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DF/HCC Protocol #: 17-380

TITLE: Phase I study of INCB039110 in combination with dabrafenib and trametinib in patients with BRAF-mutant melanoma and other solid tumors.

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IND #: 136558

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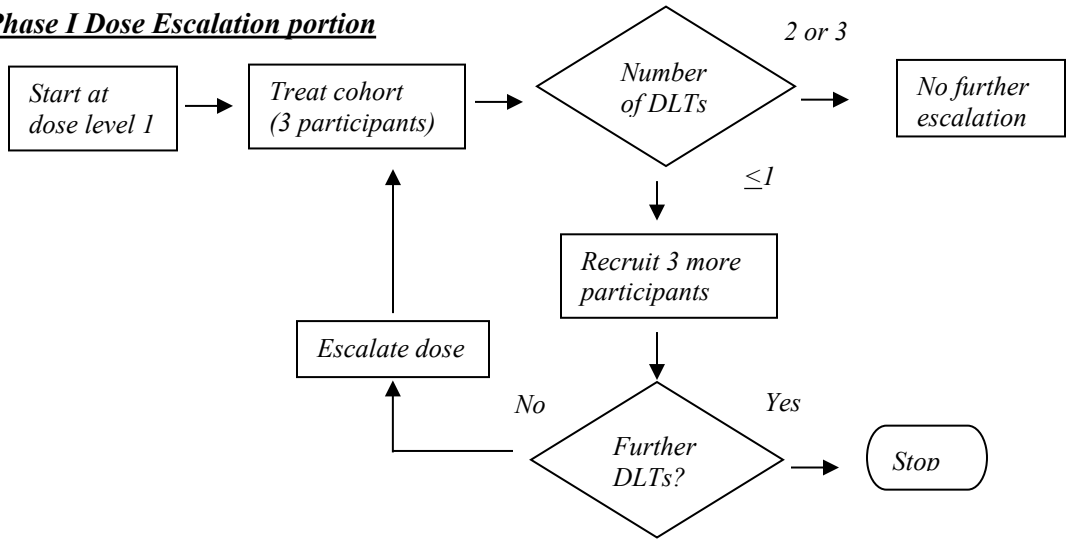
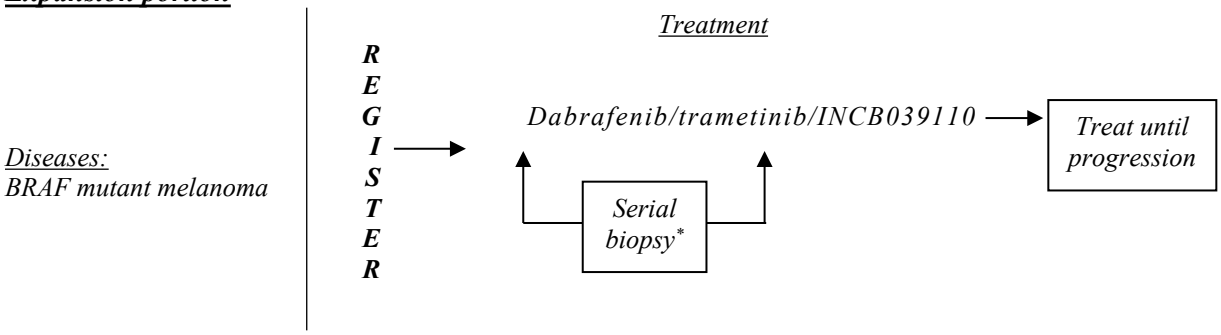
SCHEMA

Phase I Dose Escalation portion



Expansion portion



*See section 10 for additional information on serial biopsies

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1. OBJECTIVES

1.1 Phase I Objectives

Primary Objective

To determine the maximum tolerated dose (MTD), toxicity, and safety profile of INCB039110 given daily in combination with dabrafenib and trametinib in patients with BRAF-mutant unresectable or metastatic melanoma and other solid tumors.

Secondary Objectives

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 INCB039110 given daily in combination with dabrafenib and trametinib.

To describe the pharmacokinetics of treatment with dabrafenib, trametinib, and INCB039110.

1.2 Study Design

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 INCB039110. The table below summarizes the planned dose levels. 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*).

Dose Level	Dabrafenib [BID/PO]	Trametinib [QD/PO]	INCB039110 (mg QD)
-2	75 mg	1 mg	100 mg
-1	150 mg	2 mg	100 mg
1	150 mg	2 mg	200 mg
2	150 mg	2 mg	300 mg
3	150 mg	2 mg	400 mg

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 6 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. Please see section 6.1 for dose modifications of dabrafenib and trametinib. If the toxicity is determined to be related to INCB039110 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.

2. BACKGROUND

2.1 Study Disease

The incidence of melanoma has dramatically risen over the past several decades and in 2016, an estimated 76,380 new cases and 10,130 deaths are expected in the United States. (*American Cancer Society, 2016*) 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 IND Agent – INCB039110

2.2.1 Mechanisms of Action and Preclinical Data with INCB039110

INCB039110 is a potent inhibitor of JAK1 (IC50 value = 3.6 ± 2.3 nM), with 22-fold to > 500-fold selectivity over the other JAK family members (Table 1). INCB039110 does not significantly inhibit (< 30% inhibition) a broad panel of 60 other kinases, suggesting that it is a selective inhibitor of JAK1.

Table 1: Enzyme Inhibitory Activity of INCB039110 Against Different Members of the Human JAK Family

Enzyme	IC_{50} Mean ± SD (NM)[a]	Number of Experiments	Fold Selectivity for JAK1
JAK1	3.6 ± 2.3	56	-
JAK2	79.9 ± 39.5	58	22
JAK3	> 2000	6	> 500
TYK2	953 ± 330	6	265

[a] Assays were performed at 1 mM ATP concentration.

Since INCB039110 has demonstrated selectivity for the JAK1 enzyme compared with JAK2 in biochemical assays, its selectivity was examined in cell-based assay systems that measured signaling in response to stimulation with TPO or prolactin, since both have been shown to signal through a JAK2 homodimer (Murray 2007). In both systems, INCB039110 showed weak inhibition with IC50 values of approximately 1 μM or greater (Table 2), suggesting that INCB039110 is JAK2-sparing in cells.

Despite the reduced potency of INCB039110 against JAK2, INCB039110 was potent (IC50 values of approximately 10 nM to 350 nM) in cell-based assays relevant to the pathogenesis of RA, such as IL-2–stimulated phosphorylation of JAKs and STATs and IL-2–induced proliferation of T cells. This effect was not because of general cytotoxicity, as INCB039110 showed no effect on the survival of naïve T cells cultured in the absence of cytokine stimulation and did not affect the proliferation of non–JAK-dependent cell lines (< 20% inhibition at 5 μM). INCB039110 also potently inhibited the phosphorylation of STAT proteins and the production of proinflammatory factors (eg, IL-17, MCP-1) induced by other cytokines, such as IL-23 and IL-6, with IC50 values in the range of approximately 30 nM to 100 nM. These data are summarized in Table 2.

Table 2: ***In Vitro*** **Potency of INCB039110 in Cytokine and Growth Factor–Stimulated Cells**

Cytokine/ Growth Factor	Cell Type	Measured Parameter	IC_{50} for INCB039110 (Mean ± SEM)[a]	Number of Tests
IL-2	T cell	STAT3/STAT5 phosphorylation	~10-100 nM	2
IL-2	T cell	Proliferation	21 ± 11 nM	8
IL-23	T cell	STAT3 phosphorylation	59 nM	2
IL-23	T cell	IL-17 production	76 ± 40 nM	5
IL-23	T cell	IL-22 production	108 ± 41 nM	5
IL-6	PBMC	STAT3 phosphorylation	58 nM	2
IL-6	PBMC	MCP-1 production	34 ± 15 nM	4
IL-6	INA-6	Proliferation	352 ± 112 nM	18
TPO	PBMC	JAK2 phosphorylation	> 1000 nM	2
TPO	PBMC	STAT3 phosphorylation	913 ± 87 nM	4
Prolactin	CW22Rv1	STAT5 phosphorylation	~1000 nM	2

[a] Only mean value is shown for assays performed 2 times.

In addition, the human INA-6 multiple myeloma cell line was used to establish a cell-based assay system to investigate the effects of JAK1 inhibition on cell proliferation because this cancer cell line requires exogenous IL-6 for *in vitro* growth and survival (Burger et al 2001). IL-6 has been demonstrated to signal through both JAK1 and JAK2, leading to activation of STAT3. It has been previously demonstrated that activation of JAK/STAT3 in these cells is dependent on the presence of IL-6 and that inactivation of JAK/STAT3 by either withdrawal of IL-6 or inhibition of IL-6 binding to the IL-6 receptor induces cell death via apoptosis (Burger et al 2001). In this assay system, INCB039110 inhibited INA-6 cell growth with an IC50 of 352 ± 112 nM (Table 2).
In order to estimate INCB039110 potency in blocking JAK activity in hematopoietic cells *in vivo*, where serum protein binding can be a significant factor, a whole blood assay was established that measures pSTAT3 in response to cytokine stimulation. In this assay system, pSTAT3 levels in response to stimulation with IL-6 or TPO were examined, since JAK1 was shown to have a dominant role in IL-6 signaling (Guschin et al 1995) and TPO has been shown to signal through a JAK2 homodimer (Murray 2007). INCB039110 blocked IL-6– induced STAT3 phosphorylation in human whole blood, with an IC50 value of 292 ± 16 nM (n = 27), whereas the potency of INCB039110 in blocking TPO-induced pSTAT3 was significantly reduced (2322 ± 178 nM; n = 23), consistent with the JAK1 selectivity measured in the biochemical assays. No effects on total STAT3 levels were observed at any concentration tested. Similar results were obtained using whole blood from dogs and rats, confirming the species cross-reactivity and selectivity of the compound (Table 3).

Table 3: Potency Comparison of INCB039110 in Blocking IL-6 Versus TPO-Induced STAT3 Phosphorylation in Whole Blood From Different Species

Species	IL-6 pSTAT3 (IC_{50} Mean ± SEM)	Number of Tests	TPO pSTAT3 (IC_{50} Mean ± SEM)	Number of Tests	Fold Selectivity
Human	292 ± 16 nM	27	2322 ± 178 nM	23	8
Rat	274 ± 75 nM	5	8026 ± 1782 nM	3	29
Dog	114	2	1490	2	13

2.2.2 Nonclinical safety of INCB039110

INCB039110 was evaluated *in vivo* in rat adjuvant-induced arthritis (rAIA), as this model is an aggressive polyarthritis that shares characteristics with human RA (Waksman 2002). Moreover, multiple cytokines are upregulated in this model, including IL-1, IL-6, and TNF-α, and JAK1 phosphorylation has been described in the synovial lining cells and macrophages in affected rats (Shahrara et al 2003). In these experiments, administration of INCB039110 was initiated in the therapeutic mode after a robust inflammatory response was observed.

In aggregate, INCB039110 demonstrated dose-dependent effects on multiple endpoints in the rAIA model, including PD suppression of STAT3 phosphorylation and clinical and histological signs of disease. Oral doses of 1 mg/kg BID or 3 mg/kg QD are predicted to provide an AUC of approximately 0.67 to 1.0 μM·h (based on Sprague Dawley rat PK data). For comparison, the NOAEL from the 28-day rat toxicology study was 225 mg/kg, with an associated AUC of 73.6 μM·h. These data are consistent with a therapeutic margin of > 100-fold for BID administration and > 70-fold for QD administration. Significant histological improvements were observed at a dose with a predicted AUC of 2.0 μM·h, resulting in a margin of > 35-fold. The data presented above demonstrate the ability of INCB039110 to reduce joint inflammation and bone resorption in this relevant preclinical model of RA.

In the INA-6 cytokine-dependent (ie, WT JAK-dependent) model of myeloma, INCB039110 reduced tumor growth by a maximum of 6%, 45%, and 57% when continuously infused, achieving plasma concentrations of 220 nM, 470 nM, and 783 nM, respectively. Twice-daily oral administration of INCB039110 also reduced splenomegaly in a model of JAK2 V617F-driven hematological malignancy – BaF/JAK2 V617F. A BID dose of 90 mg/kg reduced splenomegaly by 67% compared with vehicle-treated mice. Plasma samples collected 1-hour postdose (near tmax) exhibited INCB039110 levels well below the concentration required to significantly inhibit JAK2 signaling in rodent whole blood samples (Table 3). In summary, pharmacological data obtained in both *in vitro* and *in vivo* model systems support the potential utility of orally administered INCB039110 in the treatment of MF and RA.

Safety Pharmacology

INCB039110 was studied in a variety of *in vitro* binding assays against panels of receptors, channels, transporters, and other kinases to evaluate specificity for the JAK family of kinases. To assess central nervous system (CNS) effects, the gross behavioral and physiological state of rats administered oral doses of INCB039110 was evaluated using parameters described in Irwin's functional observational assessment. For assessment of respiratory effects, INCB039110 was administered orally to rats followed by the measurement of respiratory rate and tidal volume. INCB039110 was also administered orally to conscious, telemetered beagle dogs to study the effects of the drug on blood pressure, heart rate, and ECG measures.

INCB039110 did not demonstrate inhibition of binding for any of 55 different receptors, channels, and transporters evaluated at 0.1 μM and 1 μM; or against approximately 60 non-JAK family kinases evaluated at 0.1 μM. Adverse findings in safety pharmacology nonclinical assessments included transient decreased motor activity in male and female rats administered a single oral dose of 1000 mg/kg, decreased respiratory function in rats administered a single oral dose of 1000 mg/kg, decreased blood pressure with a compensatory higher heart rate, and an increase in core body temperature after a single oral dose of ≥ 60 mg/kg in the dog cardiovascular evaluation. Administration of ≥ 60 mg/kg INCB039110 resulted in significantly lower arterial blood pressure (up to 5 hours postdose), compensatory higher heart rate (up to 8 hours postdose), and increased core body temperature (up to 12 hours postdose). The magnitude of change in pulse pressure was small despite INCB039110-related changes in systolic and diastolic blood pressure, suggesting no effect of INCB039110 on myocardial contractility. Although

changes in arterial blood pressure were noted at 30 mg/kg, the small magnitude and short duration of effect led to the determination of the NOAEL to be 30 mg/kg. There were no effects on ventricular repolarization at any dose in this study. The IC50 for inhibition of the hERG channel was determined to be 65.3 μM.

The toxicologic and toxicokinetic profiles of orally administered INCB039110 were characterized in single- and multiple-dose studies of up to 6 months in duration in rats and 9 months in dogs. Embryo-fetal developmental toxicity studies were conducted in rats and rabbits, and the potential genetic toxicity of INCB039110 was also assessed.

JAKs play an important role in the signaling of a number of cytokines and hematopoietic growth factors. Multiple cytokines, including IL-2 and IL-6, signal via JAK enzymes and play an important role in lymphocyte differentiation, survival, and function, and JAK2 plays a central role in erythropoiesis and thrombopoiesis in humans. INCB039110 is a potent inhibitor of JAK1 with reduced potency for JAK2. Therefore, bone marrow and the lymphoid system are the predicted target organs for INCB039110 pharmacological effects.

Two single-dose studies of orally administered INCB039110 were conducted in rats; the NOAEL was defined at the highest dose used in each study, 1200 mg/kg and 2000 mg/kg. In a single-dose study in dogs, dose levels were ≤ 1000 mg/kg. Nonadverse clinical signs, including injected sclera, prominent nictitating membrane, and emesis were noted at 1000 mg/kg.

Multiple-dose toxicity and toxicokinetic studies of INCB039110 were conducted in rats for up to 6 months and dogs for up to 9 months. A 4- or 6-week recovery group was included in the GLP studies to determine the reversibility of INCB039110-related findings.

Immunophenotyping data revealed that the cell subtypes most affected by INCB039110 administration were cytotoxic (CD8+) T cells and natural killer (NK) cells. Consistent with the lower peripheral blood lymphocyte counts, a lower proportion of bone marrow lymphoid cells was also observed. In serum chemistry evaluations, slight elevations in the liver enzymes alkaline phosphatase (ALP), aspartate aminotransferase (AST), and gamma-glutamyl transpeptidase (GGT) were noted and were more prevalent in females. In the 6-month study, where dose levels of 0 mg/kg, 30 mg/kg, 100 mg/kg, 300 mg/kg, and 1000 mg/kg daily were evaluated, increased ALP was noted for females administered ≥ 100 mg/kg daily, and increased GGT was noted in females administered 1000 mg/kg daily. There were no liver enzyme alterations in male rats in the 3- or 6-month studies. Elevations in AST were observed only in the 28-day study. These liver enzyme changes lacked a histological correlate and were deemed nonadverse. All clinical pathology and immunophenotyping changes demonstrated reversibility. Histologically, INCB039110-related alterations were similar across rat multiple-dose studies and were pharmacological in nature. Reversible lymphoid depletion of the thymus, spleen, and lymph nodes, and a reduction in bone marrow cellularity were noted. Reversible lower spleen and thymus weights correlated with the lymphoid depletion in these tissues. A reduction in adrenal gland weight was also noted in rat multiple-dose studies; however, this finding lacked a microscopic correlate and thus was considered nonadverse. Based on body weight decreases in males and the magnitude of reduction in circulating lymphocyte counts, the NOAEL for male and female rats administered INCB039110 for 6 months was determined to be 300 mg/kg daily, corresponding to AUC0-24 values of 28.8 and 86.0 μM·h for males and females, respectively, on Day 181.

In a 28-day GLP study of orally administered INCB039110 in rats, increased incidences of luteal ovarian cysts and epididymal sperm granulomas were observed. These findings were not observed in either the 3-month or 6–month study at similar plasma exposures to those in the 28-day study and, therefore, are determined to be unrelated to INCB039110 administration.

Four multiple-dose studies of orally administered INCB039110 were conducted in the dog: a 10-day non-GLP study, and 28-day, 3-month, 6-month, and 9-month GLP studies. In the 10-day, 28-day, and 3-month studies, severe clinical signs related to GI toxicity occurred in the higher dose groups, resulting in unscheduled terminations. In the 6-month GLP study in dogs, dose levels evaluated were 0 mg/kg, 10 mg/kg, 20 mg/kg, 30 mg/kg, and 60 mg/kg daily. Despite a lowering of the high-dose level to 50 mg/kg daily on Day 45, adverse clinical signs consistent with the immunosuppressive effects of INCB039110 persisted, and dose administration was subsequently terminated for this dose group during Week 17. In the 9-month study, dose levels were 10, 20, 30, and 40 mg/kg per day. A dose-related lowering of CD8+ T cells was noted in the immunophenotyping evaluations after at least 17 weeks of dose administration. INCB039110-related microscopic findings that were related to the pharmacology included decreased lymphocytes in multiply lymphoid organs (white pulp of the spleen, various lymph nodes, gut-associated lymphoid tissue) and extramedullary hematopoiesis in the spleen, at doses ≥ 30 mg/kg per day, and bone marrow hypocellularity in the sternum, at doses ≥ 20 mg/kg per day. Additional microscopic findings were observed in a few animals that were considered secondary to effects on the immune/inflammatory response. Based on the adverse findings associated with immunosuppression and development of clinical demodicosis, the NOAEL in the 9-month study was determined to be 10 mg/kg daily. At the end of the administration phase (Day 269), the NOAEL corresponded to AUC0-24 values of 3.04 μM·h and 5.00 μM·h, respectively, for males and females.

Using the exposures at the NOAELs from the 6-month toxicology studies in the rat and dog, the estimated safety margins associated with the therapeutic dose levels of 600 mg QD (estimated unbound AUC0-24 of 3.99 μM·h) and 200 mg BID (estimated unbound AUC0-24 of 2.83 μM·h) are calculated.

2.2.3 Clinical Pharmacokinetics (PK) and Activity of INCB039110

PK and metabolism of INCB039110

Study INCB 39110-101 was a single, ascending-dose escalation study of INCB039110 IR administered orally to healthy adult subjects. Pharmacokinetic data for single doses of 10 mg to 1000 mg are provided in Table 4. Curves of plasma concentration versus time are presented in Figure 1. INCB039110 was quickly absorbed when administered as IR capsules, attaining plasma Cmax within 1.5 hours after administration, and the compound was subsequently eliminated with a mean terminal-phase t½ ranging from 1.7 to 2.8 hours. Exposures were greater than dose-proportional over most of the dose range, but were dose-proportional between 500 mg and 1000 mg. For the 1000 mg dose, mean Cmax and AUC0-∞ were 7.4 μM and 20.7 μM·h, respectively. There was prolonged absorption seen after 16 hours with an apparent t½ of 9.8 hours for the higher doses.

Study INCB 39110-103 was a multiple-dose, double-blind, randomized, ascending-dose escalation study of INCB039110 administered orally over a 10-day period to healthy adult subjects. The mean t½ values ranged from 6.6 to 13 hours for SR1 (fasted or fed), and 6.5 to 8.1 hours for SR3 (fed only). The extent of systemic accumulation was similar among the 6 BID dose regimens, and on average, geometric mean Cmax, Cmin, and AUC0–τ increased by approximately 2-fold at the PK steady-state versus after the first dose. For the only QD regimen studied (800 mg SR3 fed), the geometric mean Cmax and AUC0–τ increased by 9.6% and 15%, respectively.

Table: 4 Summary of INCB039110 Single-Dose Pharmacokinetic Parameters From Study INCB 39110-101

Dose	n	C_{max} (nM)	t_{max} (h)	$t_{½}$ (h)	AUC_{0-t} (nM·h)	$AUC_{0-\infty}$ (nM·h)	CL/F (L/h)	V_z/F (L)
10 mg	8[a]	23.1 ± 12.0 20.9	1.00 (0.500-1.50)	2.70 ± 0.937 2.58	32.1 ± 18.3 28.4	ND	ND	ND
20 mg	8	57.0 ± 18.3 54.6	1.00 (0.500-1.50)	1.75 ± 0.519 1.68	98.6 ± 33.9 93.1	119 ± 38.0 113	337 ± 130 319	796 ± 192 773
50 mg	7	304 ± 146 277	0.500 (0.500-1.00)	1.73 ± 0.335 1.70	457 ± 280 406	477 ± 283 427	228 ± 83.1 211	545 ± 175 520
100 mg	8	459 ± 155 437	1.00 (0.500-2.00)	1.85 ± 0.345 1.82	929 ± 329 885	957 ± 333 914	207 ± 64.5 198	560 ± 206 519
200 mg	7	1210 ± 214 1190	1.00 (0.500-2.00)	2.02 ± 0.333 2.00	2590 ± 361 2560	2610 ± 363 2590	141 ± 20.4 139	408 ± 75.0 402
300 mg	8	1820 ± 780 1630	0.750 (0.500-2.00)	2.33 ± 0.665 2.26	4130 ± 1220 3950	4160 ± 1220 3980	143 ± 52.7 136	507 ± 359 443
500 mg	8	3750 ± 1230 3580	1.50 (0.500-3.00)	2.75 ± 1.32 2.57	9890 ± 3480 9330	9920 ± 3490 9360	103 ± 40.9 96.5	399 ± 202 358
1000 mg	8	7350 ± 3250 6770	1.50 (1.00-2.00)	9.81 ± 5.20 8.36	20500 ± 7010 19500	20700 ± 7000 19700	96.3 ± 32.0 91.7	1360 ± 886 1110
P-values from a 2-factor ANOVA of log-transformed data (factor = dose and subject)								
Dose		< 0.0001		0.319	< 0.0001	0.0008	< 0.0001	0.0003
Subject		0.0677		0.0342	0.0038	0.0938	0.0363	0.0069

ND = not determined because of large mean percentage AUC extrapolated (~43%) based on estimated $t_{½}$ values, and as a result, statistical analysis for $AUC_{0-\infty}$ excluded the data from 10 mg dose.
Note: Values are mean ± SD and geometric mean, except that t_{max} is reported as median (range).
[a] The terminal-phase $t_{½}$ could not be determined in 3 subjects who received 10 mg dose because of low plasma exposures.

Drug-drug interactions

In vitro studies indicate that CYP3A4 is the major isozyme responsible for the metabolism of INCB039110 in human liver microsomes. In the human pregnane X receptor (hPXR) reporter gene assay, INCB039110 did not induce gene activity, suggesting that the potential for INCB039110 to induce CYP3A4 in clinical studies is low. In addition, INCB039110 did not significantly inhibit the activity of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4, suggesting that the potential for INCB039110 to cause clinical drug-drug interactions through CYP inhibition is low.

Pharmacodynamic effect of INCB039110

The efficacy of INCB039110 was characterized after continuous administration using subcutaneous pumps (Table 5) or when administered orally QD or BID (Table 6). Significant dose-dependent improvements in clinical signs of disease as well as histological assessment of inflammation, pannus, bone resorption, and cartilage damage in the rat paws were achieved with continuous infusion at plasma concentrations of approximately 50 nM, with near maximal effects on clinical scores seen at approximately 95 nM. For comparison, the IC50 of INCB039110 for inhibition of IL-6–stimulated pSTAT3 is 274 nM (Table 3), suggesting modest inhibition of JAK1 can be efficacious in this model. In contrast, similar compound exposures were nearly ineffective in a JAK2-driven pharmacodynamic (PD) model in which recombinant EPO induces reticulocytogenesis. This is consistent with the enzymatic and cellular selectivity for JAK1 relative to JAK2 described above.

Table: 5 Impact of Continuously Infused INCB039110 in the rAIA – Reduction in Clinical and Histological Scores of Inflammation and Associated Plasma Drug Levels

Dose (mg/kg per day)	INCB039110 Plasma Concentration (nM)	Reduction in Clinical Score (%)	Reduction in Histological Summed Score (%)
1.0	17 ± 5	21	0
3.0	51 ± 12	74[a]	66[a]
10.0	95 ± 43	95[a]	70[a]

[a] $p < 0.05$, ANOVA.

Table: 6 Dose, Schedule, and Impact on Inflammation and Histology of INCB039110 Administered Orally in the rAIA Model

Dose (mg/kg)	Dose Schedule	Reduction in Clinical Score (%)	Reduction in Histological Summed Score (%)
1.0	QD	19	ND
3.0	QD	29[a]	ND
10.0	QD	52[a]	ND
30.0	QD	73[a]	ND
0.1	BID	32	23
0.3	BID	22	19
1.0	BID	46[a]	23
3.0	BID	56[a]	48[a]
10.0	BID	100[a]	98[a]

ND = not determined.
[a] $p < 0.05$, ANOVA.

Selection of the RP2D

Study INCB 39110-250 was a double-blind, placebo-controlled, sequential, dose-escalation study of INCB039110 administered for 28 days to subjects with chronic plaque psoriasis characterized by Static Physician's Global Assessment (sPGA) ≥ 3, PASI score > 5, and BSA of psoriatic involvement ≥ 5%. INCB039110 was evaluated in parallel with placebo in 4 treatment groups (100 mg QD, 200 mg QD, 200 mg BID, and 600 mg QD). Subjects were randomized to INCB039110 in a 3:1 ratio with placebo.

The primary efficacy endpoint was the mean percent change from baseline in sPGA at the Day 28 visit. The study met its primary endpoint, demonstrating a dose-dependent improvement in sPGA score at the Day 28 visit. At the Day 28 visit, all treatment groups had mean percent reductions in sPGA score, with 22.2%, 29.4%, 35.2%, and 42.4% reductions in the 100 mg QD, 200 mg QD, 200 mg BID, and 600 mg QD groups, respectively, compared with a reduction of 12.5% in the placebo group. The difference between the 600 mg QD group and the placebo group was statistically significant ($p = 0.003$, without adjustment for multiplicity). Similar improvement was also noted in secondary endpoints. The proportions of sPGA responders and subjects achieving 50% improvement in PASI score (PASI 50) were also dose-dependent, reaching statistical significance at 200 mg QD (PASI 50 only) and 600 mg QD (sPGA and PASI 50). Improvement in mean sPGA and PASI scores was noted as early as the first assessment (Day 8). Responses in sPGA score were observed in all INCB039110-treated groups and no placebo-treated subjects. A dose-dependent increase in the proportion of subjects achieving an sPGA

score of 1 (minimal) was seen at the Day 28 visit. The proportion of responders was statistically significant for the 600 mg QD group ($p = 0.014$, without adjustment for multiplicity). Overall, more than half of the INCB039110-treated subjects achieved at least a 50% improvement in PASI score (PASI 50) at the Day 28 visit. At the Day 28 visit, the proportion of subjects achieving PASI 50 was dose-dependent and was statistically significant for the 200 mg and 600 mg QD groups ($p = 0.016$ and $p < 0.001$, respectively). Among INCB039110 groups, 6 subjects (15.8%) achieved PASI 75 overall at the Day 28 visit compared with no subjects in the placebo group.

Antitumor Activity of INCB039110 Monotherapy

Data from INCB 39110-230, a Phase 2a, open label, multiple Simon 2-stage study of INCB039110 administered orally to subjects with PMF, PPV MF, and PET MF, were presented at the 2014 American Society of Hematology Annual Meeting in New Orleans. In brief, results showed that meaningful reductions in MF-related symptoms were achieved by Week 12 in the 200 mg BID and 600 mg QD dose groups, and symptom improvements were largely maintained through Week 24. Modest reductions in spleen volume were attained in these 2 treatment groups. Hemoglobin levels were preserved in the majority of subjects who entered the study without transfusion requirements, and subjects requiring transfusions prior to study entry achieved meaningful reductions in transfusion requirements during the study (Mascarenhas et al 2014).

2.2.4 INCB039110 Safety Profile

Summary of Treatment-Emergent Adverse Events Occurring in ≥ 5 Subjects in Study INCB 39110-230 by MedDRA Preferred Term in Decreasing Order of Frequency (Safety Population)

MedDRA Preferred Term	100 mg BID (n = 10)	200 mg BID (n = 45)	600 mg QD (n = 32)	Total (N = 87)
Anemia	3 (30.0)	20 (44.4)	11 (34.4)	34 (39.1)
Fatigue	3 (30.0)	20 (44.4)	8 (25.0)	31 (35.6)
Thrombocytopenia	3 (30.0)	11 (24.4)	8 (25.0)	22 (25.3)
Upper respiratory tract infection	4 (40.0)	10 (22.2)	7 (21.9)	21 (24.1)
Nausea	3 (30.0)	10 (22.2)	6 (18.8)	19 (21.8)
Diarrhea	2 (20.0)	9 (20.0)	6 (18.8)	17 (19.5)
Cough	1 (10.0)	10 (22.2)	5 (15.6)	16 (18.4)
Constipation	1 (10.0)	10 (22.2)	4 (12.5)	15 (17.2)
Edema peripheral	1 (10.0)	10 (22.2)	4 (12.5)	15 (17.2)
Dyspnea	1 (10.0)	6 (13.3)	7 (21.9)	14 (16.1)
Pyrexia	1 (10.0)	7 (15.6)	6 (18.8)	14 (16.1)
Dizziness	0	7 (15.6)	6 (18.8)	13 (14.9)
Pain in extremity	2 (20.0)	8 (17.8)	3 (9.4)	13 (14.9)
Night sweats	1 (10.0)	7 (15.6)	4 (12.5)	12 (13.8)
Abdominal pain	2 (20.0)	5 (11.1)	4 (12.5)	11 (12.6)
Arthralgia	0	8 (17.8)	2 (6.3)	10 (11.5)
Contusion	1 (10.0)	5 (11.1)	4 (12.5)	10 (11.5)
Headache	0	3 (6.7)	6 (18.8)	9 (10.3)
Pruritus	1 (10.0)	6 (13.3)	2 (6.3)	9 (10.3)
Vomiting	0	8 (17.8)	1 (3.1)	9 (10.3)
Hypertension	2 (20.0)	4 (8.9)	2 (6.3)	8 (9.2)
Rash	0	5 (11.1)	3 (9.4)	8 (9.2)
Urinary tract infection	1 (10.0)	3 (6.7)	4 (12.5)	8 (9.2)
Chills	1 (10.0)	4 (8.9)	2 (6.3)	7 (8.0)
Decreased appetite	0	6 (13.3)	1 (3.1)	7 (8.0)
Epistaxis	1 (10.0)	5 (11.1)	1 (3.1)	7 (8.0)

Summary of Treatment-Emergent Adverse Events Occurring in ≥ 5 Subjects in Study INCB 39110-230 by MedDRA Preferred Term in Decreasing Order of Frequency (Safety Population) (Continued)

MedDRA Preferred Term	100 mg BID (n = 10)	200 mg BID (n = 45)	600 mg QD (n = 32)	Total (N = 87)
Pneumonia	1 (10.0)	5 (11.1)	1 (3.1)	7 (8.0)
Aspartate aminotransferase increased	1 (10.0)	4 (8.9)	1 (3.1)	6 (6.9)
Asthenia	1 (10.0)	2 (4.4)	3 (9.4)	6 (6.9)
Blood creatinine increased	2 (20.0)	3 (6.7)	1 (3.1)	6 (6.9)
Bone pain	0	1 (2.2)	5 (15.6)	6 (6.9)
Confusional state	0	2 (4.4)	4 (12.5)	6 (6.9)
Dyspnea exertional	2 (20.0)	2 (4.4)	2 (6.3)	6 (6.9)
Musculoskeletal pain	0	4 (8.9)	2 (6.3)	6 (6.9)
Oropharyngeal pain	0	4 (8.9)	2 (6.3)	6 (6.9)
Stomatitis	0	4 (8.9)	2 (6.3)	6 (6.9)
Acne	0	3 (6.7)	2 (6.3)	5 (5.7)
Alanine aminotransferase increased	2 (20.0)	2 (4.4)	1 (3.1)	5 (5.7)
Back pain	1 (10.0)	4 (8.9)	0	5 (5.7)
Blood bilirubin increased	0	4 (8.9)	1 (3.1)	5 (5.7)
Bronchitis	0	2 (4.4)	3 (9.4)	5 (5.7)
Hyperbilirubinemia	1 (10.0)	4 (8.9)	0	5 (5.7)
Hyperkalemia	1 (10.0)	3 (6.7)	1 (3.1)	5 (5.7)
Leukocytosis	1 (10.0)	2 (4.4)	2 (6.3)	5 (5.7)
Muscle spasms	2 (20.0)	2 (4.4)	1 (3.1)	5 (5.7)
Sinusitis	1 (10.0)	4 (8.9)	0	5 (5.7)

2.3 Other Agents

2.3.1 Trametinib Dimethyl Sulfoxide (GSK1120212B)

- 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.

- Clinical Pharmacokinetics (PK) and Activity of Trametinib

FTIH Phase 1 Trial of Trametinib Monotherapy (MEK111054)

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

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

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

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

Drug-drug interactions:
Trametinib is metabolized predominantly via deacetylation (non-cytochrome P450 [CYP450]-mediated) with secondary oxidation or in combination with glucuronidation biotransformation pathways (Investigator's Brochure, 2012a). The deacetylation is likely mediated by hydrolytic esterases, such as carboxylesterases, or amidases. Based on *in vitro* studies, trametinib is not an inhibitor of CYP1A2, CYP2A6, CYP2B6, CYP2D6, and CYP3A4. Although trametinib was found to be an *in vitro* inhibitor of CYP2C8, CYP2C9, and 2C19; inducer of CYP3A4; and inhibitor of transporters (OATP1B1, OATP1B3,

P-glycoprotein [P-gp], and breast cancer resistance protein [BCRP]), its low efficacious dose, and low clinical systemic concentration (22.2 ng/mL or 0.04 mcM at 2 mg) relative to the *in vitro* inhibition/induction potency suggests an overall low potential for drug-drug interactions.

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

Antitumor Activity of Trametinib Monotherapy
In the FTIH phase 1 trial, 14 patients with BRAF-mutant melanoma received trametinib at 2 mg QD (2 mg/day continuously, or 2 mg for 21 days followed by a 1 week break). The overall objective response rate (ORR) was 43% (6/14), including 2 complete responses (CRs) (Investigator's Brochure, 2012a). In 9 patients with BRAF wt melanoma, 2 patients achieved a partial response (PR), and 3 stable disease (SD) (Infante *et al.,* 2010). In 26 evaluable pancreatic cancer patients, there were 2 PRs (1 PR was KRAS mutation-positive) and 11 SD (2 achieved ≥20% tumor reduction) (Messersmith *et al.,* 2011). Among the 27 CRC patients (without selection of RAS or RAF mutations), 8 SD were observed.

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

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

- Trametinib Safety Profile

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.

- Clinical Experience with the Combination of Trametinib + Dabrafenib

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).

2.3.2 Dabrafenib Mesylate (GSK2118436B)

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).

- 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.

- 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.

- 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)

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

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				

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
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				
Nasopharyngitis	10	0	3	0

[a] CI CTCAE v4.
[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						
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						

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
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

CI CTCAE v4.
[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.
ncludes the following terms: renal failure and renal failure acute.

<u>AEs of special interest</u>:

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 associated with dabrafenib (BRAF category AEs) are:	AEs of special interest that are associated with trametinib (MEK category AEs)
Skin-related toxicities Pyrexia Malignancies Renal failure (renal failure, renal failure acute) Uveitis Hyperglycemia Pancreatitis	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.

2.4 Rationale

Vemurafenib was 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.

Importantly, these early results have been corroborated by larger randomized phase 3 studies. In the coBRIM trial of 495 randomly assigned patients with unresectable locally advanced or metastatic *BRAF* V600 mutation–positive melanoma, the combination of 960mg vemurafenib twice daily and 60mg combimetinib daily produced a PFS of 9.9 months vs. 6.2 months with vemurafenib alone (*Larkin et al. 2014*). In 2014, Long *et al* demonstrated a benefit in progression-free survival and overall response rate in patients taking the combination of dabrafenib and trametinib vs. dabrafenib alone (9.3 vs. 8.3 month PFS and 67% vs. 51% overall response rate). Furthermore, an advantage in overall survival was recently reported with the combination of dabrafenib and trametinib vs. dabrafenib alone in a phase 3 randomized trial of 704 patients (*Robert et al 2015*). Those patients receiving 2mg of trametinib daily and 150mg of dabrafenib twice daily exhibited a 31% reduction in the risk of death. Consequently the FDA approved both doublet therapies – dabrafenib/trametinib and vemurafenib/combimetinib – as combination treatments for advanced BRAF-mutant melanoma November 2015.

Nevertheless, despite these exciting advances, the majority of patients on BRAF/MEK combined inhibitor therapy develop resistance, and the median PFS on combination therapy is only a few months longer than BRAF inhibitor monotherapy. Unfortunately, there is no known reliably effective, standard of care treatment for patients with BRAF inhibitor resistant melanoma. 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. Recent studies have identified the role of JAK1 in V600-BRAF mutant melanoma resistance to BRAFi-based therapy (*Kim et al., 2015*). JAK1, a member of the Janus kinase family of proteins, plays a critical and nonredundant role in biological responses downstream of a select subset of cytokine receptors, as evidenced by perinatal lethality in JAK1 knockout mice (*Rodig et al 1998*). Although the link between the Janus Kinases and the Raf/MAPK signaling pathways has been known for many years (*Stancato et al., 1997*), the connection between JAK signaling and BRAFi-resistance has only been recently illuminated (*Kim et al., 2015*). In these studies, JAK1 was found to be upregulated in BRAFi-resistant melanomas, due to attenuated expression of the ubiquitin ligase RNF125. Upregulated JAK1 was found to contribute to elevated RTK (receptor tyrosine kinase) expression, including AXL, EGFR and PDGFR. Interestingly, increased expression of these RTKs have been implicated in melanoma resistance to MAPK-directed therapies (*Muller et al 2014., Sun et al 2014*).

The newly identified regulatory axis – RNF125-JAK1-AXL/EGFR offers a unique opportunity for therapy, which was evaluated in initial preclinical assessments in these studies (*Kim et al., 2015*). First, administration of commercially available JAK inhibitors, AZD1480 or Pyridone 6, effectively attenuated growth of BRAFi-resistant melanoma in 3D soft agar culture. The effect of JAK inhibitors was pronounced on its own, although enhanced in the presence of EGFR inhibitor, Gefitinib. In vivo, growth of BRAFi-resistant melanoma was not affected by PLX4720 but when combined with JAK and EGFR inhibitors the growth of these tumors was largely attenuated. These observations provide the rationale for assessment of JAK1 inhibitors in preclinical and clinical settings in combination with dabrafenib and trametinib in BRAF-mutant melanoma specifically and, perhaps more generally, BRAF-mutant solid tumors.

2.5 Correlative Studies Background

2.5.1 Pharmacokinetic Studies

While the pharmacokinetic data of single-agent INCB039110 and the combination of dabrafenib and trametinib are known, PK data of the triple combination have not been previously studied. The lack of evidence for drug-drug interactions with INCB039110 poses little risk that the clearance of dabrafenib and/or trametinib will be altered. We will collect trough samples on the first day of certain cycles in the protocol in order to measure drug concentrations of each agent if we encounter a rate of toxicity with the combination regimen that is higher than expected

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. Therefore, sampling of trough levels on day 1 of cycle 2 and cycle 4 and 6 will provide value that correspond to steady-state concentrations of dabrafenib and trametinib.

2.5.2 Biomarkers of Drug Effect

Although we do not yet know which JAK1 targets must be modulated in order for long term anti-tumor responses to be seen to the BRAF/MEK/JAK1 inhibitor combination, preliminary studies from our group

and others suggests that the RNF125-JAK1-AXL/EGFR and EGFR-SRC family kinase–STAT3 pathways 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. Thus, levels of JAK1 protein, downstream targets such as STAT1 and STAT3 and other proteins involved in adaptive signaling responses (EGFR, PDGR, MITF, NF-kB) will be measured via immunohistochemistry, immunofluorescence or immunoblots from FNAs taken from accessible melanoma lesions before therapy initiation and following BRAF/MEK/JAK1 inhibitor treatment. The preferred time point for on-treatment biopsies is Cycle 1 Day 7 (± 3 days). Biopsies may be also taken at any time, including at the time of clinically meaningful events such as response, disease progression or adverse events of interest.

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 INCB039110 effect on relevant JAK1 target 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.

2.5.3 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.

2.5.4 Expanded Genetic/Genomic Analysis

A number of mutations in oncogenes and tumor suppressor genes have been described in melanoma (*McArthur et al,. 2012*; *TCGA*). We will plan to perform expanded genomic analysis on pretreatment samples (either from pretreatment biopsies or from archived, formalin-fixed paraffin-embedded tumor blocks [FFPE]). The exact methodology for analysis has not yet been selected, though the options include NGS for mutations/deletions, nanostring for mRNA, and IHC and RPPA for protein. As it becomes clearer which methodology is best suited for the analysis as part of this protocol, a protocol amendment will be submitted that details the rationale and specific details for which technique(s) is (are) selected.

3. PARTICIPANT SELECTION

Any labs drawn on Cycle 1, Day 1 will not be used to reconfirm eligibility. Eligibility is confirmed at screening; it is not required to be reassessed on Cycle 1, Day 1.

3.1 Eligibility Criteria

3.1.1 *For Dose-Escalation Phase:* Patients must have histologically confirmed, BRAF-mutant (V600E/K) malignancy (molecularly confirmed using Cobas assay or a comparable FDA-approved assay (for exceptions, see below*) that is metastatic or unresectable, have received and tolerated prior BRAF or BRAF and MEK inhibitor (BRAF targeted) therapy or not previously received BRAF targeted therapy, and for which standard curative or palliative 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 to the Overall Principal Investigator (PI) for approval. (FDA approved tests for BRAF V600 mutations in melanoma include: THxID BRAF Detection Kit and Cobas 4800 BRAF V600 Mutation Test).

3.1.2 *For Dose-Expansion Phase:* Patients must have histologically confirmed, BRAF-mutant (V600E/K) melanoma (molecularly confirmed using Cobas assay or a comparable FDA-approved assay (for exceptions, see below*) 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.

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

3.1.3 Patients must have measurable disease by RECIST, 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.

3.1.4 Patients may have received any number of prior lines of therapy. All prior systemic anti-cancer treatment-related toxicities must be less than or equal to Grade 1 according to the Common Terminology Criteria for Adverse Events version 4 (CTCAE version 4.0; NCI, 2009) at the time of enrollment. This does not include alopecia and Grade 1 or less peripheral neuropathy.

3.1.5 Age ≥18 years. Because no dosing or adverse event data are currently available on the use of INCB039110 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 in the opinion of the investigator.

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

– Leukocytes (WBCs)	≥3,000/uL
– absolute neutrophil count	≥1,500/uL
– hemoglobin	≥ 9 g/dl (patients may be transfused to this level)
– platelets	≥ 100,000/uL
– 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[1]
– Serum creatinine	≤1.5 mg/dL OR creatinine clearance ≥50 mL/min/1.73 m^2
– Potassium	>3 and <5.5mmol/L
– Magnesium	>1.2 and <2.5 mg/dL

[1]Therapeutic levels of anti-coagulation are permitted if clinically indicated, as per section 3.2.15. Thus PT/INR may be >1.3 if therapeutically anti-coagulated.

3.1.9 The effects of INCB039110, 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 registration 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 registration*, 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.

Men with a female partner of childbearing potential must have either had a prior vasectomy or agree to use effective contraception. Additionally, male subjects (including those who are vasectomized) whose partners are pregnant or might be pregnant must agree to use condoms for the duration of the study and for 4 months following completion of therapy.

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

3.1.11 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 radiotherapy, 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 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 JAK1 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 registration.

3.2.4 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.

3.2.5 Patients must have no clinical evidence of leptomeningeal or brain metastasis causing spinal cord compression that are symptomatic, untreated, not stable for ≥ 4 weeks prior to Day 1 of Cycle 1 (must be documented by imaging), or requiring corticosteroids to manage metastasis-related symptoms. Subjects who have been off of corticosteroids for at least 2 weeks prior to Day 1 of Cycle 1 or are on a stable dose of ≤10 mg per day of a prednisone equivalent for >1 month prior to Day 1 of Cycle 1 can be enrolled. Subjects must also be off of enzyme-inducing anticonvulsants for >4 weeks prior to Day 1 of Cycle 1.

3.2.6 History of known immediate or delayed hypersensitivity reactions attributed to compounds of similar chemical or biologic composition to INCB039110, 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 INCB039110, 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 History of interstitial lung disease or pneumonitis.

3.2.10 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.

3.2.11 History of another malignancy other than the study indication under this trial within 5 years of study enrollment, with the exception of the following:

- Subjects who underwent successful definitive 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.
- Subjects who have had a localized (i.e. no history of regional or metastatic spread) solid tumor malignancy ≥ 3 years within study enrollment and, in the opinion of the investigator, has a very low probability of recurrence.

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:
- A QT interval corrected for heart rate using the Bazett's formula QTcB ≥460 msec on the pre-study baseline single 12 lead EKG.
- History or evidence of current clinically significant uncontrolled arrhythmias (exception: patients with controlled atrial fibrillation for >30 days prior to registration are eligible).
- History of acute coronary syndromes (including myocardial infarction and unstable angina), coronary angioplasty, or stenting within 6 months prior to registration.
- 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. In patients with no history of hypertension and a pre-study baseline blood pressure of systolic >140 mmHg and/or diastolic >90 mmHg, a second reading must be taken at least 1 minute later, with the two readings averaged to obtain a final BP measurement.
- 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 For patients requiring anti-coagulation with vitamin K antagonists, 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. If clinically indicated, prophylactic low dose warfarin may be given to maintain central catheter patency.

3.2.16 Current use of a prohibited medication. The following medications or non-drug therapies are prohibited
- 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).
- 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, fluconazole
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.

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.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

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

Following registration, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. Registration cancellations must be made in OnCore as soon as possible.

4.2 Registration Process for DF/HCC Institutions

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

5. TREATMENT PLAN

Treatment will be administered on an outpatient basis. A cycle of treatment will be 28 days in length. Patients are asked to maintain a medication diary for recording each dose of Dabrafenib, Trametinib and INCB039110.

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 C) 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. Dose escalation to the next dose level will occur when the preceding cohort reaches the end of Cycle 1. Patients who do not complete treatment in cycle 1 for reasons other than dabrafenib, trametinib or INCB039110 toxicity will be replaced. Intra-patient 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) 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]	INCB039110 (mg QD)
-2	75 mg	1 mg	100 mg
-1	150 mg	2 mg	100 mg
1	150 mg	2 mg	200 mg
2	150 mg	2 mg	300 mg
3	150 mg	2 mg	400 mg

5.1 Pre-Treatment Criteria

5.1.1 Screening Visit: Patients must meet criteria at screening as outlined in Section 3.1.

- Of note, screening eligibility labs do not need to be repeated on cycle 1 day 1. If labs are drawn on Cycle 1, Day 1 will not be used to reconfirm eligibility. Eligibility is confirmed at screening; it is not required to be reassessed on Cycle 1, Day 1. Please see section 5.1.2 and the study calendar (Section 10) for information on laboratory studies and treatment parameters.

5.1.2 Hematologic Parameters for treatment:

Absolute Neutrophil Count

- Cycle 1, Day 1: ≥ 1,500/uL
- Cycle 2 and Beyond, Day 1: Per Table in Section 6.2.1.1

White Blood Cell Count

- Cycle 1, Day 1: ≥ 3,000/uL
- Cycle 2 and Beyond, Day 1: Per Table in Section 6.2.1.1

Hemoglobin

- Cycle 1, Day 1: ≥ 9g d/gl (patients may be transfused to this level)
- Cycle 2 and Beyond, Day 1: Per Table in Section 6.2.1.1

Platelets

- Cycle 1, Day 1: ≥ 100,000/uL
- Cycle 2 and Beyond, Day 1: Per Table in Section 6.2.1.1

5.1.3 Liver Function Parameters for treatment:

Transaminases (AST/ALT):

- Cycle 1, Day 1: ≤ 2.5 x institutional upper limit of normal
- Cycle 2 and Beyond, Day 1: Per Table in Section 6.1.16

Bilirubin

- Cycle 1, Day 1: < 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
- Cycle 2 and Beyond, Day 1: Per Table in Section 6.1.16

5.1.4 Renal Function Parameters for treatment:

Creatinine:

- Cycle 1, Day 1: ≤1.5 mg/mL OR creatinine clearance ≥50 mL/min/1.73 m^2
- Cycle 2 and Beyond, Day 1: Per Table in Section 6.1.9

5.1.5 All Cycles, Day 1: Patients must have all the following:

- Adequate blood pressure control as detailed in Section 6.1.11.
- ECOG performance status of 0 or 1.
- No evidence of life-threatening medical problems.

5.2 Agent Administration

5.2.1 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 more than 6 hours have lapsed from the scheduled/planned dose time (i.e. no more than 30 hours from the previous dose). Vomited doses of trametinib should not be made up.

In the event that dabrafenib, INCB039110, 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.

5.2.2 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, INCB039110, 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.

5.2.3 INCB039110

INCB039110 is administered orally and provided in 100 mg tablets.

A positive food-effect was observed for all INCB039110 formulations studied at the 300 mg (3 × 100 mg) dose level. The SR3 formulation delivered a mean relative bioavailability of 48% and is likely to be associated with a moderate food-effect. The current recommendation is to administer INCB039110 on an empty stomach, either 1 hour before or 2 hours after a meal; the recommendation to administer INCB039110 fasting may change based on emerging data. Missed doses of INCB039110 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). Vomited doses of INCB039110 should not be made up.

In the event that trametinib, dabrafenib, or both agents need to be discontinued due to toxicity reasons, treatment with INCB039110 may continue at the discretion of the investigator until time of disease progression.

5.2.4 Concomitant Administration

Dabrafenib, INCB039910 and Trametinib may be taken together.

5.3 Definition of Dose Limiting Toxicity

Dose-limiting toxicity (DLT) is based on the CTEP Active Version 4 of the NCI Common Terminology Criteria for Adverse Events (CTCAE). DLT refers to INCB039110, Dabrafenib, or Trametinib treatment-related toxicities experienced during the first cycle of treatment between days 1-28. A DLT will be defined as follows:

- Any grade 4 toxicity
- Grade 3 non-hematologic toxicity
- Grade ≥ 3 neutropenia with fever > 38.5°C
- Grade 3 thrombocytopenia with clinically significant bleeding
- Toxicity resulting in patients missing more than 20% of the doses

In general, all Grade 3, non-hematological toxicities related to INCB039110, Dabrafenib, or Trametinib 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 in less than 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.

Patients that have missed more than 20% of the doses for non-toxicity related reasons will not be considered evaluable for DLTs.

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	Proceed 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.	

5.4 General Concomitant Medication and Supportive Care Guidelines

5.4.1 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. After discussion with the Principal Investigator, agents known to affect or with the potential to affect selected CYP450 isoenzymes may be permitted, if clinically indicated.

5.4.2 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 B 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.

5.4.3 INCB039110

INCB039110 does not induce or 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. *In vitro* studies indicate that CYP3A4 is the major isozyme responsible for the metabolism of INCB039110 in human liver microsomes. INCB039110 did not significantly inhibit the activity of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4, suggesting that the potential for INCB039110 to cause clinical drug-drug interactions through CYP inhibition is low.

Supportive treatment should be given as per the institution standards and at the Investigator's discretion. Antiemetics, anti-diarrheal agents, etc. may be given to treat or prevent gastrointestinal toxicities.

5.5 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.

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF). Alternative care options will be discussed with the participant.

In the event of unusual or life-threatening complications, treating investigators must immediately (within 24 hours of awareness of the event) notify the Overall PI, David Miller, M.D., P.h.D at 617-724-4000, pager 36066.

5.6 Duration of Follow Up

Participants will be followed for 2 years after removal from study treatment or until death, whichever occurs first. Participants removed from study treatment for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. All patients will have a follow-up encounter 30 days (+ 3 days) after last study treatment for assessment of adverse effects. This encounter can be carried out in clinic or over the telephone. 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. Dermatologic skin assessments should be completed every 3 months for 6 months after discontinuation of dabrafenib or initiation of another anti-neoplastic therapy. Participants' vital status will be followed every 6 months (telephone contact is adequate) following removal from the study treatment. Date and cause of death should be provided for participants who become deceased within the 2-year interval following removal from the study treatment.

5.7 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.

The research team updates the relevant Off Treatment/Off Study information in OnCore.

6. DOSING DELAYS/DOSE MODIFICATIONS

The criteria for dose modification of dabrafenib, trametinib, and INCB039110 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 according to the specified dose modifications for 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
150 mg BID	→	100 mg BID
100 mg BID	→	75 mg BID
75 mg BID	→	50 mg BID
50 mg BID	→	Discontinue dabrafenib

Current Dose Trametinib	If Dose Reduction Required	Reduce To
2 mg QD	→	1.5 mg QD
1.5 mg QD	→	1.0 mg QD
1.0 mg 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 INCB039110. Conversely, if trametinib is permanently discontinued for trametinib-related toxicities, patients will be allowed to continue dabrafenib and INCB039110.

In the event that INCB039110 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 INCB039110 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 INCB039110 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 Treatment Related Toxicities **Not Specified** in Subsequent Sections

Table 6-1: Dabrafenib or Dabrafenib + Trametinib Dose Modification for Treatment Related Toxicities Not 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**. • If dabrafenib must be reduced to <50 mg BID, permanently **discontinue dabrafenib** • If trametinib must be reduced to < 1.0 mg QD, permanently **discontinue trametinib**
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 dabrafenib must be reduced to <50 mg BID, permanently **discontinue dabrafenib** • If trametinib must be reduced to < 1.0 mg QD, permanently **discontinue trametinib**
*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.	

6.1.2 Dabrafenib or Dabrafenib + Trametinib Dose Modification for **Pyrexia** Suspected to be Treatment-Related

- 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 Requirements for Pyrexia Suspected to be Related to Treatment

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 (must 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:	• Clinical evaluation for infection and hypersensitivity • Laboratory work-up • Hydration as required • Administer anti-pyretic treatment if clinically indicated and continue prophylactic treatment	• Interrupt dabrafenib. • Continue trametinib. • *Once pyrexia resolves* to baseline, if fever was associated with *rigors, dehydration, hypotension, or renal insufficiency*, **reduce dabrafenib by one dose level**. If it was not, restart dabrafenib at the same 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, if fever was associated with *rigors, dehydration, hypotension, or renal insufficiency*, **reduce dabrafenib by one dose level**. If it was not, restart dabrafenib at the same dose level.

Table 6-2: Dabrafenib or Dabrafenib + Trametinib dose Modification and Management Requirements for Pyrexia Suspected to be Related to Treatment

Event	Management Guideline	Dose Modification
Subsequent Events:	• Clinical evaluation for infection and hypersensitivity • Laboratory work-up • Hydration as required • 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**.[a] • If dabrafenib must be reduced to <50 mg BID, permanently **discontinue dabrafenib**.
[a] 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** Suspected to be Related to Treatment[a]

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).

Table 6-3: Dabrafenib or Dabrafenib + Trametinib Supportive care and Dose Modification for Rash Suspected to be Related to Treatment

CTCAE Grade	Adverse Event Management	Action and Dose Modification
The following Rash prophylaxis is recommended for the first 6 weeks of study treatment: • 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. ***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 dermatologist or surgeon • Infected lesions: appropriate bacterial/fungal culture-driven systemic or topical antibiotics. * Subjects who develop rash/skin toxicities should be seen by a qualified physician and should receive evaluation for symptomatic/supportive care management		
Grade 1	• If not yet started, initiate prophylactic and symptomatic treatment measures.[1] • Use moderate strength topical steroid.[2] • Reassess within 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)	• If not yet started, initiate prophylactic and symptomatic treatment measures.[1] • Use moderate strength topical steroid.[2] • Reassess within 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.**
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] 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: Triamcinolone 0.1% cream or ointment or Fluticasone propionate 0.05% cream or 0.005% ointment. 3. 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 section 1.2 for dose modification.

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

- *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. Skin exams should continue every 2-3 months for 6 months after discontinuation of dabrafenib or initiation of another anti-neoplastic therapy.

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.

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.

- *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.

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. Upon confirmed diagnosis of pancreatitis, dabrafenib should be interrupted until completely resolved. 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. • 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.

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

Serum Creatinine Level	Management Guideline	Action and Dose Modification
Serum creatinine increase >0.5 mg/dL (44 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.		

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 dabrafenib plus trametinib.

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

Clinic	LVEF-drop (%) or CTCAE grade	Dose Modification
Symptomatic[a]	• 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 with cardiologist. • Repeat ECHO after 2, 4, 8, 12, and 16 weeks or until resolution.
[a] If ECHO does not show LVEF recovery within 2 weeks, repeat ECHO 2 weeks later (prior to reaching end of 4 weeks). [b] Symptoms may include: dyspnea, orthopnea, 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. Requirements for BP monitoring and management are provided below.

- 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.

- Symptomatic hypertension is 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

Table 6-7: Dabrafenib or Dabrafenib + Trametinib Treatment Modification and Management Requirements 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 clinically 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]
(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.
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[a]	• 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.	

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 Recommendations for Diarrhea Suspected to be Related to Treatment

CTCAE Grade	Management Recommendations	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 Suspected to be Related to Treatment**

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 Considered to be Related to Study Treatment

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 until resolution. • 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 Suspected to be Related to Treatment**

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 Suspected to be Related to Treatment

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 trametinib reduced by one dose level. • Escalation to previous dose level after 4 weeks • 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 • If no recovery to grade ≤1 within 4 weeks, permanently discontinue trametinib.

CTCAE Grade	Adverse Event Management	Action and Dose Modification
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** Total Bilirubin <2x ULN, without symptoms considered related to liver injury or hypersensitivity and who can be monitored weekly for 4 weeks	• **May continue study treatment**. • If **liver chemistry stopping criteria** are met any time, proceed as described below. **MONITORING:** Repeat ALT, AST, ALK, and total bilirubin until they return to normal/baseline or stabilise
Criteria for discontinuing study drug: When any of the liver stopping criteria below is met, discontinue trametinib and dabrafenib 1. ALT ≥3xULN **and** Total bilirubin ≥2x ULN or >35% direct bilirubin[1, 2] 2. ALT ≥ 3xULN **and** INR >1.5, if INR measured and if subject is not on anticoagulation[2] 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** • 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). **MONITORING:** *In patients stopping for criteria 1-2 (with abnormal TB and INR, indicating potentially more significant liver toxicities):* • Repeat ALT, AST, ALK, and total bilirubin) and perform liver **assessment and workup** 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.

Event	Treatment modifications and assessment/monitoring
	• CBC with differential to assess eosinophilia. • Record clinical symptoms of liver injury, or hypersensitivity on AE CRF. • Record concomitant medications (including acetaminophen, herbal 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.
Footnotes 1. Serum bilirubin fractionation should be performed if testing is available. If serum bilirubin fractionation testing is unavailable, record presence of detectable urinary bilirubin on dipstick, which indicates direct bilirubin elevations and suggesting liver injury. 2. All events of ALT ≥3xULN and bilirubin ≥2xULN (>35% direct bilirubin) or ALT 3x ULN and INR >1.5 (if INR measured) may indicate severe liver injury (possible "Hy's Law"). INR measurement is not required, and the threshold value stated will not apply to subjects receiving anticoagulants. 3. New or worsening symptoms believed to be related to liver injury (such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness, or jaundice) or believed to be related to hypersensitivity (such as fever, rash or eosinophilia) 4. Includes: Hepatitis A IgM antibody; Hepatitis B surface antigen and Hepatitis B Core Antibody (IgM); Hepatitis C RNA; Cytomegalovirus IgM antibody; Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, obtain heterophile antibody or monospot testing); Hepatitis E IgM antibody 5. PK sample is desired if feasible. Record the date/time of the PK blood sample draw and the date/time of the last dose of study treatment prior to blood sample draw on the CRF. If the date or time of the last dose is unclear, provide the subject's best approximation. If the date/time of the last dose cannot be approximated OR a PK sample cannot be collected in the time period indicated above, do not obtain a PK sample. Not required for single-dose studies. 6. If hepatitis delta antibody assay cannot be performed, it can be replaced with a PCR of hepatitis D RNA virus (where needed) (Le Gal *et al.*, 2005).	

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 INCB039110 Dose modifications

INCB039110 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
400 mg	→	300 mg
300 mg	→	200 mg
200 mg	→	100 mg
100 mg	→	discontinue

In the event that dabrafenib, trametinib, or both agents need to be discontinued due to toxicity reasons, treatment with INCB039110 may continue at the discretion of the investigator until time of disease progression.

6.2.1 Management of INCB039110 toxicity

The only consistently observed effects of INCB039110 in healthy volunteers, patients with inflammatory diseases and cancer are decreases in neutrophils, hemoglobin and platelet count. For occurrences of grade 3 or 4 events by CTCAE in any of these three categories, the dose reduction table below will be followed. Patients may receive transfusions or growth factor support as indicated in cases of severe toxicity. Dabrafenib and trametinib rarely cause severe effects on blood counts. Therefore, in INCB039110 will be modified in the case of severe decreases in blood counts, assuming that these events are considered treatment related.

6.2.1.1 Dose Reduction and Interruption

Table 6-15: INCB039110 Dose Modification for Toxicities

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 **permanently discontinue study treatment**

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The expected adverse events listed in the Investigator Drug Brochure and/or Package Insert and the characteristics of an observed AE (Section 7.1) will determine whether the event requires expedited reporting **in addition** to routine reporting.

The following definitions of terms apply to this section:

Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Life-threatening adverse event or *life-threatening suspected adverse reaction.* An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

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

- Death,
- a life-threatening adverse event,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate 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. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Unexpected adverse event or *unexpected suspected adverse reaction.* An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed.

7.1 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 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 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(s) that are listed in the Investigator Drug Brochure or package insert should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.

- **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.

7.2 Expedited Adverse Event Reporting

Investigators **must** report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

7.3 DF/HCC Expedited Reporting Guidelines

Investigative sites within DF/HCC will report AEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

7.4 Expedited Reporting to the Food and Drug Administration (FDA)

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

7.5 Expedited Reporting to the company Supplying Drug (Incyte)

Incyte needs to be notified within 24 hrs of learning of an event. Inctye also needs to be provided a completed SAE form via email. SAE reporting for each subject begins the day the informed consent is signed by the patient and within 30 days after subject has completed or discontinued from the study or has taken last dose of the study drug, or as described in the IST protocol.

SAEs, occurring using Incyte Study drug, are reported in accordance with the effective protocol. SAEs occurring with any another commercial drug are reported to manufacturer of that drug in accordance with regulations and protocol.

Initial Serious Adverse events (SAEs) and/or subsequent follow-up reports should be reported via email to: ***IncytePhVOpsIST@incyte.com***. SAE reports should be for a single subject with any additional documents (i.e. discharge summary, relevant test results) included for the same subject as individual attachments to the email. One email can have multiple attachments as long as each attachment contains relevant information for the same subject.

Please email your SAE form with a cover sheet and any additional attachments to the IST email address: ***IncytePhVOpsIST@incyte.com.***

Telerx C3i is the PhV Clinical Research Organization (CRO) for Incyte. They will be contacting you for SAE follow-up requests and data clarifications by sending you a data clarification form (DCF). See follow-up section for details on this process. The following is general contact information for Telerx C3i.

TELERX C3I (Incyte PhV CRO)
US SAE fax number 1-866-726-9234

Telerx C3i tracks any critical outstanding follow-up items and questions and contacts the investigational site via a faxed DCF for the additional requested information until all outstanding queries are resolved. This includes requests for hospital discharge summaries, autopsy reports, and death certificates (if applicable), results of relevant laboratory, and diagnostic tests. The

investigational site faxes the additional information to Telerx C3i, using the Telerx C3i SAE fax number. The site personnel will update all relevant data on the appropriate SAE Report Form or equivalent to include any new or changed information. The Incyte Safety Representative (IC SR) will monitor outstanding queries and take appropriate action needed for resolution.

Any serious SAE upgraded to death or life-threatening requires that the follow up report be emailed to: ***IncytePhVOpsIST@incyte.com*** email address within 24 hours of learning of the change.

If the SAE is not previously documented in the Investigator's Brochure for INCB039110 and is thought to be related to the study drug, a manufacturer's associate may urgently require further information from the Investigator or Sponsor for reporting to Health Authorities. Incyte will be notified within 24hrs of any IND safety event and will receive a copy of the initial and follow up IND Adverse Event reports within 48hrs of submission to regulatory authorities. This will be submitted to the following email address: IncytePhVOpsIST@incyte.com

Reporting of Pregnancy

An "Initial Pregnancy Report" or equivalent must be completed in full and emailed to ***IncytePhVOpsIST@incyte.com*** within 24 hrs of discovery of a pregnancy of a subject who has taken the Incyte product or the pregnancy of a partner for a subject who has taken the Incyte product. The "Follow-up Pregnancy Report Form" or equivalent must be completed and emailed to ***IncytePhVOpsIST@incyte.com*** within 30 days after delivery, so that Incyte is provided with information regarding the outcome of the pregnancy. If the pregnancy results in any events which meet the serious criteria (i.e., miscarriage or termination), the SAE reporting process needs to be followed and the timelines associated with an SAE should be followed.

7.6 Expedited Reporting to Hospital Risk Management

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

7.7 Routine Adverse Event Reporting

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

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 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)

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 commercially supplied in bottles of #120 capsules as 50 mg and 75 mg capsules for oral administration.

- 50 mg capsules: Dark red capsule imprinted with 'GS TEW' and '50 mg'. Each bottle contains a silica gel desiccant.
- 75 mg capsules: Dark pink capsule imprinted with 'GS LHF' and '75 mg'. Each bottle contains a silica gel desiccant.

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

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

Route of Administration: Oral administration. Take dabrafenib mesylate (GSK2118436B) 1 hour prior or 2 hours after a meal. Pill cannot be crushed, chewed or dissolved in water.

Potential Drug Interactions: Dabrafenib mesylate (GSK2118436B) induces CYP3A4, 2C9, and possibly 2B6, 2C8, and 2C19 enzymes. Use caution in patients who are taking substrates that are metabolized in these enzyme pathways, such as warfarin.

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

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. Commercial drug will used in this study.

8.2 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: Trametinib is commercially supplied in bottles of #30 tablets, and is supplied as 0.5 mg and 2 mg (as free base) tablets.

- 0.5 mg tablets: Yellow, modified oval, biconvex, film-coated tablets with 'GS' debossed on one face and 'TFC' on the opposing face.
- 2 mg tablets: Pink, round, biconvex, film-coated tablets with 'GS' debossed on one face and 'HMJ' on the opposing face.

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.

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

Route of Administration: Oral. Take by mouth on an empty stomach, either 1 hour before or 2 hours after a meal. Pill cannot be crushed, chewed or dissolved in water.

Potential Drug Interactions

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

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

Availability

Trametinib dimethyl sulfoxide (GSK1120212B) is FDA-approved for the treatment of unresectable or metastatic BRAF-mutant melanoma. Commercial drug will used in this study.

8.3 INCB039110

Chemical Name: 2-(3-(4-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)-1*H*-pyrazol-1-yl)-1-(1-(3-fluoro-2-(trifluoromethyl)isonicotinoyl)piperidin-4-yl)azetidin-3-yl)acetonitrile adipate

Other Names: None

Classification: JAK inhibitor

CAS Registry Number:

Molecular Formula: C32H33F4N9O5

M.W.: 699.66

Mode of Action: INCB039110 is a potent and selective inhibitor of JAK1

How Supplied: INCB039110 will be provided as 100 mg (free-base equivalent) SR tablets. These tablets contain the active ingredient, hypromellose, microcrystalline cellulose, lactose monohydrate, and magnesium stearate. The higher strength tablet formulations (200 mg and 300 mg) also contain pregelatinized starch.

Storage: All INCB039110 drug product should be stored at ambient conditions (15°C to 30°C, or 59°F to 86°F).

Route of Administration: Oral administration. Pill cannot be crushed, chewed or dissolved in water.

Potential Drug Interactions: None identified

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 due to high binding to plasma proteins.

Availability: INCB039110 is an investigational agent and will be supplied by Incyte Pharmaceuticals.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Biomarker Studies

9.1.1 Blood BRAF Assay

Collection of Specimens

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
- Cycle 1, day 15
- Day 1 of every cycle of therapy
- At the time of progression

Handling of Specimens

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

Shipping of Specimens

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:

Flaherty Laboratory
c/o Dennie Frederick
55 Fruit Street; Jackson 9th Floor
Boston, MA 02114

Sites Performing Correlative Study

All DF/HCC study sites are expected to participate in this correlative study.

9.2 Pharmacokinetics

Little is known about the potential for drug-drug interactions (DDI) of the triple combination of dabrafenib, trametinib, and INCB039110. However, the available evidence regarding effect of each agent on cytochrome P450 isoenzymes and clearance of concomitantly administered drugs does not indicate a likely interaction.

Limited PK sampling will be performed in this protocol to enable analysis of trough levels of each agent pre-dose at the beginning of cycles 2, 4 and 6 in the case that the rate of combined toxicity is greater than expected.

Handling of Specimens

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.

<u>INCB039110 PK Samples</u>

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

Tissue analysis of pharmacodynamic effects

Collection of Specimens

Optional tumor biopsies will be performed pretreatment, one week (+/- 3 days) after commencing therapy with the combination as part of the phase I dose escalation and dose expansion cohorts, and post-treatment following completion of all study therapy. Biopsies may be also taken at any time, including at the time of clinically meaningful events such as response, disease progression or adverse events of interest. 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.

Handling of Specimens

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

Shipping of Specimens

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. Flaherty's laboratory and the remainder of samples sent to Dr. Flaherty's laboratory.

The addresses of the laboratories are:

Massachusetts General Hospital

Jackson 9
55 Fruit Street
Boston, MA 02114

Site Performing Correlative Study: Being conducted only at Massachusetts General Hospital

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 2-week prior to start of protocol therapy with the exception of scans and x-rays. Scans and x-rays must be done ≤4 weeks prior to the start of therapy.

Assessments must be performed prior to administration of any study agent. Study assessments and agents should be administered within ± 3 days of the protocol-specified date, unless otherwise noted.

Procedure	Pre-Study	Cycle 1			Cycles 2+	End of Txt Visit	30 Day F-U Encounter[3]	2-6 Month F-U
	Baseline [1]	Day 1[2,b]	Day 8[4]	Day 15[5]	Day 1[2]			
Dabrafenib and Trametinib [A]		DT ---------- DT						
INCB039110 [B]		I----------I						
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)	X [c]	X [c]						
Physical Examination	X	X		X	X	X		
Vital Signs (BP [d], HR, body temperature)	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]		
ECOG Performance Status	X	X	X	X	X	X		
Dermatology Examination [e]	X				X			X[e]
Height	X							
Weight	X	X			X	X		
Ophthalmology Examination [f]	X				X			
12 Lead EKG	X	X			X			
Hematology/Clinical Chemistry [g]	X	X	X	X	X[h]	X		
Coagulation [i]	X							
Tumor Measurements [j]	X[j]				X[j]			
PK Analysis [k]					X [k]			
Optional Tumor Biopsies [l]	X [l]		X [l]			X [l]		
Blood BRAF testing [m]		X		X	X	X		
Rash Prophylaxis [n]		X[n]		X[n]	X [n]			
AE assessment [o]	X	X	X	X	X	X	X	
Long term Follow up [p]								X[p]

Color Photography [q]	X [q]				X [q]			
Loperamide[r]	X [r]							

A. Dabrafenib and Trametinib: doses as assigned; Dabrafenib BID and Trametinib QD.
B. INCB039110: dosing as assigned per dose level.
1. All Pre-study assessments must be completed within 14 days prior to first dose, except for Scans 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. Assessment for each visit can be done within 3 days of the projected visit date.
3. Follow up encounter, which can be carried out over the phone, should be 30 days from last dose of study drugs (± 3 days).
4. Medical History, Concurrent Medications, Vital Signs, ECOG Status, Hematology and Blood Chemistry panels will be completed prior to Day 8 treatment.
5. Physical Exam, Medical History, Concurrent Medications, Vital Signs, ECOG Status, Hematology and Blood Chemistry panels will be completed prior to Day 15 treatment.

a. Informed Consent can be obtained within 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) if done within 72 hours of first dose does not need to be repeated on Day 1 of the first dose.
d. In patients with blood pressure of systolic >140 mmHg and/or diastolic >90 mmHg, a second reading should be taken at least 1 minute later, with the two readings averaged to obtain a final BP measurement.
e. Dermatological Examination done at screening and then every 8 weeks (2 cycles). For patients enrolling in the expansion cohort who may have been on DT prior to enrolling and have had a recent skin exam, skin exams within 28 days of C1D1 will suffice for eligibility. Skin exams should continue every 2-3 months for 6 months after discontinuation of dabrafenib or initiation of another anti-neoplastic therapy.
f. Ophthalmological examination is done at screening, week 4, and annually. For patients enrolling in the expansion cohort who may have been on DT prior to enrolling and have had a recent ophthalmological exam that includes all requirements listed in Section 6.1.14, eye exams within 28 days of C1D1 will suffice for eligibility. Additional ophthalmic exams will be performed only as symptomatically warranted. For further details on requirements of exam see Section 6.1.14.
g. Clinical chemistries include 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. Hematology samples will be collected before study treatment administration and at all applicable study visits
h. During Cycle 2, patients will have day 15 (+/- 3 days) clinical chemistries collected as outlined in footnote g above. After cycle 2, clinical chemistries are collected on Day 15 at the investigator's discretion.
i. Includes PT, PT-INR, PTT, repeated as indicated. These chemistries may be collected at a remote facility and the results can be faxed to the research team.
j. Tumor measurements and radiological exam done every 8 weeks 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 samples will be collected pre-dose on Day 1 of Cycles 2, 4 and 6. Also, per Section 6.1.14, 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. Also, see section 6.1.16 for PK collection in the setting of Liver Chemistry Changes.
l. Optional tumor biopsies will be performed pretreatment. Pre-treatment tissue obtained by biopsy or resection performed according to standard of care may be utilized, provided tissue was obtained within 8 weeks of study entry, and subsequent to the last systemic anticancer therapy received. Optional tumor biopsies will also be performed one week (+/- 3 days) after commencing therapy with the combination as part of the phase I dose escalation and dose expansion cohorts; however, biopsies may be taken at any time, including at the time of clinically meaningful events such as response, disease progression or adverse events of interest. Post-treatment biopsies (following completion of all study therapy) will be optional.

m. Blood BRAF analysis performed pretreatment C1D1, C1D15, at the beginning of each subsequent cycle and at disease progression (Section 9.1.1).
n. See table 6.3 for detailed prophylaxis recommended for the first 6 weeks of treatment.
o. AE assessment starting from the day 1 of dosing throughout the study
p. After 30 day follow up visit, patient is followed for survival for 2 years. See section 5.6 for full details.
q. In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended. See section 11.1.3 for full details.
r. Loperamide should be made available prior to start of study treatment so loperamide administration can begin at the first signs of diarrhea. See table 6-9 for full details.

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 (with a window of minus 1 week prior to day 1 of the cycle). 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 (with a window of minus 1 week prior to day 1 of the cycle).

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 INCB039110.

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

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

12.1.2 Responsibility for Data Submission

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

12.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Overall PI and study team.

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

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

This is a phase I trial of the combination of dabrafenib, trametinib, and INCB039110 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, up to an additional 20 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.

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	Proceed 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.	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, up to 20 patients will be enrolled in an expansion cohort of patients with melanoma 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.

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 20
0.01	0.18
0.03	0.46

True Incidence of Unexpected or Severe Toxicity	Probability of Observing One or More Patients with Toxicity among 20
0.05	0.64
0.08	0.81
0.09	0.85
0.10	0.88
0.15	0.96

Secondary endpoints that will be assessed for the 20 patients treated at the MTD in the dose-expansion cohort 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 38 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 38 patients reflects four complete dose escalation cohorts of 6 patients each and the expansion cohort of 20 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.

13.3.1 Correlatives and exploratory endpoints

- Pharmacokinetics

Pharmacokinetic parameters, including maximal plasma or serum concentration (Cmax), area under the curve to the last collection point (AUClast), area under the curve for dose interval (AUC0-t), and time of maximal concentration (Tmax), 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 – pembrolizumab, nivolumab, ipilimumab, atezolizumab, or high-dose IL-2 – 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 the RNF125-JAK1-AXL/EGFR axis

In order to characterize the changes on the tumor and its microenvironment during and following inhibition of the RNF125-JAK1-AXL/EGFR axis, we will use single-cell resolving technologies developed by our collaborators at the Dana-Farber/Harvard Cancer Center and apply them to pre-, on- and post-treatment biopsy specimens. Our collaborators in the Garraway and Regev labs at DF/HCC have developed a platform for processing single-cell isolates from patient specimens to generate single-cell transcriptome profiles (sc-RNAseq). (*Tirosh et al.,* 2016) Through a process of disaggregation into single cells, fluorescence-activated flow sorting, RNA isolation and massively paralleled sequencing of complementary DNA libraries, this technology permits the deconvolution of intra-tumoral heterogeneity and enhanced granularity of the tumor ecosystem compared to bulk RNA sequencing. With assistance from our collaborator Dr. Benjamin Izar of the Garraway/Regev labs, we will perform single-cell RNA sequencing on pre-, on- and post-treatment patient specimens to evaluate for gene expression profiles that correlate with drug-resistance or predict treatment response. Cell states will be analyzed via: 1) unbiased assessment of the intercellular transcriptional hetereogeneity using principal component analysis (PCA) followed by downstream enrichment analysis of emerging principal components (PCs); and 2) Gene Set Enrichment Analysis (GSEA) to specifically assess the activity and variability of hundreds of pathways across cells within the same tumor.

We will also utilize highly multiplexed imaging via a high-throughput cyclic immunofluorescence (CycIF) method developed by the Sorger lab at DF/HCC to perform deep-profiling at the protein level of single-cells from patient specimens. (Lin et al., 2015) Our collaborator Dr. Izar of DF/HCC has optimized this technique - which can permit up to 30 protein markers per slide - for formalin-fixed paraffin embedded patient-tumor specimens (personal communication of unpublished data). We will leverage this approach to validate that proof of mechanism and target engagement of INCB039110 by evaluating RNF125-JAK1-AXL/EGFR axis proteins implicated in therapeutic escape including BRAF, CRAF, AKT, JAK1, phospho-AKT, phospho-ERK, phospho-STAT3, EGFR and AXL. We will also use CycIF to immunophenotype tumor-infiltrated lymphocytes and tumor-associated macrophages to characterize the effects of triple JAK1i/BRAFi/MEKi targeting on the immune cell microenvironment. Multi-spectral CycIF images are segmented using freely available tools (ImageJ, CellProfiler) to generate a coordinate map against which fluorescence intensities for all channels can be aligned. Data from 10^5-10^7 cells can then be visualized as images and/or studied using dimensionality reduction methods such as principal component analysis or t-Distributed Stochastic Neighbor Embedding.

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 INCB039110 effect on relevant RNF125-JAK1-AXL/EGFR axis 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 specimens embedded in OCT will be evaluated for tumor and necrotic content.

Skyline version 1.3 will be 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 (%).

13.3.2 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) version 4.0), type of adverse event, and relationship to treatment. Deaths reportable as SAEs and non-fatal serious adverse events will be listed by patient and tabulated by primary system organ class, 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).

14. PUBLICATION PLAN

The results will be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. A full report of the outcomes will be made public no later than three (3) years after the end of the study.

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APPENDIX A PERFORMANCE STATUS CRITERIA

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

APPENDIX B INFORMATION ON POSSIBLE DRUG INTERACTIONS

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

The patient _____ is enrolled on a clinical trial using the experimental agents INCB039110, Dabrafenib mesylate and trametinib dimethyl sulfoxide. This form is addressed to the patient, but includes important information for others who care for this patient.

INCB039110, 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 **INCB039110, 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 _____.

Study Participant Self-Administration Drug Diary

DFCI Study Number:

Participant Name: _____

Your Doctor_____ Phone_____

Your Nurse_____ Phone_____

Dosing Instructions for Study Drug:

Your dose of Trametinib is _____ mg made up of _____ - _____ mg tablets.
Your dose of INCB039110 is _____ mg made up of _____ - _____ mg tablets.
Your dose of Dabrafenib is _____ mg made up of _____ - _____ mg capsules.

- You will take trametinib once per day in the morning. You will take INCB039110 once per day in the morning. You will take dabrafenib twice per day, once in the morning and then twelve hours later.
- Pills need to be taken without food on an empty stomach, at least 1 hour prior to a meal and 2 hours after a meal. Pills can be taken with water.
- Pill cannot be crushed, chewed or dissolved in water.
- Dabrafenib, INCB039910 and Trametinib may be taken together.
- Vomited doses should not be made up.
- Missed doses should not be made up if more than 6 hours have lapsed from the scheduled/planned dose time.
- Each of the study drugs should be kept in the original contained they are distributed in.
- Please remember to bring any unused drug, all empty containers, and your drug diary to your clinic visits.
- 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
- Dabrafenib and INCB039110 should be stored at room temperature. Trametinib should be refrigerated.

- Wash your hands with soap and water after taking your dose

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 Trametinib Dose	# Tablets	Time of INCB039110 Dose	# Tablets	Time of Morning Dabrafenib Dose	# Capsules	Time of Evening Dabrafenib Dose	# Capsules	Comments *If dose was vomited, missed or skipped, indicate reason below.*
1		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
2		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
3		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
4		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
5		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
6		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
7		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
8		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
9		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
10		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
11		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
12		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		

Day	Date	Time of Trametinib Dose	# Tablets	Time of INCB039110 Dose	# Tablets	Time of Morning Dabrafenib Dose	# Capsules	Time of Evening Dabrafenib Dose	# Capsules	Comments *If dose was vomited, missed or skipped, indicate reason below.*
13		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
14		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
15		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
16		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
17		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
18		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
19		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
20		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
21		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
22		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
23		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
24		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
25		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
26		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
27		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		
28		□ am □ pm		□ am □ pm		□ am □ pm		□ am □ pm		

Participant Signature or Initials _____ **Date** _____

APPENDIX D PREGNANCY REPORT FORM

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.

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APPENDIX E 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 registration 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. 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 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 dabrafenib

Table 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.

APPENDIX F 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 G 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 H 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 I 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.