-01: values for $t_{1/2}$ and C_{max} of AT13387 were similar in the 2 studies. A relatively sparse PK sampling schedule was adopted for Study AT13387-02 and this may have contributed to the higher AUC0-t values obtained. On balance, there is no clear evidence of imatinib affecting the PK of AT13387 on the data obtained so far.

Plasma samples from 5 subjects in Cohort 1 of Study AT13387-02 were analyzed for the presence of the isomeric glucuronide metabolites, AT17219 and AT17220. The semi-quantitative (non-validated) LCMS method confirmed that AT17220 was the predominant metabolite in the systemic circulation. Estimated concentrations of AT17220 were >25x those of metabolite AT17219 and >40x the concentrations of AT13387 itself, in all 3 hrs to 24 hrs postdose samples.

Drug-drug interactions

No drug drug interaction studies have been performed with AT13387. However, the potential for AT13387 to inhibit cytochrome P450 (CYP) 1A2, 3A4, 2D6, 2C9, and 2C19 was assessed in vivo and the results indicated a concentration resulting in IC50 >10 μM, suggesting a low potential for clinically significant drug-drug interactions mediated by these enzymes. The potential of AT13387 to induce liver cytochrome P450 (CYP) enzymes 1A2, 2B6, and 3A4 was also assessed in cultured human hepatocytes (n=3 donors) by measuring changes in mRNA expression. No induction of CYP1A2, CYP2B6, and CYP3A4 was seen at concentrations up to 30 μM.

In addition, studies have been performed to assess if AT13387 inhibits UGT isoforms. A small amount of inhibition (~14%) of probe substrate metabolism was observed with UGT1A1, UGT1A3 and UGT1A9 when AT13387AU was tested at 20 μM. Isoforms UGT1A6 AND UGT2B7 showed no inhibition at 20 μM. These data suggests that AT13387 has a relatively low affinity for UGT isoforms and thus it has a low risk of reducing the clearance of co-administered compounds cleared primarily by this enzyme family

Pharmacodynamic effect of AT13387

The pharmacodynamics effect of AT13387 was assessed in the FTIH study AT13387-01. HSP70 expression was assessed in plasma and PBMC lysates from samples collected for upto 24 hrs after the start of infusion in Cycle 1 and before the start of the first infusion in Cycle 2. Tumor biopsy samples were collected in screening and in Cycle 1.

Increased levels of HSP70 were detected in plasma in all dosing cohorts in the study. HSP70 induction in plasma was dose-dependent until Cohort 5 (120 mg/m^2; ~2- to 6-fold increase) in the twice-weekly regimen. In the once-weekly regimen, HSP70 induction appeared to be dose-dependent until Cohort 8 (220 mg/m^2).

HSP90 client protein levels (AKT, phospho-AKT, S6, phospho-S6, CDK4, LCK and C-Raf) were also measured in PBMCs. Due to large variability in HSP90 client knockdown in PBMCs, no meaningful conclusions could be drawn.

Pre- and post-treatment tumor biopsy samples were taken and successfully analyzed from 4 subjects treated at the MTD (120 mg/m^2) for the twice-weekly regimen. Pre-treatment samples were taken at Screening or before the start of infusion on Cycle 1, Day 1. Post-treatment samples were taken approximately 24 hrs after the end of infusion on Cycle 1, Day 18 for subjects on the twice-weekly regimen and on Cycle 1, Day 16 (+7 days) for subjects on the once-weekly regimen. HSP90 client protein depletion (B-Raf, CDK4, phospho-S6 and caspase-3) was very variable in the tumor biopsies. For paired tumor samples suitable for analysis, a consistent increase in HSP70 staining was detected in the majority of the samples.

PD analysis of samples for study AT13387-02 to AT13387-05 is ongoing.

Selection of the RP2D

The RP2D for once weekly treatment was determined to be 260mg/m^2 in study AT13387-01. No DLTs were observed in the once-weekly regimen; however, the MTD was defined based on a series of moderate toxicities (mostly Grade 2 diarrhea, nausea, vomiting, fatigue and systemic infusion reactions) observed in the 310 mg/m^2 cohort.

Antitumor Activity of AT13387 Monotherapy

The antitumor activity of AT13387 in the FTIH study AT13387 has been reported in the AT13387-01 RPED. No subjects in this study had complete response (CR) as best response to therapy. One (1) subject (2%) had partial response (PR) as best response to therapy. The PR occurred in the 220 mg/m^2 once-weekly cohort after 6 months of treatment, for a duration of 113 days (~3.8 months), in a subject with GIST who had 3 prior treatments with imatinib and who had acquired a mutation conferring resistance to imatinib prior to treatment, and was on treatment for a total of approximately 10 months.

Twenty-one (21) subjects (34%) had stable disease (SD) as best response to therapy. Of the 10 subjects with NSCLC all of whom had been heavily pretreated, 4 subjects (40%) had SD with a duration of 47-102 days. Of the 7 subjects with GIST, there was 1 subject with PR and 3 subjects (43%) with SD, two of whom had SD for 231 (~7.7 months) and 335 days (~11.2 months).

As described in the AT13387 Investigator Brochure dated March 2013 no objective responses have been reported for the Phase I monotherapy study AT13387-03. In study AT13387-02 the best responses were 1 PR (180 mg/m^2/dose) and 4 subjects with SD (1 with 150 mg/m^2/dose and 3 with 180 mg/m^2/dose). Collection of additional efficacy data from these studies and studies AT13387-04 and 05 is ongoing.

2.2.3.4 AT13387 Safety Profile

A **Comprehensive Adverse Events and Potential Risks (CAEPR)** list using NCI Common Terminology Criteria for Adverse Events (CTCAE) terms is included in Section 7.1 of the protocol.

The safety data is taken from the AT13387 March 2013 Investigator brochure. SAE data is based on information from all Astex-sponsored AT13387 studies in the Safety database as of 9 January 2013 (i.e., studies AT13387-01 and AT13387-02). All AE data are based on Astex-sponsored studies AT13387-01 and AT13387-02, which represent the studies with the most subjects treated and most substantial safety information available to date (database of 87 subjects treated).

The table below summarizes SAE data based on safety database information as of 9 January 2013.

Overview of SAEs

SAE	Number (%) of Subjects with SAEs		
	AT13387-01 (N=62)	**AT13387-02 (N=25)**	**Total (N=87)**
Any SAE	22 (35)	7 (28)	29 (33)
SAEs that led to death	6 (10)	0	6 (7)
Other SAEs (i.e., those not leading to death)	16 (26)	7 (28)	23 (26)

Source: Safety database (9 Jan 2013)

SAEs were reported for 29 of 87 subjects (33%) in Astex-sponsored studies. Most of the subjects with SAEs (22 of 29) were in Study AT13387-01.

A total of 6 deaths were reported as SAEs in AT13387 studies as of 9 January 2013. Half (3 of 6) were due to disease progression. The other 3 were due to pneumonia (2 subjects) and metastatic colon cancer (1 subject). SAEs that led to death were considered not related to study treatment by both the Investigator and the Sponsor, except for metastatic colon cancer, for which the Investigator assessed causality as possible. This subject had rapidly worsening colorectal cancer and deteriorating liver function; he died at home shortly after starting AT13387 treatment. The investigator reported the death as possibly due to progressive disease but could not exclude a contribution from AT13387. The subject's ECG was normal throughout treatment.

Other SAEs (those that did not lead to death) were reported for 23 of 87 subjects (26%). The only SAEs that were reported for >1 subject were blood CPK increased and electrocardiogram QT prolonged (2 subjects for each event, 2%).

Other SAEs (those that did not lead to death) that were considered related to study treatment were reported for 11 of 87 subjects (13%). No related SAE was reported from subjects receiving less than 120 mg/m^2/dose of AT13387. All of the related SAEs were reversible other than muscular weakness (Study AT13387-01), asthenia (Study AT13387-

01), and blood creatine phosphokinase increased (Study AT3387-02), which were ongoing at last follow up or death.

As of 31 December 2012, the most common AEs (≥10 subjects), regardless of relationship, reported in decreasing order of frequency, from Studies AT13387-01 and -02, are shown below.

Summary of AEs with Highest Incidence (≥10 Subjects) in Decreasing Order of Frequency, Regardless of Relationship or Grade, for Studies AT13387-01 and -02 (as of 31 December 2012)

Adverse Event MedDRA Preferred Term	Number (%) of Subjects					
	AT-13387-01			AT-13387-02 (n=24[b])	Overall Total AT-13387-01 + AT-13387-02 (N=86)	
	2x/week (n=28)	1x/week (n=34)	Total (n=62[a])		All AEs	CTCAE Grade 3+4 AEs
Diarrhoea	20 (71)	27 (79)	47 (76)	16 (67)	63 (73)	4 (5)
Fatigue	15 (54)	19 (56)	34 (55)	8 (33)	42 (49)	0
Nausea	8 (29)	19 (56)	27 (44)	11 (46)	38 (44)	0
Abdominal pain	9 (32)	12 (35)	21 (34)	5 (21)	26 (30)	2 (2)
Decreased appetite	7 (25)	12 (35)	19 (31)	7 (29)	26 (30)	0
Dizziness	5 (18)	15 (44)	20 (32)	6 (25)	26 (30)	1 (1)
Anaemia	9 (32)	7 (21)	16 (26)	8 (33)	24 (28)	4 (5)
Vomiting	8 (29)	7 (21)	15 (24)	9 (38)	24 (28)	0
Dry mouth	7 (25)	11 (32)	18 (29)	5 (21)	23 (27)	0
Headache	4 (14)	10 (29)	14 (23)	5 (21)	19 (22)	0
Muscle spasms	3 (11)	11 (32)	14 (23)	5 (21)	19 (22)	0
Injection site reaction	1 (4)	12 (35)	13 (21)	5 (21)	18 (21)	0
Insomnia	3 (11)	9 (26)	12 (19)	4 (17)	16 (19)	0
Visual impairment	1 (4)	13 (38)	14 (23)	2 (8)	16 (19)	0
Weight decreased	3 (11)	10 (29)	13 (21)	3 (13)	16 (19)	0
Chills	2 (7)	11 (32)	13 (21)	2 (8)	15 (17)	0
Constipation	4 (14)	8 (24)	12 (19)	2 (8)	14 (16)	0
Dehydration	3 (11)	7 (21)	10 (16)	4 (17)	14 (16)	3 (3)
Hyperhidrosis	2 (7)	9 (26)	11 (17)	3 (13)	14 (16)	0
Oedema peripheral	4 (14)	2 (6)	6 (10)	7 (29)	13 (15)	0

Activity of AT13387 in a Panel of Melanoma Cell Lines (Cont'd)

Adverse Event MedDRA Preferred Term	Number (%) of Subjects					
	AT-13387-01			AT-13387-02 (n=24[b])	Overall Total AT-13387-01 + AT-13387-02 (N=86)	
	2x/week (n=28)	1x/week (n=34)	Total (n=62[a])		All AEs	CTCAE Grade 3+4 AEs
Aspartate aminotransferase increased	8 (29)	0	8 (13)	4 (17)	12 (14)	2 (2)
Cough	6 (21)	6 (18)	12 (19)	0	12 (14)	0
Infusion	5 (18)	6 (18)	11 (17)	1 (4)	12 (14)	0
Blood creatine	7 (25)	1 (3)	8 (13)	2 (8)	11 (13)	1 (1)
Hypokalaemi	1 (4)	4 (12)	5 (8)	5 (21)	10 (12)	1 (1)
Pruritus	1 (4)	5 (15)	6 (10)	4 (17)	10 (12)	0
Rash	4 (14)	5 (15)	9 (15)	1 (4)	10 (12)	0
Vision	6 (21)	3 (9)	9 (15)	1 (4)	10 (12)	1 (1)

[a] The safety dataset includes subjects who received study treatment (i.e., 1 less than the number enrolled).
[b] Study treatment for 1 subject was missing from this preliminary database; that subject is not included in the safety data set.

The most common individual events (occurring in >25% of subjects) were diarrhea (63 subjects, 73%); fatigue (42 subjects, 49%); nausea (38 subjects, 44%); abdominal pain, decreased appetite, and dizziness (26 subjects each, 30%); anemia and vomiting (24 subjects each, 28%); and dry mouth (23 subjects, 27%). Note that under the preferred term of Visual Impairment several visual symptoms were reported, such as blurred vision, color changes, flashes, floaters, light/dark accommodation difficulties, and moving objects. Most AEs were reported as Grade 1 or 2 and were reversible.

In Studies AT13387-01 and -02, the most common related AEs (occurring in >25% of subjects) were diarrhea (58 subjects, 67%), nausea (32 subjects, 37%), and fatigue (30 subjects, 35%). Most related AEs were reported as Grade 1 or 2.

AEs of Special Interest

The following events are discussed in detail as they describe the most commonly reported class of AEs or provide more detailed information about particular types of adverse events.

Gastrointestinal (GI toxicity)

The most commonly reported GI toxicities (>15% incidence) include diarrhea, nausea, abdominal pain, vomiting, dry mouth, and constipation. These AEs are mostly Grade 1 to 2 and manageable with available supportive medications.

Infusion-Related Reactions
Treatment with AT13387 may be associated with local infusion-related irritation, as well as systemic infusion reactions, which occur either during the infusion or shortly afterwards. The local infusion site events may be formulation-related (pH of current formulation ~4.2). Systemic reactions are reversible and are often characterized by flushing, itching, rigors, chills, nausea, tachycardia/bradycardia, alterations in blood pressure and dizziness. The incidence of these reactions increases at higher dose levels and the severity of their effects may be reduced by slowing the infusion or with additional hydration or premedication with dexamethasone, antihistamines and $5HT_3$ antagonists.

Visual Disturbances and Ocular Adverse Events
A range of visual disturbances (collectively grouped under the MedDRA preferred term of Visual Impairment) have been reported in subjects receiving AT13387 in phase 1-2 trials. These include peripheral flashes (photopsia), blurred or double vision, floaters, color distortion and dimness, difficulties with light/dark accommodation, tunnel vision or other field defects, halos, apparent movement of stationary objects, and complex disturbances. The symptoms were generally Grade 1 in severity, intermittent, reversible and transient, lasting a few seconds to a few minutes and occurring on 1 to 3 days per cycle. The onset was generally in the first cycle, although in some subjects, the onset was in the first half of the second cycle. The symptoms were more frequent in the evening or at night and generally did not interfere with activities of daily living. Visual symptoms were dose-related and most frequently reported in subjects treated with 120 mg/m^2 twice-weekly in the AT13387-01 trial (11/13 subjects; 85%), compared with around half the subjects treated with 150 mg/m^2 or 180 mg/m^2 weekly and 5 of 7 subjects (71%) treated with 220 mg/m^2 weekly. Dry eyes, eye pain and keratitis have been reported but these events appear to be much less frequent with AT13387 than visual disturbances (and also less frequent than xerostomia).

Visual disturbances have been reported as side effects of treatment with a range of HSP90 inhibitors and thus appear to be a class effect. The mechanism is not well understood but nonclinical data suggest that it may be due to effects on retinal pigment epithelial cells, which are essential for the physiological function of adjacent photoreceptors. Normal conjunctival epithelium expresses abundant HSP90, and HSP90 inhibition has also been associated with dry eyes, conjunctivitis, keratitis and ocular surface disease.

Other Toxicities
Other common AEs (>15% incidence) with AT13387 include fatigue, decreased appetite, dizziness, anemia, headache, muscle spasms, insomnia, chills, dehydration, and hyperhidrosis. Two cases of renal adverse events (1 Grade 4 and another Grade 2) were reported in combination with imatinib. The subjects had borderline serum creatinine at baseline and other concomitant conditions/medications that might have contributed to these events including dehydration as a result of vomiting and diarrhea. Finally, 1 subject had asymptomatic Grade 2 QT_c prolongation (in combination with imatinib), and another subject had reversible decreased LVEF, Grade 2.

2.3 Rationale

Vemurafenib is FDA approved for the treatment of advanced, BRAF-mutant melanoma after it was shown to improve overall survival when compared with dacarbazine in patients with advanced melanoma. (*Chapman et al.,* 2011) Dabrafenib, a selective BRAF inhibitor structurally unrelated to vemurafenib, also achieves significant improvements in clinical response rate and progression-free survival compared to chemotherapy; findings that led to its FDA-approval in 2013. (*Hauschild et al.,* 2012) While these results have been significant, there remain critical limitations to the activity of these agents. Specifically, clinical resistance develops in most patients within one year, and the median progression free survival in these patients is approximately six months and durable remissions are uncommon. (*Sosman et al.,* 2012; *Chapman et al.,* 2011; *Hauschild et al.,* 2012).

Studies suggest that resistance to BRAF inhibitors is mediated by multiple mechanisms, including reactivation of the mitogen activated protein kinase (MAPK) pathway in many patients, while others show evidence of upregulation of other pro-survival signaling pathways [i.e. phosphoinositide-3-kinase (PI3K) pathway]. (*Bollag et al.,* 2010; *Johannessen et al.,* 2010; *Nazarian et al.,* 2010; *Montagut et al.,* 2008; *Poulikakos et al.,* 2011; *Shi et al.,* 2012; *Villanueva et al.,* 2010; *Emery et al.,* 2009; *Wagle et al.,* 2011) Recently, promising clinical results have been observed with the combination of BRAF and MEK inhibitors. (*Flaherty et al.,* 2012 #2) In a randomized phase II trial, 162 patients were randomized to receive either single-agent dabrafenib, the combination of dabrafenib 150 mg twice daily and trametinib 1 mg daily, or the combination of dabrafenib 150 mg twice daily and trametinib 2 mg daily. At the highest dose level, the response rate was significantly higher (76%) than the combination with the lower trametinib dose (50%) and monotherapy (54%). Interestingly, the median PFS for both combination arms was significantly longer than monotherapy (9.4 v 9.2 v 5.8 months). Supporting this proof of concept, two additional BRAF/MEK inhibitor combinations that have been evaluated in phase I clinical trials and shown promising results. The first is the combination of vemurafenib with the MEK inhibitor cobimetinib. In this phase I/II study, combination therapy has been associated with a response rate of 86% in patients with BRAF mutant melanoma. Similarly, the combination of the BRAF inhibitor LGX818 and the MEK inhibitor MEK162 is associated with a high response rate in BRAF inhibitor naïve patients (8 of 9 responders), though relatively few patients have been treated with this combination. Still, many patients develop resistance, and the median PFS on combination therapy is only a few months longer than BRAF inhibitor monotherapy.

Unfortunately, there is no known effective, standard of care treatment for patients with BRAF inhibitor resistant melanoma. In particular, the use of the other recently approved therapy, ipilumumab, appears to be uniquely ineffective in patients who have developed disease progression on vemurafenib, (*Ascierto et al 2012. Ackerman et al. 2014)* In addition, it is very difficult to enroll these patients onto clinic trials that require BRAF inhibitor washout periods due to the accelerated progression and short survival following BRAF inhibitor discontinuations. As a result, determining therapy for this hard-to-treat population of patients has become one of the most unmet clinical needs in the field.

The described mechanisms of resistance to BRAF inhibitor therapy include both reactivation of the mitogen activated protein kinase (MAPK) pathway and activation of parallel growth signaling pathways [such as the phosphoinositide-3-kinase (PI3K) pathway]. Heat shock protein 90 (HSP90) serves as a molecular chaperone to a number of mediators these growth factor pathways and is a potential therapeutic target in the setting BRAF inhibitor resistance. Included among its client proteins are insulin-like growth factor 1 receptor (IGF1R), BRAF, CRAF, AKT, cyclin D, and cyclin dependent kinase 4 (CDK4), all of which have been implicated in BRAF inhibitor resistance. Supporting this is recently published data showing that the HSP inhibitor (HSPi) XL888 blocks growth and survival, in vitro and in vivo, in BRAF-mutant melanoma cell lines that harbor acquired resistance mechanisms to BRAF inhibitor including COT overexpression, platelet derived growth factor receptor beta (PDGFR-b) overexpression, concomitant BRAF and NRAS mutations, and cyclin D amplifications (Paraiso et al. 2012). Additionally, a reduction in levels of a number of both client proteins and growth pathway mediators was seen, as was a favorable change in the pro- and anti-apoptotic molecules respectively, BIM and MCL1.

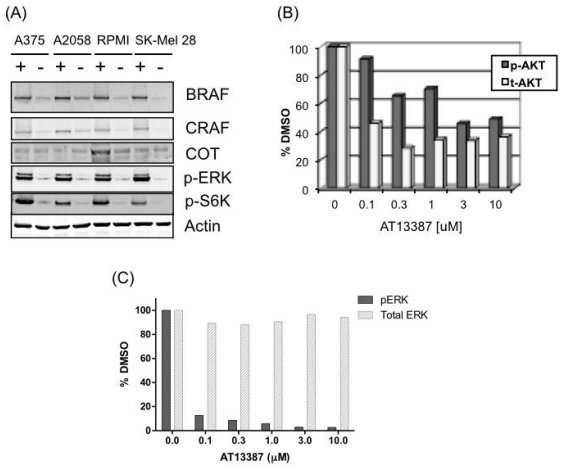

Figure 2.3.1: Effect of AT13387 treatment on HSP90 client knockdown and signaling in melanoma cell lines. A375, A2058, RPMI-7951 and SKMel-28 melanoma cells were treated with 1 μM AT13387 for 24 h. Proteins were resolved by SDS-PAGE and then immunoblotted with the indicated antibodies (A). A2058 cells were treated with varying concentrations of AT13387 for 24h and levels of AKT and pAKT determined by Mesoscale Discovery (MSD) (B). RPMI-7951 cells were treated with varying concentrations of AT13387 for 24 h and levels of ERK and pERK determined by MSD (C).

AT13387 is a small molecule inhibitor of HSP90 that has been shown to be safe given both weekly and twice weekly (either days 1 and 4 or days 1 and 2) in patients with advanced solid tumors. In melanoma cell lines, AT13387 has single agent activity in vitro in BRAF-mutant cell lines, including those that are sensitive and those that are resistant to a BRAF inhibitor, and effectively reduces HSP90 client proteins. (Figure 2.3.1) In xenograft studies, single-agent AT13387 has modest activity against BRAF-mutant melanoma cell lines. (Figure 2.3.2). In combination with a BRAF inhibitor, AT13387 is well tolerated, is neither associated with additive activity nor antagonism in BRAFi sensitive cell lines, and shows enhanced growth inhibition compared to both single-agent therapies in a BRAF inhibitor resistant cell line. (Fig 2.3.2 (B)) Lastly, a reduction in the HSP90 client proteins BRAF, CRAF, AKT, cyclin D, CDK4 was seen with AT13387 treatment in the above and additional xenograft models using BRAF-mutant melanoma cell lines (Figure 2.3.3).

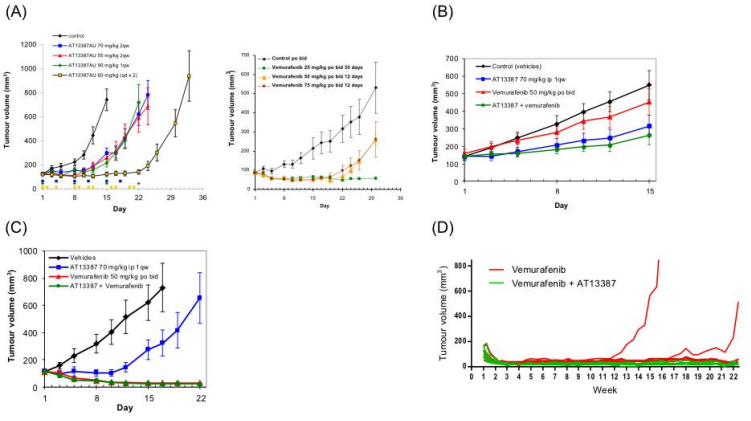

Figure 2.3.2: Effect of AT13387 and vemurafenib in melanoma xenografts. Mice had the following cell lines implanted: (A) A375; (B) A2058; (C) SK-Mel 28. Treatment with twice daily gavage (vemurafenib/vehicle) and weekly intraperitoneal injection (AT13387 or vehicle). The mice with SK-Mel 28 xenografts treated with vemurafenib or the combination with vemurafenib and AT13387 continued treatment (D). In a subset of the vemurafenib treated mice, treatment resistance develop where none of the dual treated mice developed resistance at 22 weeks.

AT13387 can overcome multiple mechanisms of BRAF inhibitor resistance, including acquired resistance, through its inhibitory effect on the AKT and ERK signalling pathways. Based on this preclinical data with AT13387 and the published data with XL888, it is clear that HSP inhibition is a rational strategy for patients with BRAFi-resistant, BRAF mutant melanoma. Importantly, in patients who are progressing on BRAF inhibitor therapy, there tends to be continued regression of some disease sites while other sites are progressing. (Long SMR) Ongoing BRAF inhibitor therapy in these patients tends to lead to continued control of some disease and can be effectively used as palliative therapy even when patients are dying of their disease. Thus, it may to be useful to continue BRAF inhibitor therapy after progression. Therefore, we propose to study the combination of dabrafenib and trametinib with AT13387 in patients who have disease progression on BRAF inhibitor therapy. Additionally, based on the xenograft data in 2.3.2 D showing that concomitant BRAF-targeted therapy with AT13387 may suppress the emergence of BRAF inhibitor resistance in tumors treated from the outset of therapy, we believe that upfront BRAF-targeted therapy plus AT13397 is a potentially useful strategy for BRAF targeted therapy naïve patients.

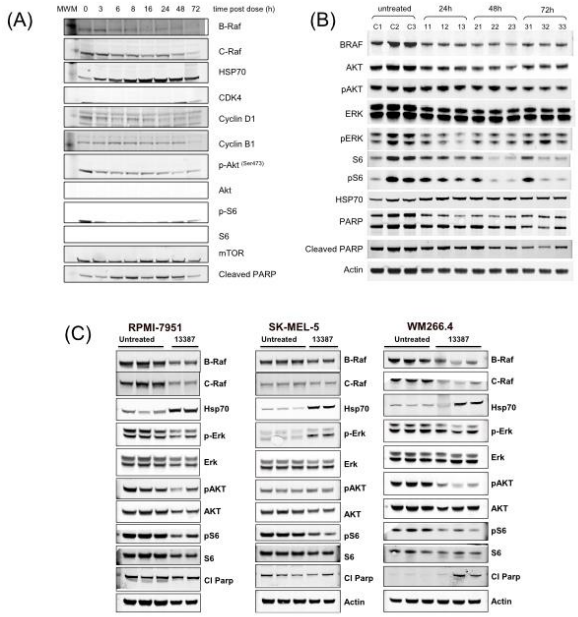

Figure 2.3.3: Effect of AT13387 on HSP90 clients in melanoma xenografts. Mice bearing (A) A375, (B) A2058, or (C) RPMI-7951, SK-MEL-5, or WM266.4 tumor xenografts were treated with single doses of 70 mg/kg AT13387.

2.4 Correlative Studies Background

2.4.1 Pharmacokinetic Studies

While the pharmacokinetic data of weekly AT13387 and the combination of dabrafenib and trametinib are known, PK data of the triple combination will be investigated as part of the Phase I portion of this study. Clinically observed toxicities will be correlated with the possibility of delayed clearance of the individual agents.

The PK sampling regimen for this study is based on the following observations; steady state for trametinib was reached by approximately Day 15 and the C_{max} and AUC for dabrafenib at the RP2D are about 40% lower on Day 15 versus Day 8. In addition, accumulation of trametinib was observed in the combination study of trametinib and dabrafenib.

Measurement of drug levels of AT13387, dabrafenib, and trametinib will be performed on Cycle 1 day 15 of three-drug treatment (pre-, 1, 2, 4, 6, and 8 hours after dosing), and Cycle 1 day 16 (24 hours after AT13387 day 15 dosing). Samples for trough level PK analysis will then be planned for each patient on day 1 of cycles 2, 4, 8, and 12.

2.4.2 Biomarkers of Drug Effect

Although we do not yet know which HSP90 client proteins must be degraded in order for long term anti-tumor responses to be seen to the BRAF/MEK/HSP90 inhibitor combination, preliminary studies from our group and others suggests that the PI3K/AKT/mTOR pathway may be critical for the short-term adaptive responses to BRAF inhibition and that prolonged inhibition of the Raf/MEK/ERK/CDK4/Cyclin D1 pathway is required for durable anti-tumor response. Levels of HSP90 client proteins, HSP chaperones and other proteins involved in adaptive signaling responses will be measured using LC-MRM from FNAs taken from accessible melanoma lesions before therapy initiation and after 7-10 days of BRAF/MEK/HSP90 treatment.

Additionally, biopsy specimens will be evaluated by Reverse Phase Protein Array (RPPA) at RPPA Core Facility, Functional Proteomics, MD Anderson Cancer Center (Texas, USA). Primary targets of interest for analysis of AT13387 effect on relevant HSP90 client proteins in tumor biopsy tissue will be evaluated. Frozen biopsy specimens obtained during collection will be sent for analysis of relevant proteins, protein post-translational modifications (phosphorylation and other modifications) and effects on relevant signaling pathways.

2.4.3 Blood Based BRAF Assay

Our group has developed a blood-based assay that can measure circulating BRAF levels in patients with BRAF mutant melanoma. (*Panka et al.,* 2010; *Panka et al.,* 2014) Our data shows that the BRAF level is reduced in patients treated with BRAF-directed therapy (both single-agent vemurafenib and the combination of DT) and, in some patients, increases in advanced of clinical or radiographic progression by 40-60 days. (*Panka et al.,* 2014) As this assay remains under clinical development, we plan to measure serial values (pre-treatment and at the beginning of each cycle) to further interrogate the utility of this assay. All samples will be initially processed in the Translational Research Laboratory at MGH and then subsequently assayed at the BIDMC.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Patients must have histologically confirmed, BRAF-mutant (V600E/K) solid tumor (molecularly confirmed using Cobas assay or a comparable FDA-approved assay) that is metastatic or unresectable, have received and tolerated prior BRAF or BRAF and MEK inhibitor (BRAF targeted) therapy at full dose or not previously received BRAF targeted therapy, and for which standard curative measures do not exist or are no longer effective.

- 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).

3.1.2 Patients 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 for non-nodal lesions and short axis for nodal lesions) as ≥20 mm with conventional techniques or as ≥10 mm with spiral CT scan, MRI, or calipers by clinical exam. See Section 11 for the evaluation of measurable disease.

3.1.3 Prior therapy is allowed. Patients may have received any number of prior lines of therapy, including treatment with a BRAF and/or MEK inhibitor.

3.1.4 All prior anti-cancer treatment-related toxicities must be less than or equal to Grade 1 according to the Common Terminology Criteria for Adverse Events version 5 (CTCAE version 5.0; NCI,2017) at the time of enrollment. **A notable exception** are endocrinopathies caused by immune checkpoint inhibitors that are appropriately treated with medical management (e.g. hormone replacement therapy, anti-diabetic agents)

3.1.5 Age ≥18 years. Because no dosing or adverse event data are currently available on the use of AT13387 in combination with dabrafenib and trametinib in patients <18 years of age, children are excluded from this study, but will be eligible for future pediatric trials.

3.1.6 ECOG performance status ≤1 (Karnofsky ≥70%, see Appendix A).

3.1.7 Life expectancy of greater than 3 months.

3.1.8 Patients must have normal organ and marrow function as defined below:

– leukocytes	≥3,000/mcL
– absolute neutrophil count	≥1,200/mcL
– hemoglobin	≥ 9 g/dl (patients may be transfused to this level)
– platelets	≥ 100,000/mcL
– total bilirubin	< 1.5 x institutional upper limit of normal OR > 1.5 x institutional upper limit of normal allowed if direct bilirubin is within normal range.
– AST(SGOT)/ALT(SGPT)	≤ 2.5 x institutional upper limit of normal
– PT/INR and PTT	≤ 1.3 x ULN
– Serum creatinine	≤1.5 mg/dL OR
– creatinine clearance	≥50 mL/min/1.73 m^2
– Potassium	>3 and <5.5mEq/L
– Magnesium	>1.2 and <2.5mEq/L
– Left ventricular ejection fraction	≥ institutional lower limit of normal (LLN) by ECHO

3.1.9 The effects of AT13387, dabrafenib, and trametinib on the developing human fetus are unknown. For this reason, women of child-bearing potential must have a *negative serum pregnancy test within 14 days prior to randomization and agree to use effective contraception* (barrier method of birth control, or abstinence; hormonal contraception is not allowed due to drug-drug interactions which can render hormonal contraceptives ineffective) *from 14 days prior to randomization*, throughout the treatment period, and for 4 months after the last dose of study treatment. Should a woman become pregnant or suspect she is pregnant while she is participating in this study, she should inform her treating physician immediately.

Based on studies in animals, it is also known that dabrafenib may cause damage to the tissue that makes sperm. This may cause sperm to be abnormal in shape and size and could lead to infertility, which may be irreversible.

Safety and efficacy of the combination of dabrafenib and trametinib in pediatric populations have not been investigated. Dabrafenib or trametinib-dabrafenib combination should not be administered to pediatric populations outside clinical trials.

3.1.10 Therapeutic level dosing of warfarin can be used with close monitoring of PT/INR by the site. Exposure may be decreased due to enzyme induction when on treatment, thus warfarin dosing may need to be adjusted based upon PT/INR. Consequently, when discontinuing dabrafenib, warfarin exposure may be increased and thus close monitoring via PT/INR and warfarin dose adjustments must be made as clinically appropriate. Prophylactic low dose warfarin may be given to maintain central catheter patency.

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

3.1.12 Able to swallow and retain oral medication, *and must not have any clinically significant gastrointestinal abnormalities that may alter absorption such as malabsorption syndrome or major resection of the stomach or bowels*

3.2 Exclusion Criteria

3.2.1 Patients who received prior systemic anti-cancer therapy (chemotherapy with delayed toxicity, extensive radiation therapy, immunotherapy, biologic therapy, or vaccine therapy) within the last 3 weeks prior to Day 1 of Cycle 1. Patients are permitted to be on dabrafenib and trametinib standard of care at start of therapy without wash-out period prior to Day 1 of Cycle 1. Dosing will change to protocol determined dose levels on Day 1 of Cycle 1.

3.2.2 Patients must not have received prior HSP90 inhibitor therapy.

3.2.3 Patients who are receiving any other investigational agents. Patients who have taken an investigational drug within 28 days or 5 half-lives (minimum 14 days), whichever is shorter, prior to randomization.

3.2.4 Patients with history of RAS mutation positive tumors regardless of interval from current study. However, patients may have concurrent BRAFV600 and RAS mutations in the tuor to be treated with protocol therapy.

3.2.5 Patients must have no clinical evidence of leptomeningeal or brain metastasis causing spinal cord compression that are symptomatic or untreated or not stable for ≥ 4 weeks (must be documented by imaging) or requiring corticosteroids. Subjects on a stable dose of corticosteroids >1 month or who have been off of corticosteroids for at least 2 weeks can be enrolled with approval of the CTEP medical monitor. Subjects must also be off of enzyme-inducing anticonvulsants for >4 weeks.

3.2.6 History of known immediate or delayed hypersensitivity reactions reactions attributed to compounds of similar chemical or biologic composition to AT13387, dabrafenib, or trametinib, or excipients or to dimethyl sulfoxide (DMSO).

3.2.7 Uncontrolled intercurrent illness including, but not limited to, ongoing or active serious infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, uncontrolled diabetes, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.8 Pregnant women are excluded from this study because AT13387, dabrafenib, and trametinib may have teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with the study drugs, breastfeeding should be discontinued *prior to* the mother being treated with the study drugs.

3.2.9 Patients known to be HIV-positive patients and on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with the study drugs. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in patients receiving combination antiretroviral therapy when indicated.

3.2.10 History of another malignancy other than the study indication under this trial within 5 years of study enrollment. Does not apply to subjects who underwent successful definititive resection of basal or squamous cell carcinoma of the skin, superficial bladder cancer, in situ cervical cancer, in situ breast cancer, or other in situ cancers.

Exception: Patients with history of RAS mutation-positive tumors are not eligible regardless of interval from the current study. Prospective RAS testing is not required. However, if the results of previous RAS testing are known, they must be used in assessing eligibility.

3.2.11 History of interstitial lung disease or pneumonitis.

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

- History of RVO or RPED, or predisposing factors to RVO or RPED (*e.g.*, uncontrolled glaucoma or ocular hypertension, uncontrolled systemic disease such as hypertension, diabetes mellitus, or history of hyperviscosity or hypercoagulability syndromes).
- Visible retinal pathology as assessed by ophthalmic exam that is considered a risk factor for RVO or RPED such as evidence of new optic disc cupping, evidence of new visual field defects, and intraocular pressure >21 mm Hg.

3.2.13 History or evidence of cardiovascular risk including any of the following:
- An average of the three most recent QT intervals corrected for heart rate using the Bazett's formula QTcB ≥460 msec.
- History or evidence of current clinically significant uncontrolled arrhythmias (exception: patients with controlled atrial fibrillation for >30 days prior to randomization are eligible).
- History of acute coronary syndromes (including myocardial infarction and unstable angina), coronary angioplasty, or stenting within 6 months prior to randomization.
- History or evidence of current ≥ Class II congestive heart failure as defined by the New York Heart Association (NYHA) functional classification system
- Treatment-refractory hypertension defined as a blood pressure of systolic >140 mmHg and/or diastolic >90 mmHg which cannot be controlled by anti-hypertensive therapy.
- Abnormal cardiac valve morphology (≥grade 2) documented by echocardiogram (subjects with grade 1 abnormalities [i.e., mild regurgitation/stenosis] can be entered on study). Subjects with moderate valvular thickening should not be entered on study.
- Prior placement of an implantable defibrillator
- History of or identification on screening imaging of intracardiac metastases

3.2.14 No known active infection with Hepatitis B Virus (HBV), or Hepatitis C Virus (HCV). Patients with chronic or cleared HBV infection and HCV infection are eligible.

3.2.15 Current use of a prohibited medication. The following medications or non-drug therapies are prohibited (Please refer to Appendix F for complete list):
- Other anti-cancer therapy while on study treatment. (note: megestrol [Megace] if used as an appetite stimulant is allowed).
- Concurrent treatment with bisphosphonates is permitted; however, treatment must be initiated prior to the first dose of study therapy. Prophylactic use of bisphosphonates in patients without bone disease is not permitted, except for the treatment of osteoporosis.
- Because the composition, PK, and metabolism of many herbal supplements are unknown, the concurrent use of all herbal supplements is prohibited during the study (including, but not limited to, St. John's wort, kava, ephedra [ma huang], ginkgo biloba, dehydroepiandrosterone [DHEA], yohimbe, saw palmetto, or ginseng).

Current use of a prohibited medication. Patients receiving any medications or substances that are strong inhibitors or inducers of CYP3A or CYP2C8 are ineligible. Current use of, or intended ongoing treatment with: herbal remedies (*e.g.*, St. John's wort), or strong inhibitors or inducers of P-glycoprotein (Pgp) or breast cancer resistance protein 1 (Bcrp1) should also be excluded. Below are a few examples of the agents.

PROHIBITED – strong inducers of CYP3A or CYP2C8, since concentrations of dabrafenib may be decreased	
Class/Therapeutic Area	**Drugs/Agents**
Antibiotics	Rifamycin class agents (e.g., rifampin, rifabutin, rifapentine),
Anticonvulsant	Carbamazepine, oxcarbazepine phenobarbital, phenytoin, s-mephenytoin
Miscellaneous	bosentan, St. John's wort
PROHIBITED – Strong inhibitors of CYP3A, or CYP2C8 since concentrations of dabrafenib may be increased	
Class/Therapeutic Area	**Drugs/Agents**
Antibiotics	Clarithromycin, telithromycin, troleandomycin
Antidepressant	Nefazodone
Antifungals	Itraconazole, ketoconazole, posaconazole, voriconazole
Hyperlipidemia	Gemfibrozil
Antiretroviral	ritonavir, saquinavir, atazanavir
Miscellaneous	Conivaptan

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/main-table/; medical reference texts such as the Physicians' Desk Reference may also provide this information. As part of the enrollment/informed consent procedures, the patient will be counseled on the risk of interactions with other agents, and what to do if new medications need to be prescribed or if the patient is considering a new over-the-counter medicine or herbal product. Appendix C is a patient information sheet that can be used for this specific protocol and presented to the patient.

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial. BRAF mutant melanoma is more common in Caucasians and women, though metastatic melanoma is more common in men.

Accrual Targets						
Ethnic Category	**Sex/Gender**					
	Females		**Males**		**Total**	
Hispanic or Latino	1	+	1	=	2	
Not Hispanic or Latino	18	+	18	=	36	
Ethnic Category: Total of all subjects	19 (A1)	+	19 (B1)	=	38	(C1)

Racial Category							
American Indian or Alaskan Native	0	+	0	=	0		
Asian	1	+	1	=	2		
Black or African American	0	+	0	=	0		
Native Hawaiian or other Pacific Islander	0	+	0	=	0		
White	18	+	18	=	36		
Racial Category: Total of all subjects	19 (A2)	+	19 (B2)	=	38 (C2)		
	(A1 = A2)		(B1 = B2)		(C1 = C2)		

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC and DF/PCC 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. Any laboratory values (chemistry and hematology) that are taken on C1D1 must re-meet eligibility in order for participants to receive treatment. 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 and DF/PCC Institutions

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

4.3 General Guidelines For Other Participating Institutions

Eligible participants will **NOT** be registered on study by the Coordinating Center. A designee from the institution will register patients with Theradex via the online slot reservation system, Interactive Web Response System (IWRS).

Prior to registration, a Site Investigator will confirm eligibility of prospectives participants for all inclusion/exclusion criteria.

Following registration, participants should begin protocol treatment. Any laboratory values (chemistry and hematology) that are taken on C1D1 must re-meet eligibility in order for participants to receive treatment. Issues that would cause treatment delays should be discussed with the Principal Investigator/Sponsor. If a participant does not receive protocol therapy following registration, the participant's protocol status must be changed. The Coordinating Center should be notified of participant status changes as soon as possible.

4.4 Registration Process For Other Participating Institutions

External sites will only be registering patients to the protocol with Theradex via IWRS and not with with DF/HCC. Sites will follow any site-specific processes as applicable.
The registration procedures are as follows:

- Obtain written informed consent from the participant prior to the performance of any protocol specific procedures or assessments.

- Verify all protocol eligibility criteria. **To be eligible for registration to the protocol, the participant must meet all inclusion and exclusion criterion as described in the protocol.**

  Reminder: Confirm eligibility for ancillary studies at the same time as eligibility for the treatment protocol. Registration to both treatment and ancillary protocols will not be completed if eligibility requirements are not met for all studies.

- Designeed will register patient with Theradex via IWRS.

5. TREATMENT PLAN

5.1 Agent Administration

Treatment will be administered on an outpatient basis. A cycle of treatment will be 28 days in length. IV administration will begin approximately the same time oral agents are dosed, but not more than 10 minutes after oral agents are taken. For Cycle days 8 and 15, patients will be instructed to bring oral agents with them to be given in the clinic. Patients are asked to maintain a medication diary for recording each dose of Dabrafenib and Trametinib (Appendix D).

Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy. The patient will be requested to maintain a medication diary of each dose of medication. The medication diary (Appendix D) and pill bottles will be returned to clinic staff at the end of each course. To document compliance, a pill count will be performed of all unused pills.

Dosing levels for the study are listed below in Table 5.1. Dose level 1 will be the first dose level. Intrapatient dose escalation will be allowed when safe and feasible once the next higher dose level has been cleared. Additional, intermediate dose levels may be enrolled under the following

conditions:

1) Toxicity is seen at a dose level where dose modification is necessary and the prior, lower dose level was treated without DLT or analysis of the PK data suggests that an intermediate dose would be appropriate;
2) An intermediate dose is conceivable based on the size of pills and nature of the toxicity at the higher dose;
3) Consensus agreement is made between investigators, industry sponsors, and CTEP;
4) CTEP and IRB approval of an amendment to the protocol that clearly describes the rationale for and the logistics of the intermediate dose level.

Table 5.1 Dose Escalation Levels for Phase I Study

Dose Level	**Dabrafenib [BID/PO]**	**Trametinib [QD/PO]**	**AT13387 [D1,8,15/IV]**
-1	75 mg	1 mg	180 mg/m2
1	150 mg	1 mg	180 mg/m2
2	150 mg	2 mg	180 mg/m2
3	150 mg	2 mg	220 mg/m2
4	150 mg	2 mg	260 mg/m2

5.1.1 Trametinib

The effect of food on trametinib absorption is unknown. The current recommendation is to administer trametinib on an empty stomach, either 1 hour before or 2 hours after a meal; the recommendation to administer trametinib fasting may change based on emerging data. Missed doses of trametinib should not be made up if there is less than 12 hours until the next scheduled dose. Vomited doses of trametinib should not be made up.

In the event that dabrafenib, AT13387, or both agents need to be discontinued due to toxicity reasons, treatment with trametinib may continue at the discretion of the investigator until time of disease progression.

5.1.2 Dabrafenib

Patients should take dabrafenib at least 1 hour prior to or 2 hours after a meal due to a potential food effect on dabrafenib absorption. Doses should be taken every 12 hours. Missed doses of dabrafenib should not be made up if it is less than 6 hours until the next scheduled dose (section 8.1.2). Vomited doses of dabrafenib should not be made up.

In the event that trametinib, AT13387, or both agents need to be discontinued due to toxicity reasons, treatment with dabrafenib may continue at the discretion of the investigator until time of disease progression.

5.1.3 AT13387

AT13387 is dosed in mg/m^2 using institutional standard of practice for dose calculations. AT13387 is administered via Infusion. Infuse over 1 hour through a central line or a well-defined peripheral vein (Note: an in-line filter is NOT required). If use a peripheral line, be sure to aspirate venous blood prior to starting the infusion. Check the

infusion site every 15 minutes and change the site of infusion should evidence of swelling or discoloration is observed. If AT13387 cannot be administered on Cycle Day 8 or 15, the dose will be skipped and not delayed.

If patient experiences pain along the infusion site, slow the infusion rate (i.e. > 1 hour duration) and/or infuse D5W through a "Y" connector. The additional volume of D5W dilutes AT13387 concentration at the local site of the infusion and alleviates the irritation.

In the event that trametinib, dabrafenib, or both agents need to be discontinued due to toxicity reasons, treatment with AT13387 may continue at the discretion of the investigator until time of disease progression.

5.2 Definition of Dose Limiting Toxicity

Dose-limiting toxicity (DLT) is based on the CTEP Active Version 5 of the NCI Common Terminology Criteria for Adverse Events (CTCAE). DLT refers to toxicities experienced during the first cycle of treatment between days 1-28. A DLT must
be considered to be at least possibly related to study drug(s) will be defined as follows:

- Any grade 4 toxicity
- Grade 3 (or grade 2 intolerable) non-hematologic toxicity
- Grade ≥ 3 neutropenia with fever > 38.5°C
- Grade 3 thrombocytopenia with clinically significant bleeding
- Furthermore, grade 2 intolerable/grade 3 fatigue that results in hospitalization should be considered a DLT.
- Intolerable AEs are toxicities deemed to lead to a missing dose of AT13387 or more than 25% of dabrafenib/trametinib in the first cycle.

In general, all Grade 3 or intolerable grade 2, non-hematological toxicities regardless of duration are considered DLT. However, the following Grade 3 toxicities will not be considered a DLT.

- The appearance of cutaneous squamous cell carcinoma or keratoacanthoma will not be considered a dose limiting toxicity as these are commonly observed with dabrafenib.
- Grade 3 fatigue persistent for ≤ 7 days
- Grade 3 nausea, vomiting, or diarrhea persisting ≤ 72 hours with maximum supportive care
- Grade 3 electrolyte events if they are resolved with replacement within 24 hours
- Lymphopenia regardless of grade
- The following grade 3 laboratory abnormalities will not be considered dose limiting if they return to baseline within 7 days: elevated bilirubin, AST, ALT, cholesterol, amylase, lipase, creatinine and hypertriglyceridemia

Management and dose modifications associated with the above adverse events are outlined in Section 6.

Dose escalation will proceed within each cohort according to the following scheme. Dose-limiting toxicity (DLT) is defined above.

Table 5.2. Dose escalation table

Number of Patients with DLT at a Given Dose Level	Dose Escalation Rule
0 out of 3	Proceed to the next dose level and enroll 3 patients
1 out of 3	Enroll and treat 3 additional patients at this dose level.
≥ 2 out of 3	Dose escalation will be stopped. The MTD will be one dose below this dose level. Three (3) additional patients will be entered at the next lower dose level if only 3 patients were treated previously at that dose.
1 out of 6	ceed to the next dose level.
≥ 2 out of 6	Dose escalation will be stopped. The MTD will be one dose below this dose level. Three (3) additional patients will be entered at the next lower dose level if only 3 patients were treated previously at that dose.
If ≥2/3 or ≥2/6 patients at dose level 1 experience dose limiting toxicities, dose level -1 will be enrolled. If dose level -1 proves too toxic, the study will stop.	

5.3 General Concomitant Medication and Supportive Care Guidelines

5.3.1 Trametinib

Because there is a 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.

5.3.2 Dabrafenib

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 should be alerted if the patient is taking any agent known to affect or with the potential to affect selected CYP450 isoenzymes. Appendix C is a patient information sheet that can be used for this specific protocol and presented to the patient.

Dabrafenib mesylate is a substrate for CYP 3A4, 2C8, p-glycoprotein (Pgp), and breast carcinoma resistance protein (Bcrp). Strong inhibitors/inducers of these enzymes and transporters are prohibited for eligibility and during the study.

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.

5.3.3 AT13387

AT13387 does not induce of inhibit the cytochrome P450 system, however the concurrent use of all other drugs, over-the-counter medications, or alternative therapies must be captured in the case report form. AT13387 is a substrate of UGT with a relatively low affinity for UGT isoforms. In vitro data demonstrate that AT13387 is a weak inhibitor of UGT1A1, UGT1A3 and UGT1A9. AT13387 is also a weak inhibitor of CYP1A2, -3A4, -2D6, -2C9 and -2C19. AT13387 metabolizes primary via the glucuronidation, sulphation and N-oxidation. Use caution in patients taking CYP1A2, -3A4, -2D6, -2C9, -2C19 and UGT1A1, -3,-9 substrates with narrow therapeutic ranges. Caution should be exercised with all medications known to cause QTc prolongation or renal impairment since these events have been reported in previous studies with AT13387.

Supportive treatment should be given as per the institution standards and at the Investigator's discretion. In the presence of systemic infusion reactions, the infusion may be diluted to 500 mL and/or premedication given before subsequent infusions based on the systemic reaction symptoms (acetaminophen/paracetamol; antihistamines, dexamethasone, or similar agents for allergic manifestations; IV ondansetron or similar agents for nausea/vomiting). Antiemetics, anti-diarrheal agents, etc. may be given to treat or prevent gastrointestinal toxicities.

Subjects are advised not to drive or operate heavy machinery until they see how they react to the injections in terms of development of visual changes (i.e., at least during the first cycle of treatment).

5.4 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

The reason for removal from study treatment will be documented in the Case Report Form.

5.5 Duration of Follow Up

Patients removed from study treatment due to progressive disease will be followed for 4 weeks (28 days) after removal from study treatment or until death, whichever occurs first. Patients removed from study treatment for any reason other than progressive disease will be followed until disease progression or death, whichever occurs first. Patients removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event or progressive disease, whichever occurs last. All patients will be asked to come to clinic for a follow-up visit 28 days (+/- 3 days) after last study treatment.

5.6 Criteria for Removal from Study

Patients will be removed from study upon completion of the follow-up phase (section 5.5), death or withdrawal of consent. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

6. DOSING DELAYS/DOSE MODIFICATIONS

The criteria for dose modification of dabrafenib, trametinib, and AT13387 are detailed below. In the setting of toxicities of sufficient severity justifying dose modification, every effort will be made to identify the related medication. In the setting where the offending agent is difficult to determine, dose modification will occur with each agent.

6.1 Dabrafenib or Dabrafenib + Trametinib Dose Modifications

The tables below outline 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	→	100mg BID
100mg BID	→	75mg BID
75mg BID	→	50 mg BID
50 mg 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

Dabrafenib + Trametinib Dose Modification Guidelines

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 50 mg BID for dabrafenib is not allowed. For

dabrafenib-trametinib combination, dose below 1 mg once daily for trametinib is not allowed; however, if dabrafenib will be permanently discontinued for dabrafenib-related toxicities, the patients will be allowed to continue trametinib and AT13387 Conversely, if trametinib is permanently discontinued for trametinib-related toxicities, patients will be allowed to continue dabrafenib and AT13387.

CTEP Medical Monitor approval is required to restart study treatment after ≥28 days of dose interruption.

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, with details stipulated in subsequent sections. CTEP drug monitors should be consulted if there are questions about the attribution of AEs and how the doses should be modified.

In the event that AT13387 needs to be discontinued due to toxicity reasons, treatment with dabrafenib and trametinib may continue at the discretion of the investigator until time of disease progression.

In the event that dabrafenib and AT13387 need to be discontinued due to toxicity reasons, treatment with trametinib may continue at the discretion of the investigator until time of disease progression.

In the event that trametinib and AT13387 need to be discontinued due to toxicity reasons, treatment with dabrafenib may continue at the discretion of the investigator until time of disease progression.

6.1.1 Dabrafenib or Dabrafenib + Trametinib Dose Modification for Toxicities **Not Specified** in Subsequent Sections

Table 6-1: Dabrafenib or Dabrafenib + Trametinib Dose Modification for ToxicitiesNot Specified in Subsequent Sections*

CTCAE Grade	Action and Dose Modification
Grade 1 **Grade 2 (Tolerable)**	• Continue study treatment at same dose level (no dose modification). • Monitor closely. • Provide supportive care according to institutional standards.
Grade 2 (Intolerable) **Grade 3**	• Interrupt study treatment. • Monitor closely. • Provide supportive care according to institutional standards. • When toxicity resolves to grade 1 or baseline, restart study treatment **reduced by one dose level**. • If the grade 2 (intolerable) or grade 3 toxicity recurs, interrupt study treatment. • When toxicity resolves to grade 1 or baseline, **restart study treatment reduced by another dose level**.
Grade 4	• Permanently discontinue, or interrupt, study treatment. • Monitor closely. • Provide supportive care according to institutional standards. • If study treatment was interrupted, restart with study treatment **reduced by one dose level** once toxicity resolves to grade 1 or baseline. •
*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 <28 days. CTEP monitor should be consulted for resumption of single agent.	

6.1.2 Dabrafenib or Dabrafenib + Trametinib Dose Modification 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, and is increased in frequency and severity in subjects receiving dabrafenib in combination with trametinib. 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. In the event of a fever, the subject should be instructed to take anti-pyretics (e.g. ibuprofen or acetaminophen/paracetamol) as appropriate to control fever. The use of oral corticosteroids should be considered in those instances in which anti-pyretics are insufficient. Monitor serum creatinine and other evidence of renal function during and following severe events of pyrexia.

Table 6-2: Dabrafenib or Dabrafenib + Trametinib dose Modification and Management Guidelines for Pyrexia[a]

Event	Management Guideline	Dose Modification

Table 6-2: Dabrafenib or Dabrafenib + Trametinib dose Modification and Management Guidelines for Pyrexia[a]

Event	Management Guideline	Dose Modification
Work up: • Clinical evaluation for infection and hypersensitivity, especially if pyrexia is complicated by rigors, severe chills, dehydration, *etc.* • Laboratory work-up (should include full-blood-count, electrolytes, creatinine, BUN, CRP, liver-function tests, blood and urine culture). ***Management***: • 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. • Oral hydration is encouraged in subjects without evidence of dehydration. Intravenous hydration is recommended if pyrexia is complicated by dehydration/hypotension. • In subject experiencing pyrexia complicated by rigors, severe chills, *etc.*, which cannot be controlled with anti-pyretic medication, oral corticosteroids should be started. • Prophylactic anti-pyretic treatment is recommended after the 2nd event if pyrexia , or after the 1st event if complicated by rigors or severe chills. Prophylactic anti-pyretics may be discontinued after three days in absence of pyrexia.		
1st Event[b]:	• Clinical evaluation for infection and hypersensitivity[c] • Laboratory work-up[c] • Hydration as required[d] • Administer anti-pyretic treatment if clinically indicated and continue prophylactic treatment[f]	• Interrupt dabrafenib. • Continue trametinib. • *Once pyrexia resolves* to baseline, restart dabrafenib at the same dose level. If fever was associated with *rigors, dehydration, hypotension, or renal insufficiency*, **reduce dabrafenib by one dose level**.
2nd Event	• Clinical evaluation for infection and hypersensitivity[c] • Laboratory work-up[c] • Hydration as required[d] • Within 3 days of onset of pyrexia: – Optimize anti-pyretic therapy. – Consider oral corticosteroids (*i.e.*, prednisone 10 mg) for at least 5 days or as clinically indicated.[f]	• **Interrupt dabrafenib.** • Continue trametinib. • *Once pyrexia resolves* to baseline, restart dabrafenib at the same dose level. If fever was associated with *rigors, dehydration, hypotension, or renal insufficiency*, **reduce dabrafenib by one dose level**.
Subsequent Events:	• Clinical evaluation for infection and hypersensitivity[c] • Laboratory work-up[c] • Hydration as required[d] • Blood sample for cytokine analysis[e] • Within 3 days of onset of pyrexia: – Optimize oral corticosteroid dose as clinically indicated for recalcitrant pyrexia.[g] – If corticosteroids have been tapered and pyrexia recurs, restart steroids. – If corticosteroids cannot be tapered, consult medical monitor.	• **Interrupt dabrafenib.** • Continue trametinib. • Once pyrexia resolves to baseline, restart dabrafenib **reduced by one dose level**.[h] • If dabrafenib must be reduced to <50 mg BID, permanently **discontinue dabrafenib**.

Table 6-2: Dabrafenib or Dabrafenib + Trametinib dose Modification and Management Guidelines for Pyrexia[a]

Event	Management Guideline	Dose Modification
[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.		

6.1.3 Dabrafenib or Dabrafenib + Trametinib Dose Modification for **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 chair or the CTEP Medical Monitor may be required.

Table 6-3: Dabrafenib or Dabrafenib + Trametinib Supportive care and Dose Modification for Rash

CTCAE Grade	Adverse Event Management	Action and Dose Modification
Supportive care: • Avoid unnecessary sun light • Apply broad-spectrum sunscreen (containing titanium dioxide or zinc oxide) with a skin protection factor (SPF) ≥15 at least twice daily. • Use thick, alcohol-free emollient cream (e.g., glycerine and cetomacrogol cream) on dry areas of the body at least twice daily. • Topical steroids and antibiotics should be applied at least twice daily starting on Day 1 of study treatment, to body areas such as face, chest, and upper back. • Use mild-strength topical steroid (hydrocortisone 1% cream) or topical antibiotic (e.g., clindamycin) or oral antibiotics (e.g., doxycycline 100 mg BID, minocycline 100 mg BID) ***Symptom Management*** • Pruritic lesions: cool compresses and oral antihistamines therapies. • Fissuring lesions: Monsel's solution, silver nitrate, or zinc oxide cream. • Desquamation: thick emollients and mild soap. • Paronychia: antiseptic bath, local potent corticosteroids in addition to oral antibiotics; if no improvement, consult dermatoligist or surgeon • Infected lesions: appropriate bacterial/fungal culture-driven systemic or topical antibiotics. *Rash prophylaxis is recommended for the first 6 weeks of study treatment * Subjects who develop rash/skin toxicities should be seen by a qualified physician and should receive evaluation for symptomatic/supportive care management		
Grade 1	• Initiate prophylactic and symptomatic treatment measures.[1] • Use moderate strength topical steroid.[2] • Reassess after 2 weeks.	• Continue study treatment. • If rash does not recover to baseline within 2 weeks despite best supportive care, **reduce study treatment by one dose level**.[3]
Grade 2 (tolerable)	• Initiate prophylactic and symptomatic treatment measures.[1] • Use moderate strength topical steroid.[2] • Reassess after 2 weeks.	• **Reduce study treatment by one dose level.** • If rash recovers to ≤ grade 1 within 2 weeks, increase dose to previous dose level. • If no recovery to ≤ grade 1 within 2 weeks, interrupt study treatment until recovery to ≤ grade 1. • **Restart study treatment at reduced dose level.**[3]
Grade ≥3 or intolerable Grade 2	• Use moderate strength topical steroids PLUS oral methyl-prednisolone dose pack.[2] • Consult dermatologist.	• Interrupt study treatment until rash recovers to ≤ grade 1. • **Restart with study treatment reduced by one dose level.**[3,4] If no recovery to ≤ grade 2 within 28 days, **permanently discontinue study treatment**.
1. Rash prophylaxis is recommended for the first 6 weeks of study treatment. 2. Moderate-strength topical steroids: Hydrocortisone 2.5% cream or fluticasone priopionate 0.5% cream. 3. Approval of CTEP Medical Monitor is required to restart study treatment after >28 days of interruption. 4. Study treatment may be escalated to previous dose level if no rash is evident 4 weeks after restarting study treatment.		

6.1.4 Dabrafenib or Dabrafenib + Trametinib Dose Modification for **palmar-plantar erythrodysesthesia syndrome (PPES)**

- Lifestyle modification: avoidance of hot water, traumatic activity, constrictive footwear, or excessive friction on the skin and the use of thick cotton socks and gloves, and shoes with padded insoles
- Symptomatic treatments: apply moisturizing creams frequently, topical keratolytics (e.g. urea 20-40 % cream, salicylic acid 6%, tazarotene 0.1% cream, fluorouracil 5% cream), clobetasol propionate 0.05% ointment for erythematous areas, topical lidocaine 2%, and / or systemic pain medication such as nonsteroidal anti-inflammatory drugs, codeine, and pregabalin for pain.

Dose modification may also be required. – Refer to table for dose modification for non-specific AEs

6.1.5 Dabrafenib or Dabrafenib + Trametinib Dose Modification for **New Primary/ Recurrent Malignancies:**

6.1.5.1 *Cutaneous SCC and New Primary Melanoma*

Dermatologic skin assessments for subjects on treatment should be performed before initiation of dabrafenib, then every 2 months through treatment. For protocols that start after June 2014, it is also recommended that skin exams should continue every 2-3 months for 6 months after discontinuation of dabrafenib or initiation of another anti-neoplastic therapy. **Report any new primary/recurrent malignancies as SAE through CTEP-AERS.**

Cutaneous SCC
Cases of cuSCC (which include those classified as keratoacanthoma or mixed keratoacanthoma subtype) have been observed in subjects treated with dabrafenib. Approximately 70 % of events occurred within the first 12 weeks of treatment with a median time to onset of 8 weeks.

These should be surgically removed according to institutional practices. Dose modification or interruption of study treatment is not required for cuSCC or KA, however cuSCC should be reported as an SAE (refer to Section 7.6). In addition, a biopsy of the lesion should be taken, where possible, and a summary of the results submitted to CTEP through the SAE reporting.

Patients should be instructed to immediately inform their physician if new lesions develop.

New Primary Melanoma

New primary melanomas have been reported in patients treated with dabrafenib. These were identified primarily within the first 5 months of therapy and did not

require treatment modification other than excision. New primary melanoma should be reported as SAE through CTEP-AERS.

6.1.5.2 *Non-Cutaneous Malignancies*

In vitro experiments have demonstrated paradoxical activation of MAP-kinase signalling in BRAF wild type cells with RAS mutations when exposed to BRAF inhibitors, which may lead to increased risk of non-cutaneous malignancies in patients treated with dabrafenib. Cases of RAS-driven malignancies have been seen with BRAF inhibitors. Patients should be monitored as clinically appropriate.

Permanently discontinue dabrafenib in patients who develop RAS mutation-positive non-cutaneous malignancies. If used in combination with trametinib, trametinib may continue.

Following discontinuation of dabrafenib, monitoring for non-cutaneous secondary/recurrent malignancies should continue for up to 6 months or until initiation of another anti-neoplastic therapy.

New non-cutaneous malignancies should be reported as a SAE. A biopsy of the new malignancy should be taken, where possible, and submitted for further analyses with the results provided to CTEP via SAE reporting. Testing of these biopsies should include RAS mutation analysis and may include analysis of genomic alterations, which include but not limited to DNA, RNA and protein analysis of these biopsy specimens, and would analyze the biological pathways known to be associated with, and relevant to, BRAF-mutant tumor activation.

6.1.6 Dabrafenib-Trametinib Dose Modification for **Hemorrhages**

Table 6-4: Dabrafenib or Dabrafenib-Trametinib Treatment Modifications for Hemorrhage

Grade 3	• Hold dabrafenib or dabrafenib-trametinib for up to 3 weeks • If improved, resume the drugs at one dose reduction • If no improvement, permanently discontinue dabrafenib or dabrafenib-trametinib
Grade 4	• Permanently discontinue dabrafenib or dabrafenib-trametinib

6.1.7 Dabrafenib or Dabrafenib-Trametinib Dose Modification for **Pancreatitis**

In the event of abdominal pain or suspected pancreatitis, amylase and lipase laboratory samples should be collected for confirmation of the diagnosis. Patients should be closely monitored when re-starting dabrafenib after an episode of pancreatitis.

6.1.8 Dabrafenib or Dabrafenib-Trametinib Dose Modification for **Hyperglycemia**

Hyperglycemia requiring an increase in the dose of, or initiation of insulin or oral therapy can occur with dabrafenib. Monitor serum glucose levels as clinically appropriate during treatment with dabrafenib in subjects with pre-existing diabetes or hyperglycemia. Advise patients to report symptoms of severe hyperglycemia such as excessive thirst or any increase in the volume or frequency of urination.

6.1.9 Dabrafenib + 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 modified if clinically possible.

Table 6-5: Dabrafenib or Dabrafenib + Trametinib Dose Modification for Renal Insufficiency

Serum Creatinine Level	Management Guideline	Action and Dose Modification
Serum creatinine increase >0.2 mg/dL (18 mcmol/L) ***BUT*** ≤0.5 mg/dL (44 mcmol/L) above baseline	• Recheck serum creatinine within 1 week. • Serum creatinine increase >1 week: contact CTEP Medical Monitor. If elevation persists beyond 4 weeks, recommend evaluation (consider renal biopsy) for etiology; consider nephrology consultation. • 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) ***OR*** >2 mg/dL (>177 mcmol/L)	• Monitor serum creatinine ≥2-times per week. • Hospitalization may be necessary if serum creatinine cannot be monitored frequently. • If pyrexia is present, treat pyrexia per guidelines.[a] • Consult nephrologist if clinically indicated. • Perform renal biopsy if clinically indicated, for example: – Renal insufficiency persists despite volume repletion. – Patient has new rash or signs of hypersensitivity (such as elevated eosinophil count).	• Interrupt study treatment until serum creatinine recovers to baseline. • 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. See guidelines for pyrexia Section 6.1.2.
[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 CTEP Medical Monitor is required before restarting study treatment if there is evidence of thrombotic microangiopathy.

6.1.10 Dabrafenib + Trametinib Dose Modification for **Reduced Left Ventricular Ejection Fraction**

Decreases of the left ventricular ejection fraction (LVEF) have been observed in patients receiving dabrafenic plus trametinib. Therefore, ECHOs/MUGAs must be performed in regular intervals outlined in the Study Calendar. The same procedure (either ECHO or MUGA, although ECHO is preferred) should be performed at baseline and at follow-up visit(s).

Table 6-6: Dabrafenib + Trametinib Treatment Modification and Management Guidelines 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 trametinib and repeat ECHO within 2 weeks.[a], dabrafenib may continue • If the LVEF **recovers** within 4 weeks (defined as LVEF ≥LLN and absolute decrease ≤10% compared to baseline): – Consult with the CTEP Medical Monitor and request approval for restart. – Restart trametinib at reduced doses by one dose level. Continue dabrafenib at current dose. – Repeat ECHO 2, 4, 8, and 12 weeks after re-start; continue in intervals of 12 weeks thereafter. • If LVEF **does not recover** within 4 weeks: – Consult with cardiologist. – **Permanently discontinue trametinib**. – Once LVEF recovers, resumption of dabrafenib may be considered after consultation with CTEP. – Repeat ECHO after 2, 4, 8, 12, and 16 weeks or until resolution. – Report as SAE
Symptomatic[b]	• Grade 3: resting LVEF 39-20% or >20% absolute reduction from baseline • Grade 4: Resting LVEF ≤20%.	• **Permanently discontinue trametinib. Report as SAE** • **Hold dabrafenib until LVEF improves. Consult CTEP drug monitor for resumption of dabrafenib.** • Consult with cardiologist. • 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. [b] Symptoms may include: dyspnea, orthopenea, and other signs and symptoms of pulmonary congestion and edema.		

6.1.11 Dabrafenib or Dabrafenib + Trametinib Dose Modification for **Hypertension**

Increases in blood pressure (BP) have been observed in patients receiving dabrafenib plus trametinib. Recommendations for BP monitoring and management are provided below.

Monitoring: All BP assessments should be performed under the following optimal conditions:

- The subject has been seated with back support, ensuring that legs are uncrossed and flat on the floor.
- The subject is relaxed comfortably for at least 5 minutes.
- Restrictive clothing has been removed from the cuff area, and the right cuff has been selected.
- The subject's arm is supported so that the middle of the cuff is at heart level.
- The subject remains quiet during the measurement.
- 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 collected as described above.
- Asympomatic 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 Dabrafenib + Trametinib Treatment Modification and Management Guidelines for Hypertension

Event	Management Guideline	Dose Modification
(Scenario A) Asymptomatic and persistent[a] SBP of ≥140 and <160 mmHg, or DBP ≥90 and <100 mmHg OR linically significant increase in DBP of 20 mmHg (but still below 100 mmHg)	• Adjust current or initiate new antihypertensive medication(s). • Titrate antihypertensive medication(s) during the next 2 weeks to achieve well-controlled[b] BP. • If BP is not well-controlled within 2 weeks, consider referral to a specialist and go to scenario (B).	• Continue protocol therapy.
(Scenario B) Asymptomatic SBP ≥160 mmHg, or DBP ≥100 mmHg, OR Failure to achieve well-controlled BP within 2 weeks in Scenario A.	• Adjust current or initiate new antihypertensive medication(s). • Titrate antihypertensive medication(s) during the next 2 weeks to achieve well-controlled BP.	• Interrupt study treatment if clinically indicated. • Once BP is well-controlled[b], restart study treatment **reduced by one dose level**.
(Scenario C) Symptomatic hypertension OR 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). • Titrate antihypertensive medication(s) during the next 2 weeks to achieve well-controlled BP. • Referral to a specialist for further evaluation and follow-up is recommended.	• Interrupt study treatment if clinically indicated. • Once BP is well controlled, restart study treatment **reduced by one dose level**.[c]

Table 6-7: Dabrafenib or Dabrafenib + Trametinib Treatment Modification and Management Guidelines for Hypertension

Event	Management Guideline	Dose Modification
(Scenario D) Refractory hypertension unresponsive to above interventions or hypertensive crisis.	• Continue follow-up per protocol.	• Permanently discontinue study treatment.
a. Hypertension detected in two separate readings during up to three consecutive visits b. Well-controlled blood pressure defined as SBP ≤140 mm Hg and DBP ≤90 mm Hg in two separate readings during up to three consecutive visits. c. Escalation of trametinib to previous dose level can be considered if BPs remain well-controlled for 4 weeks after restarting of trametinib. Approval from CTEP Medical Monitor is required. • d. Symptomatic hypertension defined as hypertension aggravated by symptoms (e.g., headache, light-headedness, vertigo, tinnitus, episodes of fainting) that resolve after the blood pressure is controlled within the normal range		

6.1.12 Dabrafenib or Dabrafenib + Trametinib Dose Modification for **QTc Prolongation**

Table 6-8: Dabrafenib + Trametinib modification for QTc Prolongation

Prolongation	Action and Dose Modification
• QTcB ≥501 msec	• Interrupt study treatment until QTcB prolongation resolves to grade 1 or baseline. • Test serum potassium, calcium, phosphorus and magnesium. If abnormal, correct per routine clinical practice to within normal limits. • Review concomitant medication usage for agents that prolonged QTc. • If the event resolves, restart study treatment at current dose level.[b] • If the event does not resolve, permanently discontinue study treatment. **Consider evaluation with cardiologist.** • If the event recurs, permanently discontinue study treatment. **Consider evaluation with cardiologist.**
Abbreviations: msec = milliseconds; QTcB = QT interval on electrocardiogram corrected using the Bazett's formula a. Based on average QTc value of triplicate ECGs. For example, if an ECG demonstrates a prolonged QT interval, obtain two or more ECGs over a brief period, and then use the averaged QTc values of the three ECGs to determine if study treatments should be interrupted or discontinued. b. If the QTc prolongation resolves to grade 1 or baseline, the subject may resume study treatment if the investigator and CTEP medical monitor agree that the subject will benefit from further treatment.	

6.1.13 Dabrafenib or Dabrafenib + 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 Dabrafenib + Trametinib Treatment Modification and Management Guidelines for Diarrhea

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

6.1.14 Dabrafenib or Dabrafenib + Trametinib Dose Modification for **Vision Changes**

Episodes of vision changes have been observed in patients receiving dabrafenib, trametinib, or the combination of both therapies. An ophthalmologist should be consulted if changes in vision

develop. However, if the visual changes are clearly unrelated to study treatment (e.g., allergic conjunctivitis), then monitor closely as it may be reasonable to defer ophthalmic examination.

Uveitis and iritis have been associated with dabrafenib, while RPED and RVO have been associated with trametinib therapy. Monitor patients for visual signs and symptoms (such as change in vision, photophobia, and eye pain) during therapy. Special attention should be given to retinal findings (e.g., retinal pigment epithelial detachment (RPED) or retinovascular abnormalities (i.e., branch or central retinal vein occlusions [RVO]). For events of visual changes regardless of severity but for which an ophthalmic examination is conducted, a blood sample for PK analysis is encouraged when feasible, and the blood sample should be drawn as close as possible to the time of the event.

The ophthalmology exam will include best corrected visual acuity, visual field examination, tonometry, slit lamp biomicroscopic examination of the anterior segment (with special attention to inflammation) and the posterior segment, and dilated indirect fundoscopy with special attention to retinal abnormalities. Optical coherence tomography is strongly recommended at scheduled visits and if retinal abnormalities are suspected. Other types of ancillary testing including color fundus photography, and fluorescein angiography may also be indicated as determined by clinical exam.

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 Dabrafenib-Trametinib Treatment Modification for Visual Changes

CTCAE Grade	Management Guideline	Action and Dose Modification
Grade 1	• Consult ophthalmologist immediately.	• If dilated fundus examination cannot be performed within 7 days of onset, hold trametinib until RPED and RVO can be excluded by retina specialist/ ophthalmologist. Dabrafenib may be continued. • If RPED and RVO excluded, continue (or restart) trametinib at same dose level • If Uveitis/Iritis, refer to table below for Iritis/Uveitis • If RPED suspected or diagnosed, refer to RPED dose modification table below; **report as SAE if diagnosed**. • If RVO suspected or diagnosed: **Permanently discontinue trametinib and report as SAE if diagnosed.**

CTCAE Grade	Management Guideline	Action and Dose Modification
Grade 2 and 3	• Consult ophthalmologist immediately.	• Hold trametinib. Dabrafenib may be continued. • If RPED and RVO excluded, restart trametinib at same dose level • If Uveitis/Iritis, refer to table below for Uveitis/Iritis • If RPED diagnosed, see RPED dose modification table below; **report as SAE**. • If RVO diagnosed: **Permanently discontinue trametinib and report as SAE**.
Grade 4	• Consult ophthalmologist immediately.	• Interrupt trametinib. Dabrafenib may be continued. • If RPED and RVO excluded, may consider restarting trametinib at same or reduced dose after discussion with study medical monitor. • If Uveitis/Iritis, refer to table below • **If RVO or RPED diagnosed, permanently discontinue trametinib** and report as SAE.
Abbreviations: RPED = retinal pigment epithelial detachments; RVO = retinal vein occlusion; SAE = serious adverse event *If visual changes are clearly unrelated to study treatment (e.g., allergic conjunctivitis), monitor closely but ophthalmic examination is not required		

Table 6-11: Dose Modification for RPED

Event CTCAE Grade	Action and Dose Modification
Grade 1 RPED (Asymptomatic; clinical or diagnostic observations only)	• Continue trametinib with retinal evaluation monthly until resolution. If RPED worsens, follow instructions below. • Dabrafenib treatment is not affected
Grade 2-3 RPED (Symptomatic with mild to moderate decrease in visual acuity; limiting instrumental ADL)	• Interrupt trametinib. Continue dabrafenib • Retinal evaluation monthly. • If improved to ≤ Grade 1, restart trametinib with one dose level reduction (reduced by 0.5 mg) or discontinue in patients taking trametinib 1 mg daily. • If no recovery within 4 weeks permanently discontinue trametinib

Table 6-12: Dabrafenib or Dabrafenib-Trametinib Dose Modification for Uveitis and Iritis

CTCAE Grade	Action and Dose Modification
Uveitis and Iritis	• Continue study treatment • Control ocular inflammation with local therapies • If not improved to grade ≤1 within 1 week, interrupt dabrafenib until resolution of ocular inflammation and then restart dabrafenib reduced by one dose level • If no recover within 4 weeks, permanently discontinue dabrafenib. Trametinib may be continued.

6.1.15 Dabrafenib + Trametinib Dose Modification for **Pneumonitis**

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

Table 6-13: Dabrafenib-Trametinib Treatment Modification for Pneumonitis

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

6.1.16 Dabrafenib or Dabrafenib + Trametinib Dose Modification for **Liver Chemistry Changes**

Table 6-14: Dabrafenib or Dabrafenib-Trametinib Dose Modification for Liver Chemistry Changes

Event	Treatment modifications and assessment/monitoring
• ALT ≥3x ULN but <5x ULN and TB <2x ULN, without symptoms considered related to liver injury or hypersensitivity and who can be monitored weekly for 4 weeks	• **May continue study treatment**. • Report as SAE if CTEP-AERS reporting criteria is met. • If **liver chemistry stopping criteria** are met any time, proceed as described below. **MONITORING:** Repeat LFT (ALT, AST, ALK, bilirubin) until they return to normal/baseline or stabilise (LFT may be every 2 weeks after 4 weeks if ALT <3x ULN and TB <2 ULN).
Criteria for discontinuing study drug: When any of the liver stopping criteria below is met, discontinue trametinib and dabrafenib 1. ALT ≥3xULN **and** bilirubin ≥2x ULN or >35% direct bilirubin[1, 2] 2. ALT ≥ 3xULN **and** INR >1.5, if INR measured[2] (INR threshold does not apply if subject is on anticoagulant) 3. ALT ≥5x ULN 4. ALT ≥3x ULN persists for ≥4 weeks 5. ALT ≥3x ULN **and** cannot be monitored weekly for 4 weeks 6. ALT ≥3x ULN associated with symptoms[3] (new or worsening) believed to be related to liver injury or hypersensitivity	• **Immediately discontinue study treatment.** • **Do not restart/rechallenge** unless approved by CTEP medical monitor. *[Do not include for single-dose studies.]* • Report as SAE if: 1) CTEP-AERS reporting criteria are met, or 2) patients meet criteria 1-2. • Perform liver event **ASSESSMENT AND WORKUP (see below)**. • Monitor the subject until liver chemistries resolve, stabilize, or return to baseline (see **MONITORING** below). If applicable, provide details on required follow up assessments (*e.g.*, follow up for overall survival or disease recurrence or progression). *[Do not include for single-dose studies]* **MONITORING:** *In patients stopping for criteria 1-2 (with abnormal TB and INR, indicating potentially more significant liver toxicities):* • Repeat liver chemistries (ALT, AST, ALK, bilirubin) and perform liver event **follow-up assessments** within **24 hours**. • Monitor subjects twice weekly until LFT return to normal/baseline or stabilize. • A specialist or hepatology consultation is recommended. *In patients stopping for criteria 2-6:* • Repeat LFT and perform liver event **follow up assessments** within **24-72 hours** • Monitor subjects weekly until LFTs return to normal/baseline or stabilize. **ASSESSMENT and WORKUP:** • Viral hepatitis serology.[4] • If possible, obtain blood sample for PK analysis.[5] • Serum CPK and LDH. • Fractionate bilirubin, if total bilirubin ≥2x ULN. • CBC with differential to assess eosinophilia. • Record clinical symptoms of liver injury, or hypersensitivity on AE CRF. • Record concomitant medications (including acetaminophen, herbal

Event	Treatment modifications and assessment/monitoring
	remedies, other over the counter medications). • Record alcohol use. *Additional work up for patient stopping for criteria 1-2 (with abnormal TB and INR, indicating potentially more significant liver toxicities)*: • Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies, and quantitative total immunoglobulin G (IgG or gamma globulins). • Serum acetaminophen adduct HPLC assay (in subjects with likely acetaminophen use in the preceding). • If there is underlying chronic hepatitis B (e.g. positive hepatitis B surface antigen): quantitative hepatitis B DNA and hepatitis delta antibody.[6] • Liver imaging (ultrasound, MRI, CT) and /or liver biopsy.

6.1.17 Dabrafenib or Dabrafenib-Trametinib Dose Modification for Venous Thromembolism (VTE)

	Dabrafenib	Trametinib (When Used in Combination)
Uncomplicated DVT or PE	Do not modify the dose.	Withhold trametinib for up to 3 weeks. • If improved to Grade 0-1, resume at a lower dose level. • If not improved, permanently discontinue.
Life Threatening PE	Permanently discontinue dabrafenib	Permanently discontinue trametinib.

6.2 AT13387 Dose modifications

AT13387 dosing may be reduced in the setting of toxicity. The below table denotes the possible dose levels based on the dose escalation schema.

Current Dose	If Dose Reduction Required	Reduce To
260 mg/m2 days1, 8, and 15	→	220 mg/m2 days 1, 8, and 15
220 mg/m2 days 1, 8, and 15	→	180 mg/m2 days 1, 8, and 15
180 mg/m2 days 1, 8, and 15	→	150 mg/m2 days 1, 8, and 15
150 mg/m2 days 1, 8, and 15	→	Discontinue AT13387

Further dose modifications to 120 mg days 1, 8, and 15 are permitted at the discretion of the Investigator but should be discussed with CTEP and the study sponsors. In the event that dabrafenib, trametinib, or both agents need to be discontinued due to toxicity reasons, treatment with AT13387 may continue at the discretion of the investigator until time of disease progression.

6.2.1 Management of AT13387 toxicity

Dexamethasone and/or antihistamines could be given to treat or prevent the systemic infusion reactions. Antiemetics, anti-diarrheal agents, etc. may be given to treat or prevent gastrointestinal toxicities.

6.2.1.1 Dose Reduction and Interruption

Table 6-15: AT13387 Dose Modification for Toxicities Not Specified in Subsequent Sections

CTCAE Grade	Action and Dose Modification
Grade 1	• Continue study treatment at same dose level (no dose modification). • Monitor closely. • Provide supportive care according to institutional standards.
Grade 2 (Tolerable)	• Interrupt study treatment if clinically indicated. • Monitor closely. • Provide supportive care according to institutional standards. • When toxicity resolves to grade 1 or baseline, restart study treatment at current dose level.
Grade 2 (Intolerable) **Grade 3**	• Interrupt study treatment. • Monitor closely. • Provide supportive care according to institutional standards. • When toxicity resolves to grade 1 or baseline, restart study treatment **reduced by one dose level**. • If the grade 3 toxicity recurs, interrupt study treatment. • When toxicity resolves to grade 1 or baseline, **restart study treatment reduced by another dose level**.
Grade 4	• Interrupt study treatment. • Monitor closely. • Provide supportive care according to institutional standards. • Restart with study treatment **reduced by one dose level** once toxicity resolves to grade 1 or baseline. • 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 CTEP Medical Monitor.
In the event of abdominal pain or suspected pancreatitis, amylase and lipase laboratory samples should be collected for confirmation of the diagnosis.	

Dose reductions for QTc elevations
A 12-lead EKG will be performed in triplicate prior to the start of each AT13387 infusion. If a subject's QTc is >480 msec but ≤500 msec, the infusion will be withheld until the subject's QTc has decreased to ≤480 msec and then the subject may be dosed at the same dose level. If a subject's QTc is ≥500 msec, the treatment will be withheld until the subject's QTc has decreased to <480 msec and then the subject will be dosed at one dose level below their assigned treatment.

Dose modifications for ocular toxicity
While ocular toxicity is an adverse event of special interest, dose reductions and modifications will be made based on the table above (Table 6-13). Additionally, patients will be required to

have an ophthalmology visit within 24 hours of first event of visual disturbance.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting (via CTEP-AERS) **in addition** to routine reporting.

7.1 Comprehensive Adverse Events and Potential Risks Lists (CAEPRs)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset of AEs, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with ***bold*** and ***italicized*** text. The SPEER is a list of events that are protocol-specific exceptions to expedited reporting to NCI via CTEP-AERS (except as noted below).

Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification.

The CAEPR may not provide frequency data; if not, refer to the Investigator's Brochure for this information.

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required

7.1.1.1 CAEPR for Trametinib

Comprehensive Adverse Events and Potential Risks list (CAEPR)
for
Trametinib dimethyl sulfoxide (GSK1120212B, NSC 763093)

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 1111 patients.* Below is the CAEPR for Trametinib dimethyl sulfoxide (GSK1120212B).

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the

AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.4, October 7, 2016[1]

Adverse Events with Possible Relationship to Trametinib dimethyl sulfoxide (GSK1120212B) (CTCAE 4.0 Term) [n= 1111]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia (Gr 2)***
CARDIAC DISORDERS			
		Heart failure	
		Left ventricular systolic dysfunction	
	Sinus bradycardia		
EYE DISORDERS			
	Blurred vision		
	Dry eye		
		Eye disorders - Other (chorioretinopathy also known as retinal pigment epithelial detachment)	
		Eye disorders - Other (retinal vein occlusion)	
	Eye disorders - Other (visual disorders)[2]		
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 2)***
		Colitis	
		Colonic perforation	
	Constipation		***Constipation (Gr 2)***
Diarrhea			***Diarrhea (Gr 3)***
	Dry mouth		***Dry mouth (Gr 2)***
	Dyspepsia		***Dyspepsia (Gr 2)***
	Mucositis oral		***Mucositis oral (Gr 2)***
Nausea			***Nausea (Gr 3)***
	Vomiting		***Vomiting (Gr 3)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills		***Chills (Gr 2)***
	Edema face		
Fatigue			***Fatigue (Gr 3)***
	Fever		***Fever (Gr 2)***
IMMUNE SYSTEM DISORDERS			
	Allergic reaction[3]		
INFECTIONS AND INFESTATIONS			
	Lung infection		
	Paronychia		***Paronychia (Gr 2)***
	Skin infection		***Skin infection (Gr 2)***
INVESTIGATIONS			
	Alanine aminotransferase increased		***Alanine aminotransferase increased (Gr 2)***

Adverse Events with Possible Relationship to Trametinib dimethyl sulfoxide (GSK1120212B) (CTCAE 4.0 Term) [n= 1111]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Alkaline phosphatase increased		***Alkaline phosphatase increased (Gr 2)***
	Aspartate aminotransferase increased		***Aspartate aminotransferase increased (Gr 2)***
	CPK increased		
	Ejection fraction decreased		
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 2)***
	Dehydration		***Dehydration (Gr 3)***
	Hypoalbuminemia		
	Hypomagnesemia		***Hypomagnesemia (Gr 2)***
	Hyponatremia		***Hyponatremia (Gr 3)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		
	Back pain		***Back pain (Gr 2)***
		Musculoskeletal and connective tissue disorder - Other (rhabdomyolysis)	
	Pain in extremity		***Pain in extremity (Gr 2)***
NERVOUS SYSTEM DISORDERS			
	Dizziness		***Dizziness (Gr 2)***
	Headache		***Headache (Gr 2)***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		***Cough (Gr 2)***
	Dyspnea		***Dyspnea (Gr 2)***
		Pneumonitis	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Alopecia		***Alopecia (Gr 2)***
	Dry skin		***Dry skin (Gr 2)***
		Palmar-plantar erythrodysesthesia syndrome	
	Periorbital edema		
	Pruritus		***Pruritus (Gr 2)***
	Skin and subcutaneous tissue disorders - Other (folliculitis)		***Skin and subcutaneous tissue disorders - Other (folliculitis) (Gr 2)***
Skin and subcutaneous tissue disorders - Other (rash)[4]			***Skin and subcutaneous tissue disorders - Other (rash)[4] (Gr 3)***
VASCULAR DISORDERS			
	Hypertension		***Hypertension (Gr 2)***
Vascular disorders - Other (edema)[5]			***Vascular disorders - Other (edema)[5] (Gr 2)***
	Vascular disorders - Other (hemorrhage)[6]		

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting

PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Visual disorders include visual disturbance that can be associated with conjunctival hemorrhage, corneal graft rejection, cyclitis, eye nevus, halo vision, iritis, macular edema, retinal hemorrhage, visual acuity reduced, visual impairment, and vitreous detachment.

[3]Hypersensitivity (allergic reactions) may present with symptoms such as fever, rash, increased liver function tests, and visual disturbances.

[4]Skin and subcutaneous tissue disorders - Other (rash) may include rash, rash acneiform, rosacea, erythematous rash, genital rash, rash macular, exfoliative rash, rash generalized, erythema, rash papular, seborrhoeic dermatitis, dermatitis psoriasiform, rash follicular, and skin fissures.

[5]Edema includes edema, lymphedema, and edema limbs.

[6]The majority of hemorrhage events were mild. Major events, defined as symptomatic bleeding in a critical area or organ (e.g., eye, GI hemorrhage, GU hemorrhage, respiratory hemorrhage), and fatal intracranial hemorrhages have been reported.

Adverse events reported on Trametinib dimethyl sulfoxide (GSK1120212B) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that Trametinib dimethyl sulfoxide (GSK1120212B) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Disseminated intravascular coagulation; Febrile neutropenia; Leukocytosis
CARDIAC DISORDERS - Atrial fibrillation; Cardiac arrest; Myocardial infarction; Restrictive cardiomyopathy; Sinus tachycardia
EYE DISORDERS - Corneal ulcer; Eyelid function disorder; Flashing lights; Floaters; Glaucoma; Papilledema; Photophobia; Retinal detachment
GASTROINTESTINAL DISORDERS - Anal hemorrhage; Ascites; Duodenal ulcer; Enterocolitis; Esophageal necrosis; Esophageal ulcer; Esophagitis; Gastric hemorrhage; Gastric ulcer; Gastritis; Gastrointestinal disorders - Other (intestinal obstruction); Gastrointestinal disorders - Other (oropharyngeal pain); Gastrointestinal disorders - Other (pneumatosis intestinalis); Gastrointestinal fistula; Gingival pain; Hemorrhoidal hemorrhage; Ileus; Lower gastrointestinal hemorrhage; Obstruction gastric; Pancreatitis; Rectal hemorrhage; Small intestinal obstruction; Upper gastrointestinal hemorrhage
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Flu like symptoms; General disorders and administration site conditions - Other (axillary pain); Localized edema; Malaise; Non-cardiac chest pain; Pain
HEPATOBILIARY DISORDERS - Cholecystitis; Hepatic failure; Hepatic pain; Hepatobiliary disorders - Other (hepatic encephalopathy)
INFECTIONS AND INFESTATIONS - Biliary tract infection; Catheter related infection; Device related infection; Endocarditis infective; Enterocolitis infectious; Hepatitis viral; Infections and infestations - Other (abscess limb); Infections and infestations - Other (necrotizing fasciitis); Infections and infestations - Other (oral infection); Pharyngitis; Rash pustular; Sepsis; Upper respiratory infection; Urinary tract infection
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Bruising
INVESTIGATIONS - Blood bilirubin increased; Creatinine increased; Electrocardiogram QT corrected interval prolonged; GGT increased; Investigations - Other (blood lactate dehydrogenase increased); Lipase increased; Lymphocyte count decreased; Platelet count decreased; Serum amylase increased; White blood cell decreased
METABOLISM AND NUTRITION DISORDERS - Hyperglycemia; Hyperkalemia; Hyperuricemia; Hypocalcemia; Hypoglycemia; Hypokalemia; Metabolism and nutrition disorders - Other (hyperphosphatemia)

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Generalized muscle weakness; Musculoskeletal and connective tissue disorder - Other (compression fracture); Musculoskeletal and connective tissue disorder - Other (muscle spasm); Myalgia; Neck pain
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (tumor hemorrhage); Tumor pain
NERVOUS SYSTEM DISORDERS - Dysgeusia; Encephalopathy; Intracranial hemorrhage; Lethargy; Nervous system disorders - Other (diplopia); Seizure; Somnolence; Stroke; Syncope; Transient ischemic attacks
PSYCHIATRIC DISORDERS - Anxiety; Confusion; Delirium; Depression; Hallucinations; Insomnia; Personality change
RENAL AND URINARY DISORDERS - Acute kidney injury; Cystitis noninfective; Hematuria; Proteinuria; Renal and urinary disorders - Other (dysuria); Urinary incontinence
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Vaginal fistula; Vaginal hemorrhage
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Bronchopulmonary hemorrhage; Epistaxis; Hypoxia; Laryngeal edema; Pleural effusion; Pneumothorax; Productive cough; Pulmonary hypertension; Respiratory failure; Sinus disorder
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Bullous dermatitis; Photosensitivity; Purpura; Skin and subcutaneous tissue disorders - Other (erythema nodosum); Skin and subcutaneous tissue disorders - Other (nail disorder); Skin and subcutaneous tissue disorders - Other (skin fissures); Skin ulceration; Urticaria
VASCULAR DISORDERS - Hematoma; Hot flashes; Hypotension; Thromboembolic event (venous)

Note: Trametinib dimethyl sulfoxide (GSK1120212B) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.1.1.2 CAEPR for Dabrafenib

Comprehensive Adverse Events and Potential Risks list (CAEPR) for Dabrafenib mesylate (GSK2118436B, NSC 763760)

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 1291 patients.* Below is the CAEPR for Dabrafenib mesylate (GSK2118436B).

> **NOTE**: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.3, May 20, 2016[1]

Adverse Events with Possible Relationship to Dabrafenib mesylate (GSK2118436B) (CTCAE 4.0 Term) [n= 1291]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia[2,3]		***Anemia[2,3] (Gr 2)***
EYE DISORDERS			
		Eye disorders - Other (iritis)[4]	
		Uveitis[4]	
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 3)***
	Constipation		***Constipation (Gr 3)***
	Diarrhea		***Diarrhea (Gr 3)***
Nausea			***Nausea (Gr 3)***
		Pancreatitis	
	Vomiting		***Vomiting (Gr 3)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills		***Chills (Gr 2)***
	Edema limbs[5]		***Edema limbs[5] (Gr 2)***
Fatigue			***Fatigue (Gr 3)***
Fever[6]			***Fever[6] (Gr 2)***
	Flu like symptoms		***Flu like symptoms (Gr 2)***
	General disorders and administration site conditions - Other (hemorrhage)[7]		
IMMUNE SYSTEM DISORDERS			
		Allergic reaction[8]	
INFECTIONS AND INFESTATIONS			
	Infections and infestations - Other (nasopharyngitis)		
INVESTIGATIONS			
	Creatinine increased[3]		***Creatinine increased[3] (Gr 2)***
	Neutrophil count decreased[3]		***Neutrophil count decreased[3] (Gr 2)***
	Platelet count decreased[3]		***Platelet count decreased[3] (Gr 2)***
	White blood cell decreased[3]		***White blood cell decreased[3] (Gr 2)***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 2)***
Hyperglycemia[3]			***Hyperglycemia[3] (Gr 2)***
	Hypokalemia[3]		***Hypokalemia[3] (Gr 2)***
	Hyponatremia[3]		***Hyponatremia[3] (Gr 2)***
Hypophosphatemia[3]			***Hypophosphatemia[3] (Gr 2)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
Arthralgia			***Arthralgia (Gr 3)***
	Back pain		***Back pain (Gr 3)***
	Myalgia		***Myalgia (Gr 3)***
	Pain in extremity		***Pain in extremity (Gr 3)***
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS)			

Adverse Events with Possible Relationship to Dabrafenib mesylate (GSK2118436B) (CTCAE 4.0 Term) [n= 1291]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (squamous cell carcinoma or keratoacanthoma)[9]		***Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (squamous cell carcinoma or keratoacanthoma)[9] (Gr 2)***
	Treatment related secondary malignancy (non-SCC)[10]		
NERVOUS SYSTEM DISORDERS			
	Dizziness		***Dizziness (Gr 2)***
Headache			***Headache (Gr 2)***
		Syncope	
RENAL AND URINARY DISORDERS			
		Renal and urinary disorders - Other (renal failure)	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		***Cough (Gr 2)***
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
Alopecia			***Alopecia (Gr 2)***
	Dry skin		***Dry skin (Gr 2)***
	Hyperhidrosis		***Hyperhidrosis (Gr 2)***
	Palmar-plantar erythrodysesthesia syndrome		***Palmar-plantar erythrodysesthesia syndrome (Gr 2)***
	Pruritus		***Pruritus (Gr 3)***
Rash[11]			***Rash[11] (Gr 2)***
	Skin and subcutaneous tissue disorders - Other (abnormal hair texture)		***Skin and subcutaneous tissue disorders - Other (abnormal hair texture) (Gr 2)***
Skin and subcutaneous tissue disorders - Other (hyperkeratosis)			***Skin and subcutaneous tissue disorders - Other (hyperkeratosis) (Gr 2)***
		Skin and subcutaneous tissue disorders - Other (neutrophilic panniculitis)[12]	
Skin and subcutaneous tissue disorders - Other (skin papilloma)			***Skin and subcutaneous tissue disorders - Other (skin papilloma) (Gr 2)***
VASCULAR DISORDERS			
	Vascular disorders - Other (venous thromboembolic event)[13]		

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]The incidence of anemia is increased when dabrafenib mesylate (GSK2118436B) is used in combination with trametinib dimethyl sulfoxide (GSK1120212B).

[3]The frequencies of these events are based upon laboratory findings rather than being due to patient-

reported outcomes.

[4]Dabrafenib mesylate (GSK2118436B) has been associated with ocular toxicities including chorioretinitis, retinitis, iridocyclitis, iritis, and uveitis.

[5]Edema limbs (peripheral edema) is a risk associated when dabrafenib mesylate (GSK2118436B) is used in combination with trametinib dimethyl sulfoxide (GSK1120212B) compared to dabrafenib mesylate (GSK2118436B) alone.

[6]Fever (pyrexia) can be associated with hypotension and/or (in rare cases) syncope. The frequency of fever and serious febrile events is increased when dabrafenib mesylate (GSK2118436B) is used in combination with trametinib dimethyl sulfoxide (GSK1120212B).

[7]Treatment with dabrafenib mesylate (GSK2118436B) in combination with trametinib dimethyl sulfoxide (GSK1120212B) resulted in an increased incidence and severity of hemorrhagic events compared to patients treated with dabrafenib mesylate (GSK2118436B) as a single agent. Sites of hemorrhage may include, but are not limited to, intracranial, reproductive tract, respiratory tract, and gastrointestinal hemorrhage.

[8]Manifestations of allergic reactions (hypersensitivity) to dabrafenib mesylate (GSK2118436B) may include bullous rash (bullous dermatitis).

[9]Squamous cell carcinoma (SCC), including SCC of the skin, SCC in situ (Bowen's disease), and keratoacanthoma have been observed.

[10]New non-SCC malignancies have been reported including primary melanoma, basal cell carcinoma, and non-cutaneous malignancies.

[11]Rash includes the terms: rash, rash acneiform, rash papular, rash maculo-papular, and erythema.

[12]Recurrent neutrophilic panniculitis has been observed in at least one patient treated with dabrafenib mesylate (GSK2118436B) in combination with the MEK inhibitor trametinib dimethyl sulfoxide (GSK1120212B).

[13]Venous thromboembolic events (including deep vein thrombosis and pulmonary embolism) is a risk associated when dabrafenib mesylate (GSK2118436B) is used in combination with trametinib dimethyl sulfoxide (GSK1120212B).

Adverse events reported on Dabrafenib mesylate (GSK2118436B) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that Dabrafenib mesylate (GSK2118436B) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (agranulocytosis); Blood and lymphatic system disorders - Other (pancytopenia); Disseminated intravascular coagulation; Febrile neutropenia; Hemolysis
CARDIAC DISORDERS - Acute coronary syndrome; Atrial fibrillation; Atrial flutter; Heart failure; Left ventricular systolic dysfunction; Mitral valve disease; Myocardial infarction; Sinus tachycardia
ENDOCRINE DISORDERS - Hyperthyroidism; Hypothyroidism
EYE DISORDERS - Blurred vision; Eye disorders - Other (amaurosis fugax); Eye disorders - Other (visual acuity reduced); Eye disorders - Other (visual impairment); Eye disorders - Other (vitreous detachment); Floaters; Photophobia; Retinopathy
GASTROINTESTINAL DISORDERS - Colitis; Colonic perforation; Dry mouth; Dyspepsia; Gastritis; Stomach pain
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Localized edema; Non-cardiac chest pain; Pain

HEPATOBILIARY DISORDERS - Cholecystitis; Hepatic pain
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Bruising
INVESTIGATIONS - Alanine aminotransferase increased; Alkaline phosphatase increased; Aspartate aminotransferase increased; Blood bilirubin increased; CD4 lymphocytes decreased; Ejection fraction decreased; Electrocardiogram QT corrected interval prolonged; GGT increased; Lipase increased; Lymphocyte count decreased; Weight loss
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hypercalcemia; Hypernatremia; Hypocalcemia; Hypoglycemia; Hypomagnesemia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthritis; Generalized muscle weakness; Musculoskeletal and connective tissue disorder - Other (muscle spasms); Neck pain
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Leukemia secondary to oncology chemotherapy; Myelodysplastic syndrome; Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (mycosis fungoides)
NERVOUS SYSTEM DISORDERS - Ataxia; Cognitive disturbance; Dysgeusia; Intracranial hemorrhage; Lethargy; Nervous system disorders - Other (intracranial pressure increased); Paresthesia; Seizure; Somnolence
PSYCHIATRIC DISORDERS - Anxiety; Confusion; Depression
RENAL AND URINARY DISORDERS - Hematuria; Renal calculi; Urinary frequency
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Menorrhagia; Reproductive system and breast disorders - Other (hematospermia)
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Nasal congestion; Respiratory, thoracic and mediastinal disorders - Other (oropharyngeal pain); Sore throat; Stridor; Voice alteration
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Photosensitivity; Purpura; Skin and subcutaneous tissue disorders - Other (palmoplantar keratoderma)
VASCULAR DISORDERS - Flushing; Hot flashes; Hypertension; Hypotension

Note: Dabrafenib mesylate (GSK2118436B) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.1.1.3 CAEPR for AT13387

Comprehensive Adverse Events and Potential Risks list (CAEPR) for AT13387 (Onalespib, NSC 749712)

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 119 patients.* Below is the CAEPR for AT13387 (Onalespib).

> **NOTE**: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.0, April 6, 2017[1]

Adverse Events with Possible Relationship to AT13387 (Onalespib) (CTCAE 4.0 Term) [n= 119]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			***Anemia (Gr 2)***
EYE DISORDERS			
	Blurred vision		
	Eye disorders - Other (visual impairment)		***Eye disorders - Other (visual impairment) (Gr 2)***
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 2)***
	Constipation		***Constipation (Gr 2)***
Diarrhea			***Diarrhea (Gr 2)***
	Dry mouth		***Dry mouth (Gr 2)***
	Dyspepsia		***Dyspepsia (Gr 2)***
	Flatulence		***Flatulence (Gr 2)***
	Gastrointestinal hemorrhage[2]		
	Hemorrhoids		***Hemorrhoids (Gr 2)***
Nausea			***Nausea (Gr 2)***
	Vomiting		***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Edema limbs		
Fatigue			***Fatigue (Gr 2)***
	Fever[3]		***Fever[3] (Gr 2)***
	Infusion related reaction[3]		***Infusion related reaction[3] (Gr 2)***
Injection site reaction[4]			***Injection site reaction[4] (Gr 2)***
	Malaise		***Malaise (Gr 2)***
INFECTIONS AND INFESTATIONS			
	Infection[5]		***Infection[5] (Gr 2)***
INVESTIGATIONS			
	Alanine aminotransferase increased		***Alanine aminotransferase increased (Gr 2)***
	Alkaline phosphatase increased		***Alkaline phosphatase increased (Gr 2)***
	Aspartate aminotransferase increased		***Aspartate aminotransferase increased (Gr 2)***
	CPK increased		***CPK increased (Gr 2)***
	Electrocardiogram QT corrected interval prolonged		
	Lymphocyte count decreased		***Lymphocyte count decreased (Gr 2)***
	Platelet count decreased		***Platelet count decreased (Gr 2)***
	Weight loss		***Weight loss (Gr 2)***
	White blood cell decreased		
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 2)***
	Dehydration		***Dehydration (Gr 2)***
	Hypocalcemia		***Hypocalcemia (Gr 2)***
	Hypokalemia		
	Hypomagnesemia		
	Hyponatremia		***Hyponatremia (Gr 2)***

Adverse Events with Possible Relationship to AT13387 (Onalespib) (CTCAE 4.0 Term) [n= 119]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Musculoskeletal and connective tissue disorder - Other (muscle spasms)		***Musculoskeletal and connective tissue disorder - Other (muscle spasms) (Gr 2)***
	Myalgia		***Myalgia (Gr 2)***
NERVOUS SYSTEM DISORDERS			
	Dizziness		***Dizziness (Gr 2)***
	Dysgeusia		
	Headache		***Headache (Gr 2)***
PSYCHIATRIC DISORDERS			
	Insomnia		***Insomnia (Gr 2)***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		***Cough (Gr 2)***
	Dyspnea		***Dyspnea (Gr 2)***
	Hiccups		***Hiccups (Gr 2)***
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Dry skin		***Dry skin (Gr 2)***
	Hyperhidrosis[3]		***Hyperhidrosis[3] (Gr 2)***
	Rash acneiform		
	Rash maculo-papular		***Rash maculo-papular (Gr 2)***
VASCULAR DISORDERS			
	Flushing		***Flushing (Gr 2)***

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Gastrointestinal hemorrhage includes Anal hemorrhage, Cecal hemorrhage, Colonic hemorrhage, Duodenal hemorrhage, Esophageal hemorrhage, Esophageal varices hemorrhage, Gastric hemorrhage, Hemorrhoidal hemorrhage, Ileal hemorrhage, Intra-abdominal hemorrhage, Jejunal hemorrhage, Lower gastrointestinal hemorrhage, Oral hemorrhage, Pancreatic hemorrhage, Rectal hemorrhage, Retroperitoneal hemorrhage, and Upper gastrointestinal hemorrhage under the GASTROINTESTINAL DISORDERS SOC.

[3]Infusion-related reactions may include, tachycardia/bradycardia, hypotension/hypertension, flushing, chills, fever, hyperhidrosis, itching, rigors, and abdominal cramps.

[4]Injection site reaction may include injection site irritation, injection site pain, injection site inflammation or redness, or erythema.

[5]Infection may include all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

Adverse events reported on AT13387 (Onalespib) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that AT13387 (Onalespib) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Febrile neutropenia
CARDIAC DISORDERS - Cardiac disorders - Other (atrioventricular block NOS); Left ventricular systolic dysfunction; Palpitations
EYE DISORDERS - Dry eye; Eye disorders - Other (color distortion); Eye disorders - Other (diplopia); Eye disorders - Other (halos); Eye disorders - Other (loss of visual acuity during changes in ambient light levels); Eye disorders - Other (tunnel vision); Eye disorders - Other (visual color darkening); Eye disorders - Other (visual disturbances); Eye pain; Flashing lights; Floaters; Keratitis; Night blindness; Papilledema; Photophobia; Retinopathy
GASTROINTESTINAL DISORDERS - Colitis; Mucositis oral; Oral dysesthesia; Oral pain; Salivary duct inflammation
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills[3]; Flu like symptoms
HEPATOBILIARY DISORDERS - Hepatic hemorrhage
INVESTIGATIONS - Activated partial thromboplastin time prolonged; Blood bilirubin increased; Creatinine increased; Ejection fraction decreased; Neutrophil count decreased
METABOLISM AND NUTRITION DISORDERS - Hyperglycemia; Hypoalbuminemia; Hypophosphatemia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Back pain; Bone pain; Generalized muscle weakness
NERVOUS SYSTEM DISORDERS - Seizure; Syncope; Tremor
PSYCHIATRIC DISORDERS - Anxiety
RENAL AND URINARY DISORDERS - Acute kidney injury; Proteinuria
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Pneumonitis
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Palmar-plantar erythrodysesthesia syndrome; Pruritus; Skin hyperpigmentation
VASCULAR DISORDERS - Hypertension[3]

Note: AT13387 (Onalespib) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting beginning April 1, 2018. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs for the agent that are ***bold and italicized*** in the CAEPR (*i.e.*, those listed in the SPEER column, Section 7.1.1) should be reported through CTEP-AERS only if the grade is above the grade provided in the SPEER.
  - Other AEs for the protocol that do not require expedited reporting are addressed in section 7.4.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.

- Probable – The AE *is likely related* to the study treatment.
- Possible – The AE *may be related* to the study treatment.
- Unlikely – The AE *is doubtfully related* to the study treatment.
- Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Expedited Adverse Event Reporting

7.3.1 Expedited AE reporting for this study must use CTEP-AERS (Adverse Event Expedited Reporting System), accessed via the CTEP Web site (https://eapps-ctep.nci.nih.gov/ctepaers). The reporting procedures to be followed are presented in the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" which can be downloaded from the CTEP Web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm. These requirements are briefly outlined in the tables below (Section 7.3.3).

In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, the 24-hour notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site.

7.3.2 CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Study Coordinator of the Lead Organization, Principal Investigator, and the local treating physician, and the Reporter and Submitter. CTEP-AERS provides a copy feature for other e-mail recipients.

7.3.3 Expedited Reporting Guidelines

Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

Note: A death on study requires both routine and expedited reporting regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.

Death due to progressive disease should be reported as **Grade 5 "Disease progression"** in the system organ class (SOC) "General disorders and administration site conditions." Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention [1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)

NOTE: Investigators MUST immediately report to the sponsor (NCI) ANY Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)

An adverse event is considered serious if it results in ANY of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours

4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria MUST be immediately reported to the NCI via electronic submission within the timeframes detailed in the table below.

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days	24-Hour 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR.

Expedited AE reporting timelines are defined as:

- "24-Hour; 5 Calendar Days" - The AE must initially be electronically submitted within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "10 Calendar Days" - A complete expedited report on the AE must be submitted electronically within 10 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:

Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 3, 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

[2]For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

7.4 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions. **AEs reported expeditiously through CTEP-AERS must also be reported in routine study data submissions.**

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. AEs are reported in a routine manner at scheduled times during the trial using Medidata Rave. For this trial the Adverse Event Case Report Form is used for routine AE reporting in Rave.

7.5 Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported expeditiously via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (*e.g.*, acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

7.6 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine reporting unless otherwise specified. For dabrafenib-containing protocols, all second malignancies should be reported through CTEP-AERS.

7.7 Pregnancy, Fetal Death, and Death Neonatal

NOTE: When submitting CTEP-AERS reports for "Pregnancy", "Pregnancy loss", or "Neonatal loss", the **Pregnancy Information Form** should be completed and faxed along with any additional medical information to **301-230-0159 (see Appendix F).** The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agent(s) should be documented in the "Description of Event" section of the CTEP-AERS report.

7.7.1.1 Pregnancy

- Because patients who become pregnant on study risk intrauterine exposure of the fetus to agents which may be teratogenic, DCTD/DCP is requesting that pregnancy should be reported in an expedited manner via CTEP-AERS as **Grade 3** "***Pregnancy, puerperium and perinatal conditions - Other (pregnancy)***" under the ***Pregnancy, puerperium and perinatal conditions*** SOC.
- The pregnancy outcome for patients on study should be reported via CTEP-AERS at the time the outcome becomes known, accompanied by the same Pregnancy Report Form used for the initial report.

7.7.1.2 Pregnancy Loss

• Pregnancy loss is defined in CTCAE as "*Death in utero*."

• Any Pregnancy loss should be reported expeditiously, as Grade 4 "*Pregnancy loss*" under the Pregnancy, puerperium and perinatal conditions SOC.

• A Pregnancy loss should NOT be reported as a Grade 5 event under

• the Pregnancy, puerperium and perinatal conditions SOC, as currently CTEPAERS

• recognizes this event as a patient death.

7.7.1.3 **Death Neonatal**

- Neonatal death, defined in CTCAE as "**A disorder characterized by cessation of life occurring during the first 28 days of life**" that is felt by the investigator to be at least possibly due to the investigational agent/intervention, should be reported expeditiously.
- A neonatal death should be reported expeditiously as **Grade 4** "***Death neonatal***" under the ***General disorders and administration*** SOC.
- Neonatal death should **NOT** be reported as "***Death neonatal"*** under the ***General disorders and administration SOC***, a Grade 5 event. If reported as such, the CTEP-AERS interprets this as a death of the patient being treated.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational agents administered in this study can be found in Section 7.1.

8.1 CTEP IND Agent(s)

8.1.1 Trametinib dimethyl sulfoxide (GSK1120212B) (NSC 763093)

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

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

CAS Registry Number: 1187431-43-1

Classification: MEK inhibitor

Molecular Formula: $C_{26}H_{23}FIN_5O_4 \bullet C_2H_6OS$

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

Approximate Solubility: Trametinib dimethyl sulfoxide is almost insoluble in water

(<0.0001 mg/mL at 25° C)

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.

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

How Supplied: Novartis supplies and CTEP, NCI, DCTD distributes 0.5 mg and 2 mg (as free base) tablets.

Each commercially-labeled bottle contains 30 tablets with a desiccant.

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 yellow, modified oval, biconvex and film-coated with 'GS' debossed on one face and 'TFC' on the opposing face. Aqueous film coating consists of hypromellose, titanium dioxide, polyethylene glycol, iron oxide yellow.

- 2 mg tablets are pink, round, biconvex and film-coated with 'GS' debossed on one face and 'HMJ' on the opposing face. Aqueous film coating consists of hypromellose, titanium dioxide, polyethylene glycol, polysorbate 80, iron oxide red.

Storage: Store tablets at 2°C - 8°C in the original bottle. Do not repackage tablets or remove desiccant. Bottles should be protected from light and moisture.

If a storage temperature excursion is identified, promptly return trametinib to 2°C -8°C and quarantine the supplies. Provide a detailed report of the excursion (including documentation of temperature monitoring and duration of the excursion) to PMBAfterHours@mail.nih.gov for determination of suitability.

Stability: Shelf life studies of trametinib dimethyl sulfoxide are ongoing. Refer to the package label for expiration.

Route of Administration: Oral. Take by mouth on an empty stomach, either 1 hour before or 2 hours after a meal. If a dose of trametinib is missed, the dose can be taken if it is more than 12 hours until the next scheduled dose.

Potential Drug Interactions

In vitro studies suggest that trametinib dimethyl sulfoxide is not a substrate of CYP enzymes or of human BCRP, MRP2, OATP1B1, OATP1B3, OATP2B1, OCT1 or

MATE1 transporters. Trametinib elimination by deacetylation to metabolite M5 is dependent on carboxylesterases (CES1b, CES1c and CES2). M5 is eliminated by CYP3A4 and other pathways, presenting the clinically relevant, albeit low, potential for drug-drug interaction. Trametinib is a substrate for P-gp and BSEP, but this is not expected to be clinically relevant due to trametinib's high permeability.

Trametinib dimethyl sulfoxide is an *in vitro* inhibitor of CYP 2C8, and is anticipated to have overall low potential for drug interactions as a perpetrator. It is also a weak CYP3A4 inducer and expected to have little clinical effect on sensitive substrates. Trametinib is not an inhibitor of CYP 1A2, 2A6, 2B6, 2C9, 2C19, 2D6 and 3A4 and not an inhibitor of P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT2 MRP2 and MATE1.

Availability

Trametinib dimethyl sulfoxide (GSK1120212B) is FDA-approved for the treatment of unresectable or metastatic BRAF-mutant melanoma. It will be supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI.

Trametinib dimethyl sulfoxide (GSK1120212B) is provided to the NCI under a Collaborative Agreement between the Pharmaceutical Collaborator and the DCTD, NCI (see Section 12.3).

8.1.2 Dabrafenib mesylate (GSK2118436B) (NSC 763760)

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

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

Classification: BRAF inhibitor

CAS Registry Number: 1195768-06-9

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

M.W.: 615.68 (mesylate salt)

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.

How Supplied: Dabrafenib mesylate (GSK2118436B) capsules are supplied by Novartis and distributed by the DCTD, NCI, as 50 mg and 75 mg capsules (equivalent to the free-base) for oral administration.

Each commercially-labeled bottle contains 120 capsules and a silica gel desiccant.

- 50 mg capsule is dark red and imprinted with 'GS TEW' and '50 mg.'
- 75 mg capsule is dark pink and imprinted with 'GS LHF' and '75 mg.'

Capsule excipients include microcrystalline cellulose, magnesium stearate (vegetable source), and colloidal silicon dioxide. Capsule shells contain hypromellose, red iron oxide (E172), and titanium dioxide (E171).

Storage: Store at 25°C (77°F); excursions permitted to 15°C to 30°C (59°F to 86°F) [see USP Controlled Room Temperature].

Stability: Shelf-life studies of dabrafenib mesylate (GSK2118436B) are ongoing. Refer to the package label for expiration.

If a storage temperature excursion is identified, promptly return dabrafenib mesylate (GSK2118436B) to room temperature and quarantine the supplies. Provide a detailed report of the excursion (including documentation of temperature monitoring and duration of the excursion) to PMBAfterHours@mail.nih.gov for determination of suitability.

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.

Potential Drug Interactions: *In vivo* data shows that metabolism of dabrafenib mesylate (GSK2118436B) and its metabolites is mediated by CYP3A4 and CYP2C8. Use caution if strong inducers or inhibitors of CYP2C8 or 3A4 are co-administered with dabrafenib. Although *in vitro* studies indicate dabrafenib mesylate and its metabolites are substrates for P-glycoprotein (P-gp) and BCRP transporters, the apparent high oral bioavailability and permeability indicate modulation of efflux transporters will have minimal impact on dabrafenib pharmacokinetics.

Dabrafenib mesylate (GSK2118436B) and its metabolites showed moderate inhibition of CYP 2C8, 2C9, 2C19 and 3A4 in human microsomes studies. Use caution in patients who are taking sensitive substrates of these enzymes. Additionally, dabrafenib and its metabolites showed inhibition of OATP1B1, OATP1B3, OAT1, OAT3 and OCT2 transporter systems and weak to moderate inhibition of BCRP. Neither dabrafenib nor its metabolites show inhibition of P-gp *in vitro*.

Dabrafenib mesylate (GSK2118436B) induces CYP3A4, 2C9 and possibly 2B6, 2C8, 2C19 enzymes, UDP glucuronosyltransferase and P-gp. Use caution in patients who are taking substrates of these pathways, such as warfarin or hormonal contraceptives.

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.

Patient Care Implications: In the case of overdose, patients should be treated symptomatically since there is no specific antidote. Hemodialysis is likely to be ineffective since dabrafenib mesylate is highly bound to plasma proteins.

Availability

Dabrafenib mesylate (GSK2118436B) is FDA-approved for the treatment of unresectable or metastatic BRAF-mutant melanoma. It is supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI.

Dabrafenib mesylate (GSK2118436B) is provided to the NCI under a Collaborative Agreement between the Pharmaceutical Collaborator and the DCTD, NCI (see Section 12.3).

8.1.3 AT13387

Chemical Name: (2,4-dihydroxy-5-isopropyl-phenyl)-[5-(4-methyl-piperazin-1-ylmethyl)-1,3-dihydro-isoindol-2-yl]-methanone, *L*-lactic acid salt

Other Name: AT13387AU, Onalespib

Classification: Heat shock protein 90 (HSP90) inhibitor

Molecular Formula: $C_{24}H_{31}N_3O_3.C_3H_6O_3$ **M.W.:** 499.61

Mode of Action: AT13387 is a synthetic non-ansamycin small molecule that inhibits heat shock protein 90 (HSP90. HSP90 seems to affect multiple aberrant signaling pathways and therefore may be of clinical benefit in several cancer treatments.

How Supplied: AT13387 is supplied by Astex Pharmaceuticals Inc. and distributed by CTEP, NCI as a 265-mg free base equivalent (L-lactic acid salt) vial, containing white to off-white lyophilized powder. The agent is formulated in **pH 5.0 (red cap).**

Preparation: Reconstitute the 265-mg lyophilized powder with 10 mL of Sterile Water for Injection (SWFI) resulting in 25.7 mg/mL concentration (10.3 mL total volume). A sticky mass will be formed. Vigorously shake the vial. Agitate until the contents are fully dissolved (about 5 minutes). Leave the diluted vial at ambient temperature for 15-30 minutes to allow any foam to dissipate. If not used immediately, store the reconstituted vial(s) at 2^0 to 8^0 C not to exceed 8 hours.

Withdraw the calculated dose of AT13387 and further dilute it in 250 mL of D5W or 0.9% NS. The prepared IV solution is compatible in PVC or non-PVC infusion bags.

Store the prepared IV solution at 2^0 to 8^0 C (not to exceed 8 hours) if not used immediately. When removed from the refrigerator, allows the prepared IV solution to sit at room temperature between 15 to 30 minutes before administering to patients. The prepared IV solution must be used within 8 hours -i.e., from the time the drug vial is diluted to the time the IV administration is complete. Protection from light during the infusion period is not required.

Storage: Store the intact vials at 15^0 to 25ºC (59 to 77ºF). Protect from light.

If a storage temperature excursion is identified, promptly return AT13387 to 15^0 to 25ºC (59 to 77ºF) and quarantine the supplies. Provide a detailed report of the excursion (including documentation of temperature monitoring and duration of the excursion) to PMBAfterHours@mail.nih.gov for determination of suitability.

Stability: Shelf life surveillance of the intact vials is ongoing**.**
Route of Administration: Intravenous

Method of Administration: Infuse over 1 hour through a central line or a well-defined peripheral vein (Note: an in-line filter is NOT required). If use a peripheral line, be sure to aspirate venous blood prior to starting the infusion. Check the infusion site every 15 minutes. Change infusion site should evidence of swelling or discoloration is observed.

Potential Drug Interactions: AT13387 is a substrate of UGT with a relatively low affinity for UGT isoforms. In vitro data demonstrate that AT13387 is a weak inhibitor of UGT1A1, UGT1A3 and UGT1A9. AT13387 is also a weak inhibitor of CYP1A2, -3A4, -2D6, -2C9 and -2C19. AT13387 appears to metabolize via the glucuronidation, sulphation and N-oxidation.

Pre-clinical studies suggest that AT13387 is a substrate of P-gp, the efflux ratios was above 2 (ranging from 3.4 to 4.6); a moderate inhibitor of BCRP (35.9% +/- 2%, p=0.0001) and P-gp (31.3% +/- 1.2%, p= 0.0009), and a strong inhibitor of MATE1 (94.6% +/- 0.2%, p=0.0001) and MATE2-K (91.2% +/- 1.2%, p= 0.0002).

Patient Care Implications: There are no genotoxicity, carcinogenicity, developmental and reproductive studies conducted with AT13387. Women of childbearing potential should not become pregnant or breastfeed and men should not father a child during the study. All subjects must use acceptable contraceptive measures during the treatment of AT13387 and 3 months after the last dose of the investigational drug.

Systemic infusion reactions (e.g., vomiting, itching skin, or swelling), slow the infusion and/or administer NS or D5W through a "Y" connector in parallel to AT13387 IV infusion. Pre-medication (e.g., dexamethasone, H-1 and H-2 antagonist) may be given before subsequent infusions and/or administer additional volume of 500 mL over 1 hour if medically appropriate.

Avoid extravasation. For local irritation, apply cold compress or topical pain medication. Change infusion site if any evidence of swelling or discoloring is observed.

8.1.4 Agent Ordering and Agent Accountability

8.1.4.1 NCI-supplied agents may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained). The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees can submit agent requests through the PMB Online Agent Order Processing (OAOP) application (https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx). Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account (https://eapps-ctep.nci.nih.gov/iam/) and the maintenance of an "active" account status and a "current" password.

For questions about drug orders, transfers, returns, or accountability, call (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET) or email PMBAfterHours@mail.nih.gov anytime.

8.1.4.2 Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Drug Accountability Record Form (DARF). (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.

8.1.4.3 Investigator Brochure Availablilty— The current versions of the IBs for PMB-supplied agents will be accessible to site investigators and research staff through the PMB Online Agent Order Processing (OAOP) application. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account and the maintenance of an "active" account status and a "current" password. Questions about IB access may be directed to the PMB IB coordinator at IBCoordinator@mail.nih.gov

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Biomarker Studies

9.1.1 PK Analysis

Little is known about the potential for drug-drug interactions (DDI) of the triple combination of dabrafenib, trametinib, and AT13387. For this reason, extensive PK analysis will be performed during this study.

9.1.2 Tissue anaysis of HSP90 inhibition

Subjects enrolled into the trial that have tumor tissue which is accessible for biopsy via FNA, core, or excisional will have tumor biopsies at the timepoints described in the study calendar. Specimens (approximately 50-100 mg/wet weight) will be analyzed in the lab of Keiran Smalley, PhD at the Moffitt Cancer Center, 12902 Tampa, FL 33602. Biopsy specimens and blood specimens will be evaluated via a novel mass spectrometry based assay to validate the role of increased HSP70 expression as a pharmacodynamic marker of HSP90 inhibition. Correlative studies will address whether HSP90 inhibition blocks the signaling pathways implicated in therapeutic escape including receptor tyrosine kinases, components of the mitogen activated protein kinase (MAPK) and PI3K/AKT signaling pathways.

Correlative studies will be performed to assess how changes in the expression of the key signaling proteins relate to patient response. The HSP client proteins and compensatory signaling molecules to be studied in the proteomic assay include Pathways and processes involved in the cell cycle (CDK1, CDK2, CDK4, cyclin D1, CHK1), receptor tyrosine kinases (ERBB2, IGF1R,), RAS/MAPK signaling (NRAS, HRAS, KRAS, SOS1, SHC1, BRAF, ARAF, CRAF, MEK1/2, c-Myc), PI3K/AKT signaling (AKT1, AKT2, AKT3, mTOR, CSK, FAK1, GSK3 β), B-catenin/WNT signaling (APC, Axin, β-catenin, α-catenin), NF κ B, Src and MITF. In parallel, pre and post-treatment tumor biopsy specimens will be processed for immunohistochemistry and stained for HSP client proteins implicated in therapeutic escape including BRAF, CRAF, AKT, phospho-AKT and phospho-ERK. All IHC work will be performed in Dr. Sullivan's laboratory.

Additionally, biopsy specimens will be evaluated by Reverse Phase Protein Array (RPPA) at RPPA Core Facility, Functional Proteomics, MD Anderson Cancer Center (Texas, USA). Primary targets of interest for analysis of AT13387 effect on relevant HSP90 client proteins in tumor biopsy tissue will be evaluated. Frozen biopsy specimens obtained during collection will be sent for analysis of relevant proteins, protein post-translational modifications (phosphorylation and other modifications) and effects on relevant signaling pathways.

For FNA specimens, an 18-gauge needle should be used to perform multiple passes per needle, per sample under sterile conditions. For each pass, negative pressure was applied via the plunger on a 10 ml syringe to introduce adequate amounts of tissue into the needle bore. Following sampling, ~500 uL of preservcyt was aspirated into the syringe and the contents of the syringe were expelled into a sterile 1.5 ml eppindorf tube. Samples were kept on dry ice for transport. Then the sample is centrifuged at 0.2 rcf to form a pellet, aspirated off the preservcyt using a 200 uL pipet and a 200 uL capillary/gel-loading

pipette tip. Aspirated preservcyt will be re-spun at 0.2 rcf, pelleting any remaining tissue/cells and remaining liquid was again aspirated off as waste. All samples are then placed at -20C for undergoing urea lysis the following day. For excisional specimens the same process will take place except 10-20 passes with a 18 gauge needle will be performed on the same in sterile conditions. Specimens will then be snap frozen in liquid nitrogen and stored in cryotubes before being transported to Dr. Smalley's lab at Moffitt.

It is not necessary to do FNAs if core needle biopsies or excisions can be performed instead. Furthermore, while liquid nitrogen is the preferred method of freezing specimens, using dry ice is allowable if it improves the chances of getting biopsies. Samples can also be transferred t oSullivan Lab at Massachusetts General Hospital for freezing and storing.

9.1.3 Blood BRAF test

Our group has developed a blood-based assay that can measure circulating BRAF levels in patients with BRAF mutant melanoma. (*Panka et al.,* 2010; *Panka et al.,* 2014) Our data shows that the BRAF level is reduced in patients treated with BRAF-directed therapy (both single-agent vemurafenib and the combination of DT) and, in some patients, increases in advanced of clinical or radiographic progression by 40-60 days. (*Panka et al.,* 2014) As this assay remains under clinical development, we plan to measure serial values (pre-treatment and at the beginning of each cycle) to further interrogate the utility of this assay. All samples will be initially processed in the Translational Research Laboratory at MGH and then subsequently assayed at the BIDMC.

9.2 Laboratory Correlative Studies

9.2.1 *PK Analysis*

9.2.1.1 Collection of Specimen(s)

Specimens will be collected as follows:

While the pharmacokinetic data of single-agent AT13387 and the combination of dabrafenib and trametinib are known, PK data of the triple combination will be done during this study. Clinically observed toxicities will be correlated with the possibility of delayed clearance of individual agents. Drug levels of AT13387, dabrafenib, and trametinib will be performed on day 1 of three-drug treatment (pre-, 1, 2, 4, 6, and 8 hours after dosing), day 2 (24 hours after day 1 dosing) as well as day 15 (pre-, 1, 2, 4, 6, and 8 hours after dosing), day 16 (24 hours after day 1 dosing). For the purpose of this study, post-treatment refers to post-infusions. All PK samples, inclusive of those drawn for oral agents, will be drawn at the same time relative to the end of infusion (+ 10 min).

Samples for trough level PK analysis will then be planned for each patient on day 1 of cycles 2, 4, 8, and 12.

Cycle(s)	Day of Collection	Planned Collection Time
1	1	Pre-treatment
	1	1.0 hr. post treatment
	1	2.0 hr. post treatment
	1	4.0 hr. post treatment
	1	6.0 hr. post treatment
	1	8.0 hr. post treatment
	2	24.0 hr. post treatment (C1D1)
	15	Pre-treatment
	15	1.0 hr. post treatment
	15	2.0 hr. post treatment
	15	4.0 hr. post treatment
	15	6.0 hr. post treatment
	15	8.0 hr. post treatment
	16	24.0 hr. post treatment (C1D15)
2, 4, 8, 12	1	Pre-treatment

9.2.1.2 Handling of Specimens(s)

For each sample, the date and time of the dose and the date and time of the PK sample draw will be recorded and keep as Source Documentation in each individual patients chart.

Details for AT13387 PK collection and process are provided in the "AT13387 Pharmacokinetic Laboratory Manual.". Note: This manual will be disseminated with the clinical protocol and is also available to investigators on request from the coordinating center.

AT13387 PK Samples

If AT13387 was not administered at Cycle day 8 or 15, PK samples will only be drawn for dabrafenib/trametinib for that time point.

1. At each time point, collect 4 mL whole blood PK sample into a properly labeled 4 mL Lithium Heparin blood collection tube. Record the date and time each sample is collected. **Note the sampling time and volume of blood collection may differ with specific protocols and/or regimens.**

2. Immediately after collection, gently invert (do not shake) the evacuated blood collection tube for 30 seconds, then place on a standard roller for a minimum of 5 minutes. If a blood roller is not available, continue to invert the tube a further 8 – 10 times. Blood samples should be processed into plasma within 1 hour of sample collection

3. Centrifuge the tube at 1100 - 1500g for 10-15 minutes at 4-8° C within 1 hour of

sample collection.

4. Immediately transfer plasma into two 2.0 mL Matrix TrackMate ScrewTop tubes, each containing approximately 0.75 mL of plasma.

5. Store at -20°C until shipped.

6. Ship all frozen plasma samples on dry ice

Details for dabrafenib and trametinib PK collection and process are provided in the "Pharmacokinetic Laboratory Manual". Note: This manual will be disseminated with the clinical protocol and is also available to investigators on request from the coordinating center.Dabrafenib/Trametinib PK Samples

Due to the potential instability of trametinib, pharmacokinetic blood samples collected for the determination of trametinib/dabrafenib concentration in plasma will be collected in 2 mL K2-EDTA blood tubes.

1. At each time point, collect 2 mL whole blood PK sample into a properly labeled 2mL K2EDTA evacuated blood collection tube. Record the date and time the sample is collected. (**Note the sampling time and volume of blood collection may differ with specific protocols and/or regimens.)**

2. Immediately after collection, gently invert (DO NOT SHAKE) the evacuated blood collection tube 8-10 times to mix the K2EDTA anticoagulant with the whole blood. Blood samples MUST be placed on wet ice immediately after mixing the blood with the anticoagulant. Blood samples should be processed into plasma within 15 minutes of collection (30 minutes maximum).

3. Centrifuge the sample at 2500 to 3000 rpm for 10 to 15 minutes in refrigerated centrifuge to achieve a clear plasma layer over the red cells. The speed and time may be varied according to the make and model of centrifuge used.

4. Immediately transfer plasma into a corresponding pre-labeled 1.8 mL NUNC tubes (each containing approximately 0.75 mL of plasma).

5. Store at -20 °C until shipped.

6. Ship all frozen plasma samples on dry ice, overnight.

9.2.1.3 Shipping of Specimen(s)

AT13387 PK Samples

The frozen plasma samples for AT13387 will be packed in dry ice sufficient to last during transport for 3 days and shipped to:

BASi Sample Management
2701 Kent Avenue
West Lafayette, IN 47906
USA

An inventory of the samples shipped will accompany the package. Samples should be shipped so they **arrive before 4pm, Monday to Friday only. Weekend deliveries are not permitted.**

Dabrafenib/Trametinib PK Samples

The frozen plasma samples for dabrafenib/trametinib will be packed in dry ice (sufficient to last during transport for 3 days) and shipped to:

Carolyn Dugan
Covance Laboratories Inc.
Bioanalytical Chemistry Sample Accession Room, 1S - 160
3301 Kinsman Boulevard
Madison, WI 53704
Phone: 608-242-2720
Fax: 608-242-2735
Carolyn.Dugan@covance.com
Madison.SA@covance.com
Also cc Mark Hoffmann at mark.hoffmann@covance.com

An inventory of the samples shipped will accompany the package. Samples should be shipped so they **arrive before 4pm, Monday to Friday only. Weekend deliveries are not permitted.**

9.2.1.4 Site(s) Performing Correlative Study

All study sites involved in the study will perform PK analysis.

9.2.2 Tissue analysis of HSP90 clients

9.2.2.1 Collection of Specimen(s)

Optional tumor biopsies will be performed pretreatment and one week (+/- 3 days) after commencing therapy with the combination as part of the phase I dose escalation and dose expansion cohorts. Only lesions that are deemed biopsiable through minimal risk procedures will be selected. For each biopsy, if possible, four core biopsy passes will be made. The first and third cores of tissue will be placed in formalin and then fixed in paraffin. The second and fourth cores of tissue will be flash frozen in liquid nitrogen and embedded in OCT. The first core will be used for IHC and primary outcome assessment. The second core will be used for gene and protein expression analysis. The third and fourth cores will be used to augment testing of cores 1 and 2, should the first and/or second cores be exhausted, and will be stored for future

analysis.

9.2.2.2 Handling of Specimens(s)

As described above, four cores will be obtained and fixed. The SOP for collection, handling, and processing is attached (Appendix I). 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.

9.2.2.3 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. One flash frozen sample will be sent on dry ice to Dr. Smalley's laboratory and the remainder of samples sent to Dr. Sullivan's laboratory. If only one frozen sample is available, this will be sent to Dr. Smalley's laboratory.

The addresses of the laboratories are:

Sullivan Laboratory
c/o Mark Hammond
55 Fruit Street; Jackson 9th Floor
Boston, MA 02114

Smalley Laboratory
Moffitt Cancer Center
12902 Magnolia Drive
Tampa, FL 33602

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

9.2.3. Blood-BRAF Assay

9.2.3.1 Collection of Specimen(s)

Samples will be obtained via venipuncture at the time of safety lab collection. One 10 mL green top (heparinized) tube and 1 serum separator will be required to be collected at the following time points:

- Pretreatment
- Following the one week lead-in of AT13387
- Cycle 1, day 15 (in phase I study)
- Day 1 of every cycle of therapy

- At the time of progression

9.2.3.2 Handling of Specimens(s)

Specimens will either be processed on site using the collection and processing SOP (Appendix I) or shipped to the Sullivan Laboratory overnight for processing and storage. Following processing, samples will be stored in a -80 degree freezer until batch analysis is performed.

9.2.3.3 Shipping of Specimen(s)

Unprocessed specimens should be shipped overnight on wet ice (i.e. Freezer packs). Previously processed samples may be sent in batches on dry ice via overnight shipping. The address of the laboratory is:

Sullivan Laboratory
c/o Mark Hammond
55 Fruit Street; Jackson 9th Floor
Boston, MA 02114

9.2.3.4 Site(s) Performing Correlative Study

All study sites are expected to participate in this correlative study.

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 14 days prior to start of protocol therapy. Scans and x-rays must be done ≤4 weeks (28 days) prior to the start of therapy. Patients who are not being treated with AT13387 do not need to have Day 8 and Day 15 assessments done. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy. Participants will be required to maintain a diary (Appendix D) during each cycle and to bring the diary, unused pills, and all medication bottles to the day 1 visit of each cycle.

Procedure	Pre-Study	Cycle 1			Cycles 2+			End of Txt Visit	28 Day F-U Visit [3]
	Baseline [1]	Day 1[2,b]	Day 8[4]	Day 15[4]	Day 1[2]	Day 8	Day 15		
Dabrafenib and Trametinib [A]		DT ---------- DT							
AT13387 [B]		X	X	X	X	X	X		
Informed Consent [a]	X								
Demographic	X								
Medical History	X	X	X	X	X				
Concurrent Medication	X	X	X	X	X				
Serum pregnancy test (B-Hcg) [c]	X								
Physical Examination	X	X	X	X	X			X	
Vital Signs (BP, HR, body temperature)	X	X	X	X	X	X	X	X	
ECOG Performance Status	X	X	X	X	X	X	X	X	
Dermatology Examination [d]	X				X				
Height	X								
Weight	X	X			X			X	
Ophthalmology Examination [e]	X				X				
12 Lead EKG [f]	X	X	X	X	X	X	X		
Hematology/Clinical Chemistry [g, p]	X	X	X	X	X	X[p]	X[p]	X	
Echo/MUGA [h]	X				X				
Coagulation [i]	X								
Tumor Measurements [j]	X								
Radiological Examination [j]	X							X [j]	
PK Analysis [k]		X		X					
Biopsies[l]	X		X					X	
Blood BRAF testing[m]		X		X	X			X	
AE assessment [n]	X	X	X	X	X	X	X	X	X
Long term Follow up [o]									

A. Dabrafenib and Tramatinib: doses as assigned; Dabrafenib BID and Trametinib QD.
B. AT13387: dosing as assigned per dose level.
1. All Prestudy assessments must be completed within 14 days prior to first dose, except for Scans and Echo that must be done within 28 days prior to first dose.

2. Assessment throughout the study is calendar based starting from first day of dosing (Day 1) in the first cycle of treatment. Dose interruptions should not alter the assessment schedule for any subsequent treatment cycle. Assessments and administration of study medication for each visit can be done within 3 days of the projected visit date. However, there must always be at least 6 days between AT13387 infusions.
3. Follow up visits should be 28 days from last dose of study drugs (± 3 days).

a. Informed Consent can be obtained 28 days prior to the first dose
b. If within 3 days of screening, this assessment does not need to be repeated on Day 1 of the first dose
c. Serum Pregnancy test (Woman of childbearing potential only) done within 72 hours of first dose.
d. Dermatological Examination done at screening and then every 8 weeks (2 cycles)
e. Ophthalmological examination is done at screening, week 4, and annually; additional ophthalmic exams will be performed only as symptomatically warranted
f. Triple EKG will be collected at at intervals according to the treating institution's standards prior to each AT13387 infusion
g. sodium, potassium, carbon dioxide/bicarbonate, BUN creatinine, glucose, calcium, magnesium, phosphorus, AST, ALT, total Bilirubin, Alkaline phosphatase, total protein and albumin, LDH, hematology includes CBC with differentials and platelets
h. Echo/MUGA is done at screening, week 4, week 12, week 24 and every 12 weeks thereafter.
i. Includes PT, PT-INR, PTT, repeated as indicated
j. Tumor measurements and radiological exam done every other (odd) cycle within 7 days of day 1 treatment. Documentation (radiologic) must be provided for patients removed from the study for progressive disease. Brain CT/MRI is required at baseline for patients with melanoma.
k. PK for phase 1: Cycle 1 Days 1 and 15; pre, 1, 2, 4, 6, 8, 24 hour post dose, Day 1 in Cycles 2, 4, 8, and 12.
l. In patients with biopsiable disease; pre treatment, Cycle 1 Day 8 (+/- 3 days), at disease progression (if possible)
m. Blood BRAF analysis performed C1 D1, C1 D15, at the beginning of each subsequent cycle and at disease progression.
n. AE assessment starting from the day 1 of dosing throughout the study
o. After 28 day follow up visit, patient is followed for survival only. Survival status is to be collected every 6 months (+/- 2 weeks) for up to 2 years and can be completed by phone. Patients removed from study treatment for any reason other than progressive disease will be followed until disease progression or death, whichever occurs first.
p. After cycle 6, hematology/clinical chemistry are collected on Day 8 and Day 15 at the investigator's discretion.

4. Physical Exam, Medical History, Concurrent Medications, Vital Signs, ECOG Status, Hematology and Blood Chemistry panels will be completed prior to Day 8 and Day 15 treatment. Day 8 and Day 15 assessments are not required for patients who are not receiving AT13387 infusions.

11. MEASUREMENT OF EFFECT

Although response is not the primary endpoint of the phase I portion of this trial, patients with measurable disease will be assessed by standard criteria. For the purposes of this study, patients should be re-evaluated every *8* weeks. In addition to a baseline scan, confirmatory scans will also be obtained at least 4 weeks following initial documentation of an objective response. Every attempt should be made to use a consistent imaging modality for each individual participant.

11.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every *8* weeks. In addition to a baseline scan, confirmatory scans should also be obtained not less than 4 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

11.1.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with trametinib, dabrafenib, and AT13387.

Evaluable for objective response. Only those patients 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 response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Patients 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 as ≥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. *If the investigator thinks it appropriate to include them, the conditions under which such lesions should be considered must be defined in the protocol*.

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, and abdominal masses (not followed by CT or MRI), are considered as 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 patient, 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 Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. 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 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. In the case that skin lesions are followed, documentation of pathologic CR of that lesion will be made via biopsy in the event that a previously raised lesion becomes flat.

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 slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for 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.

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 date 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 at 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 patient 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.

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.

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 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 Patients 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: Patients 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 Patients 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).

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.

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

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Method

This study will be monitored by the Clinical Trials Monitoring Service (CTMS). Information on CTMS reporting is available at http://www.theradex.com/CTMS. Data collection for this study will be done exclusively through Medidata Rave. Data will be submitted by all participating institutions to CTMS at least once every two weeks via Medidata Rave.

Users that have not previously activated their iMedidata/Rave account will receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU web site, Rave tab under Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU website under the Rave tab at http://www.ctsucontact@westat.com. For CTMS monitored stuidies, after users have activated their accounts, please contact the Theradex Help Desk at (609) 799-7580 or by email at ctms@theradex.com for additional support with Rave and completion of CRFs.

12.1.2 Responsibility for Data Submission

Study participants are responsible for submitting data every two weeks to CTMS via Medidata Rave.

12.2 CTEP Multicenter Guidelines

This protocol will adhere to the policies and requirements of the CTEP Multicenter Guidelines. The specific responsibilities of the Principal Investigator and the Coordinating Center (Study Coordinator) and the procedures for auditing are presented in Appendix B.

- The Principal Investigator/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports received from CTEP to all participating institutions for submission to their individual IRBs for action as required.

- Except in very unusual circumstances, each participating institution will order DCTD-supplied agents directly from CTEP. Agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO (PIO@ctep.nci.nih.gov) except for Group studies.

12.3 Collaborative Agreements Language

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A

suitable model agreement can be downloaded from: http://ctep.cancer.gov.

2. For a clinical protocol where there is an investigational Agent used in combination with (an)other Agent(s), each the subject of different Collaborative Agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data"):

   a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NCI, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

   b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own Agent.

   c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order as described in the IP Option to Collaborator (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm). Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy

review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

Email: ncicteppubs@mail.nih.gov

The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

This is a phase I trial of the combination of dabrafenib, trametinib, and AT13387 in patients with BRAF-mutant advanced solid tumors. The primary objective of the study is to determine the maximum tolerated dose (MTD) of the combination; secondary objectives will provide preliminary estimates of efficacy and toxicity. The escalation and stopping rules imply that the incidence rate for DLT will be less than 33% at the MTD. Following the identification of the MTD, an additional 14 patients will be enrolled in a dose expansion cohort at the MTD.

Toxicities and lab values will be graded according to the NCI Common Toxicity Criteria for Adverse Events (v4.0). Dose-limiting toxicities will be scored and will be defined as in Section 5.2.

Dose escalation will use a standard "3+3" approach, beginning in dose level 1, with dosing and rules for escalation and de-escalation described in the tables below. Tolerability assessments will be based on cycle 1 alone. The MTD is defined as the highest dose level at which 0 or 1 of six patients has experienced a DLT. If an excessive number of toxicities occur at the initial dose level 1, the dose will be reduced to the fall-back dose of -1. If dose level -1 has excessive toxicity, the study will stop.

Dose Level	Dabrafenib [BID/PO]	Trametinib [QD/PO]	AT13387 [D1,8,15/IV]
-1	**75 mg**	**1 mg**	**180 mg/m2**
1	**150 mg**	**1 mg**	**180 mg/m2**
2	**150 mg**	**2 mg**	**180 mg/m2**
3	**150 mg**	**2 mg**	**220 mg/m2**
4	**150 mg**	**2 mg**	**260 mg/m2**

Number of Patients with DLT at a Given Dose Level	Dose Escalation Rule
0 out of 3	Proceed to the next dose level and enroll 3 patients
1 out of 3	Enroll and treat 3 additional patients at this dose level
	Dose escalation will be stopped. The MTD will be one dose below this dose

≥ 2 out of 3	level. Three (3) additional patients will be entered at the next lower dose level if only 3 patients were treated previously at that dose.
1 out of 6	Proceed to the next dose level.
≥ 2 out of 6	se escalation will be stopped. The MTD will be one dose below this dose level. Three (3) additional patients will be entered at the next lower dose level if only 3 patients were treated previously at that dose.
If ≥2/3 or ≥2/6 patients at dose level 1 experience dose limiting toxicities, dose level -1 will be enrolled. If dose level -1 proves too toxic, the study will stop.	Dose escalation will be stopped. The MTD will be one dose below this dose level. Three (3) additional patients will be entered at the next lower dose level if only 3 patients were treated previously at that dose.

The following table shows the probability of escalating the dose for various true, but unknown, rates of unacceptable toxicity.

True DLT Rate	Probability of Dose Escalation
0.10	0.91
0.20	0.71
0.30	0.49
0.40	0.31
0.50	0.17
0.60	0.08

A patient will be replaced if determination of DLT cannot be adequately assessed because of rapid disease progression or if treatment is stopped during the first cycle of therapy for reasons other than toxicity.

When the MTD had been determined, a total of 14 patients will be enrolled in an expansion cohort of patients with BRAF-mutant advanced solid tumors to obtain preliminary toxicity and efficacy estimates. Patients treated at the MTD during the dose escalation phase may be included in the expansion cohort. Toxicity and efficacy rates will be summarized with 90% exact binomial confidence intervals. For a sample of size 14, the confidence intervals will be no wider than 0.48.
The table below summarizes the probabilities of observing at least one patient with a severe or unexpected toxicity in the expansion cohort for a range of incidence rates.

True Incidence of Unexpected or Severe Toxicity	Probability of Observing One or More Patients with Toxicity among 14
0.01	0.13
0.03	0.35
0.05	0.51
0.08	0.69
0.09	0.73
0.10	0.77
0.15	0.90

Secondary endpoints that will be assessed for the 14 patients treated at the MTD are: objective response rate, progression-free survival (PFS), 6-month PFS, and 1-year overall survival (OS).

Objective response rate is defined as the proportion of patients with complete or partial response as their best response to therapy. At the time of each restaging, patients will be classified as achieving complete response (CR), partial response (PR), stable disease (SD), progressive disease (PD), or non-evaluable for response according to RECIST (Version 1.1) criteria. Objective response will be determined by the best overall response designation recorded between the date of first dose of trial therapy and the date of objectively documented disease progression or cessation of trial therapy, whichever occurs first. For patients without documented progression or cessation of trial therapy, all available response designations will contribute to the objective response determination.

The distributions of progression-free survival (PFS) and overall survival (OS) will each be summarized using the product-limit method of Kaplan-Meier. Median times for each endpoint will be presented with two-sided, 90% confidence intervals estimated using log(-log(survival)) methodology. Kaplan-Meier estimates of 6-month PFS and 1-year OS will also be presented with two-sided, 90% confidence intervals.

13.2 Sample Size/Accrual Rate

The total enrollment of this trial will be between 4 and 32 evaluable patients. The minimum would occur if the triple combination proved too toxic at dose levels 1 and -1 and the trial was stopped during the dose escalation phase. A maximum enrollment of 32 patients reflects four complete dose escalation cohorts of 6 patients each and the expansion cohort of 14 total patients at the MTD. Assuming an accrual rate of 2-3 patients per month, we estimate a maximum enrollment period of 20 months.

13.3 Analysis of Secondary, Correlative, and Exploratory Endpoints

The response rate will be presented as a point estimate with a 90% exact binomial confidence interval. The one-year disease-free and overall survival after treatment will be estimated using the product-limit methods of Kaplan-Meier, and presented with 90% confidence intervals. Standard errors will be estimated using log(-log(survival)) methodology.

Correlatives and exploratory endpoints

Pharmacokinetics
Pharmacokinetic parameters, including maximal plasma or serum concentration (C_{max}), area under the curve to the last collection point (AUC_{last}), area under the curve for dose interval (AUC_{0-t}), and time of maximal concentration (T_{max}), will be determined. Descriptive statistics including mean, standard deviation, coefficient of variation, geometric mean, median, minimum and maximum will be computed for each pharmacokinetic variable; descriptive statistics for natural-log transformed pharmacokinetic variables will also be provided.

Exploratory Analyses
Factors related to prior therapy and features of therapy will be collected and explored with respect to efficacy outcomes. These include whether or not participants had prior treatment with any of the following agents – ipilimumab, lambrolizumab, MPDL-3280, or nivolumab – and the time of last dose for each. Subset analyses will be performed to assess for indications of differential outcomes. Additionally, on-study features such as dose reductions or discontinuations (of any or all study drugs) will also be taken into consideration.

Evaluation of HSP90 clients

Correlative studies will be performed to assess how changes in the expression of the key signaling proteins relate to patient response. The HSP client proteins and compensatory signaling molecules to be studied in the proteomic assay include Pathways and processes involved in the cell cycle (CDK1, CDK2, CDK4, cyclin D1, CHK1), receptor tyrosine kinases (ERBB2, IGF1R,), RAS/MAPK signaling (NRAS, HRAS, KRAS, SOS1, SHC1, BRAF, ARAF, CRAF, MEK1/2, c-Myc), PI3K/AKT signaling (AKT1, AKT2, AKT3, mTOR, CSK, FAK1, GSK3β), B-catenin/WNT signaling (APC, Axin, β-catenin, α-catenin), NFκB, Src and MITF.

Furthermore, biopsy specimens will be evaluated by Reverse Phase Protein Array (RPPA) at RPPA Core Facility, Functional Proteomics, MD Anderson Cancer Center (Texas, USA). Primary targets of interest for analysis of AT13387 effect on relevant HSP90 client proteins in tumor biopsy tissue will be evaluated. Frozen biopsy specimens obtained during collection will be sent for analysis of protein expression, protein phosphorylation and effects on relevant signaling pathways. 10-15 mg of tumor tissue should be provided for protein extraction and all tumor tissue must be supplied in Precellys tubes with Precellys beads for homogenization. Evaluation will depend upon availability of validated antibodies at MD Anderson Cancer Center. Equivalent biopsy speciments embedded in OCT will be evaluated for tumor and necrotic content.

In parallel, pre and post-treatment tumor biopsy specimens will be processed for immunohistochemistry and stained for HSP client proteins implicated in therapeutic escape including BRAF, CRAF, AKT, phospho-AKT and phospho-ERK.

Skyline version 1.3 will used for data evaluation. Peaks will be evaluated by comparison of their elution time and fragment ion signal ratios to their matched internal standards. All transitions above 10% of the base peak were used for quantification. Data will be exported to Excel for calculations of protein quantity, standard deviation, and CV (%).

Adverse Events and Toxicity Data

Adverse events (AEs): All adverse events recorded during the trial will be summarized. The incidence of treatment-emergent adverse events will be summarized according to primary system organ class, severity (based on the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) version5.0), 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, and type of adverse event. Any other information

collected (e.g. start/end dates, cumulative dose/number of cycles of therapy, duration of adverse event, severity, or relatedness to trial medication) will be listed, as appropriate.

Laboratory abnormalities: The frequency of notable lab abnormalities (i.e., newly occurring CTCAE grade-3 or -4 laboratory toxicities), will be displayed by parameter and treatment cycle. Similarly, the frequency of all laboratory abnormalities will be displayed by parameter, worst CTCAE grade experienced, and treatment cycle. A separate listing will display notable laboratory abnormalities (i.e., newly occurring CTCAE grade-3 or -4 laboratory toxicities).

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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 CTEP MULTICENTER GUIDELINES

If an institution wishes to collaborate with other participating institutions in performing a CTEP sponsored research protocol, then the following guidelines must be followed.

Responsibility of the Protocol Chair

- The Protocol Chair will be the single liaison with the CTEP Protocol and Information Office (PIO). The Protocol Chair is responsible for the coordination, development, submission, and approval of the protocol as well as its subsequent amendments. The protocol must not be rewritten or modified by anyone other than the Protocol Chair. There will be only one version of the protocol, and each participating institution will use that document. The Protocol Chair is responsible for assuring that all participating institutions are using the correct version of the protocol.
- The Protocol Chair is responsible for the overall conduct of the study at all participating institutions and for monitoring its progress. All reporting requirements to CTEP are the responsibility of the Protocol Chair.
- The Protocol Chair is responsible for the timely review of Adverse Events (AE) to assure safety of the patients.
- The Protocol Chair will be responsible for the review of and timely submission of data for study analysis.

Responsibilities of the Coordinating Center

- Each participating institution will have an appropriate assurance on file with the Office for Human Research Protection (OHRP), NIH. The Coordinating Center is responsible for assuring that each participating institution has an OHRP assurance and must maintain copies of IRB approvals from each participating site.
- Prior to the activation of the protocol at each participating institution, an OHRP form 310 (documentation of IRB approval) must be submitted to the CTEP PIO.
- The Coordinating Center is responsible for central patient registration. The Coordinating Center is responsible for assuring that IRB approval has been obtained at each participating site prior to the first patient registration from that site.
- The Coordinating Center is responsible for the preparation of all submitted data for review by the Protocol Chair.
- The Coordinating Center will maintain documentation of AE reports. There are two options for AE reporting: (1) participating institutions may report directly to CTEP with a copy to the Coordinating Center, or (2) participating institutions report to the Coordinating Center who in turn report to CTEP. The Coordinating Center will submit AE reports to the Protocol Chair for timely review.
- Audits may be accomplished in one of two ways: (1) source documents and research records for selected patients are brought from participating sites to the Coordinating Center for audit, or (2) selected patient records may be audited on-site at participating sites. If the NCI chooses to have an audit at the Coordinating Center, then the Coordinating Center is responsible for having all source documents, research records, all IRB approval documents, NCI Drug Accountability Record forms, patient registration lists, response assessments scans, x-rays, etc. available for the audit.

Inclusion of Multicenter Guidelines in the Protocol

- The protocol must include the following minimum information:
  - The title page must include the name and address of each participating institution and the name, telephone number and e-mail address of the responsible investigator at each participating institution.
  - The Coordinating Center must be designated on the title page.
  - Central registration of patients is required. The procedures for registration must be stated in the protocol.
  - Data collection forms should be of a common format. Sample forms should be submitted with the protocol. The frequency and timing of data submission forms to the Coordinating Center should be stated.
  - Describe how AEs will be reported from the participating institutions, either directly to CTEP or through the Coordinating Center.
  - Describe how Safety Reports and Action Letters from CTEP will be distributed to participating institutions.

Agent Ordering

- Except in very unusual circumstances, each participating institution will order DCTD-supplied investigational agents directly from CTEP. Investigational agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO.

APPENDIX C 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 AT13387, Dabrafenib mesylate and trametinib dimethyl sulfoxide. This clinical trial is sponsored by the National Cancer Institute. This form is addressed to the patient, but includes important information for others who care for this patient.

AT13387, 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 and 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 agents **AT13387, dabrafenib mesylate, and trametinib dimethyl sulfoxide.** This clinical trial is sponsored by the NCI. Dabrafenib mesylate 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, 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.	Dabrafenib mesylate interact with specific liver enzymes called **CYP 3A4, CYP2C9, and CYP 2C8**, and must be used very carefully with other medicines that interact with these enzymes. ➢ 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**." ➢ 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 D DRUG DIARY

Study Participant Self-Administration Drug Diary

DFCI Study Number: 14-186

Participant Name: _____

Your Doctor_____ Phone_____

Your Nurse_____ Phone_____

Dosing Instructions for Dabrafenib:

Your dose of dabrafenib is _____ mg made up of _____ - _____ mg tablets. You take two doses of dabrafenib each day.

Dosing Instructions for Trametinib:

Your dose of trametinib is _____ mg made up of _____ - _____ mg tablets. You take one dose of trametinib each day.

- You will take trametinib once per day. You will take dabrafenib twice per day.
- You should take your first dose of dabrafenib and your dose of trametinib in the morning.
- You should not eat for two hours before taking dabrafinib and trametinib.
- You should not eat for one hour after taking dabrafenib and trametinib.
- Approximately 12 hours after your morning dose of dabrafenib, you should take an evening dose of dabrafenib. You again should not eat two hours before and one hour after taking dabrafenib.
- Dabrafenib should be stored at room temperature. Trametinib should be refrigerated.
- Vomited doses should not be made up. Missed doses of trametinib should not be made up if more than 6 hours have lapsed from the scheduled/planned dose time (i.e. no more than 30 hours from the previous dose). Missed doses of dabrafenib should not be made up if it is less than 6 hours until the next scheduled dose.
- Please contact your study staff immediately if you develop a fever, which is defined as a temperature of 38.5° Celsius or 101.3° Fahrenheit
- Each of the study drugs must be kept in the original container they are distributed in
- Please remember to bring back any unused study drug, all empty containers, and your diary to the next clinic visit
- Wash your hands with soap and water after taking your dose

Diary Page **126** of **3**

DOSING LOG: Cycle ____ Date of Day 1: _____

Dosing Log Instructions

- Make sure to indicate the date, time, amount taken and any comments immediately following each dose.
- Bring your study drug containing all remaining medication, including empty bottles, to each visit.
- Once complete, provide this signed or initialed and dated dosing log to your study doctor or nurse.

Day	**Date**	**Time of Dose**	**Dabrafenib** Dose (mg)	**Time of Dose**	**Dabrafenib** Dose (mg)	**Time of Dose**	**Trametinib** Dose (mg)	**Comments** ***If dose was vomited, missed or skipped, indicate reason below.***
1		am		pm		am		
2		am		pm		am		
3		am		pm		am		
4		am		pm		am		
5		am		pm		am		
6		am		pm		am		
7		am		pm		am		
8		am		pm		am		
9		am		pm		am		
10		am		pm		am		
11		am		pm		am		
12		am		pm		am		
13		am		pm		am		

Day	**Date**	**Time of Dose**	**Dabrafenib** Dose (mg)	**Time of Dose**	**Dabrafenib** Dose (mg)	**Time of Dose**	**Trametinib** Dose (mg)	**Comments** ***If dose was vomited, missed or skipped, indicate reason below.***
14		am		pm		am		
15		am		pm		am		
16		am		pm		am		
17		am		pm		am		
18		am		pm		am		
19		am		pm		am		
20		am		pm		am		
21		am		pm		am		
22		am		pm		am		
23		am		pm		am		
24		am		pm		am		
25		am		pm		am		
26		am		pm		am		
27		am		pm		am		
28		am		pm		am		

Participant Signature or Initials **Date**

Attach to AdEERS Report

PREGNANCY INFORMATION FAX
FACSIMILE TRANSMISSION
Ticket Number:_____

Study #:
SAE FAX NO: (301) 230-0159
ALTERNATE FAX NO: (301) 897-7404

Initial Report Date: ___ - ___ - ___ (DD - MMM - YY)	Follow-up Report Date: ___ - ___ - ___ (DD - MMM - YY)
Principal Investigator:	Reporter:
Reporter Telephone #:	Reporter FAX #:

Investigator Number | Subject Number

Complete all of the investigator and subject number boxes provided. Use leading zeros, when necessary, to complete all expected boxes.

Example: Investigator #407 would be filled in as: 0 0 4 0 7

Subject Initials

Record the first letter of the subject's first, middle and last name, in that sequence. If the subject has no middle name, enter a dash.

Example: A - C

Subject's Sex: ☐ Female ☐ Male

Subject's Weight: _____ kg

Subject's Date of Birth: ___ - ___ - ___ (DD - MMM - YYYY)

Subject's Ethnicity (check one only): ☐ Hispanic or Latino ☐ Not Hispanic or Latino ☐ Not Available

Subject's Race (check all that apply): ☐ American Indian or Alaska Native ☐ Asian ☐ Black or African American ☐ Native Hawaiian or Other Pacific Islander ☐ White ☐ Not Available

Study Drug:

Study Drug Start Date: ___ - ___ - ___ (DD - MMM - YY)

Study Drug Stop Date: ___ - ___ - ___ (DD - MMM - YY) OR ☐ Study Drug Continuing

Dose: | Route: **ORAL** | Frequency: **QD** | Kit #:

First Day of Last Menstrual Period: ___ - ___ - ___ (DD - MMM - YY) Estimated Date of Delivery: ___ - ___ - ___ (DD - MMM - YY)

Method of Contraception (check all that apply):
☐ Oral Contraceptive Pills ☐ Condoms ☐ Periodic Abstinence ☐ Progestin Injection or Implants ☐ Spermacide
☐ Diaphragm ☐ Intrauterine Device (IUD) ☐ Tubal Ligation ☐ Other, specify:_____

Reproductive History: ☐ Gravida _____ ☐ Para _____

Tests performed during pregnancy: ☐ None ☐ Unknown
☐ CVS Results: ☐ Normal ☐ Abnormal
☐ Amniocentesis Results: ☐ Normal ☐ Abnormal
☐ Ultrasound Results: ☐ Normal ☐ Abnormal

Pregnancy Outcome
Was pregnancy interrupted? ☐ Yes ☐ No
If yes, specify: ☐ Elective Termination ☐ Spontaneous Abortion ☐ Ectopic
Date of Termination: ___ - ___ - ___ (DD - MMM - YY)
If pregnancy was **not** terminated, specify pregnancy outcome (and provide infant outcome information)
☐ Vaginal Birth: ☐ Premature ☐ Term OR ☐ C-Section: ☐ Scheduled ☐ Emergency Date of Delivery: ___ - ___ - ___ (DD - MMM - YY)
Infant outcome information: ☐ Normal ☐ Abnormal

Additional Case Details (if needed):

NOTE: For an initial reporting fax both the Pregnancy Report CRF and Additional Pregnancy Information Fax Page. For follow-up reporting, fax only the Additional Pregnancy Information Fax Page.

FAXP_CS 1 8/14/08 1.0

NOTE: The patient should have appropriate follow-up as deemed necessary by their physician.
If the the baby is born with a birth defect or anomaly, then a second AdEERS report is required.

APPENDIX F CONCOMITANT MEDICATIONS AND NON-DRUG THERAPIES

1.1. Permitted Medications and Non-Drug Therapies

The investigator must be informed as soon as possible about any medication taken from the time of screening until 30 days after the last dose of study treatment. Any concomitant medication(s), including dietary supplements, taken during the study will be recorded in the eCRF. The minimum requirement is that drug name, dose, and the dates of administration are to be recorded. Additionally, a complete list of all prior surgical procedures will be recorded in the eCRF. Subjects should receive full supportive care during the study, including transfusions of blood and blood products, and treatment with antibiotics, anti-emetics, anti-diarrheals, and analgesics, and other care as deemed appropriate, and in accordance with their institutional guidelines. Use of anticoagulants such as warfarin is permitted provided that INR is monitored in accordance with local institutional practice.

1.2. Prohibited Medications and Non-Drug Therapies

The use of certain medications and illicit drugs within 28 days or 5 half lives, whichever is shorter, prior to randomization and for the duration of the study will not be allowed.
The following medications or non-drug therapies are also prohibited while on treatment in this study:

- Other anti-cancer therapies;
- Other investigational drugs;
- Antiretroviral drugs (Note: Subjects with known HIV are ineligible for study participation);
- Herbal remedies (e.g., St. John's wort);
- Dabrafenib is metabolized primarily by Cytochrome P450 (CYP) 2C8 and CYP3A4. Co-administration of dabrafenib with ketoconazole, a CYP3A4 inhibition, or with gemfibrozil, a CYP2C8 inhibitor, resulted in increases in dabrafenib AUC of 71% and 47%, respectively. Drugs that are strong inhibitors or inducers of CYP3A and CYP2C8 (see list in Table 1) may only be used under special circumstances (e.g. as a single use for a procedure) while treatment with study drug is interrupted as they may alter dabrafenib concentrations; consider therapeutic substitutions for these medications. Approval of the CTEP medical monitor is required in these situations. The list may be modified based on emerging data. Refer to the SPM for the most current list.

Table 1 Prohibited Medications

PROHIBITED – strong inducers of CYP3A or CYP2C8, since concentrations of dabrafenib may be decreased	
Class/Therapeutic Area	**Drugs/Agents**
Antibiotics	Rifamycin class agents (e.g., rifampin, rifabutin, rifapentine),
Anticonvulsant	Carbamazepine, oxcarbazepine phenobarbital, phenytoin, s-mephenytoin
Miscellaneous	bosentan, St. John's wort
PROHIBITED – Strong inhibitors of CYP3A, or CYP2C8 since concentrations of dabrafenib may be increased	
Class/Therapeutic Area	**Drugs/Agents**
Antibiotics	Clarithromycin, telithromycin, troleandomycin
Antidepressant	Nefazodone
Antifungals	Itraconazole, ketoconazole, posaconazole, voriconazole
Hyperlipidemia	Gemfibrozil
Antiretroviral	ritonavir, saquinavir, atazanavir
Miscellaneous	Conivaptan

1.3. Medications to be Used with Caution

The following medications should be used with caution as their concentrations may be altered by dabrafenib or they may alter dabrafenib concentrations:

- Drugs that are moderate inhibitors or inducers of CYP3A and CYP2C8 as they may alter concentrations of dabrafenib.
- Dabrafenib has been shown to induce CYP3A4 and CYP2C9 in vivo using midazolam (CYP3A4 substrate) and S-warfarin (CYP2C9 substrate). Dabrafenib is an in vitro inducer of CYP2B6 and other enzymes such as CYP2C8, CYP2C19, UDP-glucuronyl transferases. Transporters may also be affected. Co-administration of dabrafenib and medications which are affected by the induction of these enzymes (including warfarin) and transporters may result in loss of efficacy. If co-administration of these medications is necessary, investigators should monitor subjects for loss of efficacy or consider substitutions of these medications. A partial list of these medications is provided in Table 2 and in the SPM.
- Therapeutic level dosing of warfarin can be used with approval by the CTEP Medical Monitor and close monitoring of PT/INR by the site. Exposure decreased by 37% due to enzyme induction when on treatment, thus warfarin dosing may need to be adjusted based upon PT/INR. Consequently, when discontinuing dabrafenib, warfarin exposure may be increased and thus close monitoring via PT/INR and warfarin dose adjustments must be made as clinically appropriate. Prophylactic low dose warfarin may be given to maintain central catheter patency.

Dabrafenib solubility is pH-dependent with decreased solubility at higher pH. Drugs such as proton pump inhibitors that inhibit gastric acid secretion to elevate gastric pH may decrease the solubility of dabrafenib and reduce its bioavailability. No clinical study has been conducted to evaluate the

effect of pH on dabrafenib pharmacokinetics. In an ad-hoc analysis, no differences in C_{max} and AUC were noted between subjects who reported taking pH-elevating products relative to other subjects. Due to the theoretical risk that pH-elevating agents may decrease oral bioavailability and exposure to dabrafenib, these medicinal products that increase gastric pH should be used with caution when administered with dabrafenibTable 2 Medications to be used with Caution

USE WITH CAUTION: Moderate inhibitors of CYP3A, or CYP2C8 since concentrations of dabrafenib may be increased	
Class/Therapeutic Area	**Moderate CYP3A and CYP2C8 Inhibitors**
Antiarrhythmics	Diltiazem, verapamil
Antibiotic	Erythromycin
Antifungal	Fluconazole
Miscellaneous	Aprepitant
USE WITH CAUTION: Co-administration of these drugs with study treatment may result in loss of efficacy. Monitor subjects for loss of efficacy or substitute with another medication.	
Class/Therapeutic Area	**CYP3A4, CYP2B6, CYP2C8, CYP2C9, or CYP2C19 Substrates that May be Affected by Induction**
Analgesics	Alfentanil, buprenorphine, celecoxib, codeine, fentanyl, methadone, oxycodone
Antiarrhythmics	Disopyramide, dronedarone, mexiletine, propafenone, quinidine
Antibiotics	Chloramphenicol, doxycycline, erythromycin, moxifloxacin
Anticoagulants/ Antiplatelets	Cilostazole, warfarin
Anticonvulsants	Divalproex, lamotrigine, valproate, zonisamide
Antidepressants and Antipsychotics	Aripiprazole, bupropion, buspirone, desipramine, haloperidol, mirtazapine, pimozide, quetiapine, trazodone, amitriptyline, clomipramine, imipramine
Antidiabetics	Glyburide, saxagliptin, tolbutamide, nateglinide, pioglitazone, repaglinide, rosiglitazone
Antifungals	Caspofungin, fluconazole, terbinafine
Antihistamines	Astemizole, chlorpheniramine, ebastine
Antihypertensives	Amlodipine, diltiazem, felodipine, nifedipine, nilvadipine, nisoldipine, verapamil
Antimigraine Agents	Diergotamine, eletriptan, ergotamine
Corticosteroids	Dexamethasone, methylprednisolone, oral budesonide
Erectile Dysfunction Agents	Sildenafil, tadalafil, vardenafil
HMG-CoA Reductase Inhibitors	Atorvastatin, lovastatin, simvastatin
Hypnotics and Sedatives	Alprazolam, brotizolam, diazepam, estazolam, midazolam, triazolam, zolpidem, zopiclone
Immunosuppressants	Everolimus, sirolimus, tacrolimus
Miscellaneous	Aprepitant, cisapride, darifenacin, disopyramide, leflunomide, methohexital, oral contraceptives, quinine, ranitidine, solifenacin, sulfasalazine, tramadol, tolvaptan, chloroquine, zopiclone
Selective Aldosterone Blockers	Eplerenone

USE WITH CAUTION: Co-administration of drugs that increase gastric pH should be used with caution when administered with dabrafenib..	
pH altering agents	dexlansoprazole. esomeprazole, famotidine, ilaprazole, lansoprazole, omeprazole, pantoprazole, rabeprazole, ranitidine

Abbreviations: CYP = cytochrome P450; HMG-CoA = 3-hydroxy-3-methylglutaryl-coenzyme A.

Questions regarding concomitant medications should be directed to the CTEP Medical Monitor for clarification.

APPENDIX G BIOASSAY TEMPLATES 1

ISOLATION OF PERIPHERAL BLOOD LYMPHOCYTES

Materials.

1. Histopaque 1077 (Sigma)-for creating Ficoll gradients for cell separation.

2. 10X Phosphate buffered saline (PBS)

3. Fetal bovine serum (FBS)-for making freezing media.

4. Dimethyl sulfoxide (DMSO)-for making freezing media.

5.Freezing media: 5% DMSO, 95% fetal bovine serum.

6. Polypropylene centrifuge tubes (50cc).

7. Nunc freezing vials (1.5 ml).

Method for the isolation of peripheral blood lymphocytes

Peripheral blood lymphocytes are isolated by Ficoll density centrifugation (47) in a laminar flow hood (BL2). If a BL2 cabinet is not available can be done on the lab bench but try to remove Histopaque and freezing media under sterile conditions to avoid bacterial contamination.

1. Wearing gloves pour contents of green top heparinized blood collection tube into a 50 cc centrifuge tube.
2. Add 10 ml of PBS to green top tubes and pool with the blood in the 50 cc centrifuge tube. If there are two green tops pool the blood and PBS into a single 50 cc centrifuge tube.
3. Add 10 ml Histopaque to a new 50 cc centrifuge tube.
4. Carefully layer the blood on top of the Histopaque (Hold the tube with the Histopaque as horizontal as possible as you slowly layer the blood, trying not to mix the layers. Alternately you can try to underlay the Histopaque below the blood but this can be messy).
5. Centrifuge at 700 x g at room temperature for 12 min.
6. At the interface of the layers will be the PBLs (should be a white layer of cells). At the bottom of the tube will be the red cells. Remove some of the plasma above the interface with a 10 ml pipette. Remove the cells at the interface with the same pipette, trying not to remove anything below the interface. Transfer to a new centrifuge tube.
7. Add enough PBS to fill the 50 cc tube.
8. Centrifuge at 400 x g at room temperature for 5 min.
9. Aspirate the liquid above the cellular pellet.
10. Add 2 ml freezing media to the pellet. Gently pipette up and down and aliquot into two Nunc freezing vials.
11. Store at -80 short term or in liquid nitrogen long term.

APPENDIX H BIOASSAY TEMPLATES 2

BLOOD BRAF ASSAY PROTOCOL

RNA from ficoll purified PBMCs is isolated by the trizol method (Invitrogen) (46) and (1 μg) reverse transcribed to cDNA by standard methods using M-MLV reverse transcriptase (Invitrogen) and oligo $(dt)_{15}$ (Promega). The cDNA is subjected to real time PCR for 18S RNA in order to normalize the quantity, as well as quality of the input RNA prior to the next step (ABI for oligo/probe set).

The equilibrated cDNA is PCR amplified using PCR master mix (Promega) and oligonucleotides [5′(CCATATCATTGAGACCAAATTTGAGATG)3′ and 5′(GGCACTCTGCCATTAATCTCTTCATGG)3′] that produces a product of 466 bp including the mutation site at position 600. The PCR conditions are 94° for 2 min followed by 40 cycles of 94° for 1 min, 60° for 2 min and 72° for 2 min with a final incubation of 72° for 7 min.

After cleanup using a nucleospin extract column (Clontech), a portion of the PCR product is digested with TSPR1 (restriction site=NNCASTGNN, New England Biolabs, Beverly, Massachusetts, USA) at 65° for 16h. Only wild-type Braf and not V600E mutant Braf PCR product is digested by this enzyme. This digestion is added to reduce the amount of contaminating normal Braf from surrounding and infiltrating normal tissue in the blood samples. The TspR1 digestion is not complete resulting in some PCR product containing wild-type sequence at position 600.

A 1/100 dilution of the TSPR1 digested material is then PCR amplified a second time using nested oligonucleotides 5′(ACGCCAAGTCAATCATCCACAGAG)3′ and 5′(CCGTACCTTACTGAGATCTGGAGACAGG)3′ producing a product of 331 bp, which is enriched in PCR products containing the position 600 mutation. The conditions of the PCR are the same as the first PCR except instead of 40 cycles, the amplification is 45 cycles for PBLs.

After a second cleanup using a nucleo-spin extract column, the DNA (1/1000 dilution) is subjected to a BRAF V600E real time PCR as described (german group).

Purified Braf V600E first round PCR product with a known concentration is also run through the assay and is used to create a standard curve. Using the standard curve the amount of end product is determined.

APPENDIX I BIOASSAY TEMPLATES 3

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 Skin: Flush with water for at least 15 minutes 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:* *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: *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 Considerations	Use only containers specifically designed for holding 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 1cm^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).

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APPENDIX J BIOASSAY TEMPLATES 4

RPPA ANALYSIS

Preparation of tumor lysates for reverse phase protein array (RPPA) analysis

1. Reagents and materials:
    a. Frozen tumor tissue set on dry ice, scalpel, weighing dish, tweezers, lysis buffer with protease inhibitors set on ice, 5ml tubes (round bottom) labeled with sample number and set on ice.

    b. Lysis Buffer: 1% Triton X-100, 50mM HEPES, pH 7.4, 150mM NaCl, 1.5mM $MgCl_2$, 1mM EGTA, 100mM NaF, 10mM Na pyrophosphate, 1mM Na_3VO_4, 10% glycerol, containing freshly added protease and phosphatase inhibitors from Roche Applied Science Cat. # 04693116001 and 04906845001, respectively.

    c. 4 × SDS Sample Buffer: 40% Glycerol, 8% SDS, 0.25M Tris-HCL, pH 6.8. Before use, add 2-mercaptoehtanol at 1/10 of volume.

2. Preparation of tumor:
    a. OCT-embedded tumor tissue
        i. Review H&E slide of OCT-embedded tumor with pathologist to select area for protein extraction.
        ii. Using the H&E slide as a guide, macrodissect the desired region, keeping the OCT base intact. Submit to histology for preparation of tumor shears, which should be cut using a DNAse/RNAse-free blade at -30°C. The number and thickness of shears must be determined by review of the available surface area of the tumor (i.e. 4 X 20 um shears). Shears should be transferred into a sterile tube on dry ice, and stored at -80°C until use.
        iii. Add ice-cold lysis buffer to the tube. The volume of lysis buffer is 200 ul per 5 mg of tumor tissue
    b. Snap-frozen tumor tissue
        i. Remove the tumor tissue from cryovials and set in weighing dish at room temperature for a short while (Do not wait for complete thaw). Cut a small piece of the tumor and weigh by analytical balance. Try to put the remaining tumor tissue back on dry ice as soon as possible.
        ii. Put the small piece of tumor tissue into a 5ml tube on ice. Add ice-cold lysis buffer to the tube. The volume of lysis buffer is 200 ul per 5 mg of tumor tissue

3. Generation of protein lysate
    a. Homogenize the tumor tissue by electric homogenizer for 8 seconds. The tumor tissue should be set on ice while homogenizing to prevent heating. Wash the homogenizer probe twice with ice-cold water in between samples and dry the probe with Kimwipe.

b. Optional: Set the samples on ice for 10 minutes.

c. Transfer the samples to microcentrifuge tubes and centrifuge at 4 °C, 14,000rpm for 10 minutes.

d. Collect supernatant (tumor lysates) and transfer to another set of microcentrifuge tubes.

e. Determine protein concentration by BCA or Bradford reaction and adjust protein concentration to 1.3 mg/ml by lysis buffer.

f. Mix the cell lysate with 4 × SDS sample buffer without bromophenol blue (3 parts of cell lysate plus one part of 4 × SDS sample buffer). Boil the samples for 5 minutes. The samples are ready for RPPA processing. If the samples need to be stored for later use, store them in –80 °C.

4. Submit samples for RPPA analysis at the MD Anderson Functional Proteomics Core Facility. Provide at least 35 □l of the denatured protein lysate for each sample. Each tube should be clearly labeled. Also include a Microsoft Excel file list of the sample names, sample order, protein concentration and sample volume. Results for each sample will be reported as load-corrected, $\log_2$ relative protein concentrations for each protein.