SUMMARY OF CHANGES

NCI Protocol #: 9455
Local Protocol #: OSU-13117

NCI Version Date: January 30, 2018
Protocol Version Date: January 30, 2018

#	Section	Page	Comments
I.	Header	ALL	HEADER Updated version date to January 30, 2018
II.	Cover Page	5	PROTOCOL TYPE Added version January 30, 2018
III	Cover Page	3	MULTI SITE CONTACT The multi-center trial coordinator has been changed from Jennifer Sexton to **Julie Agriesti, CCRC**
IV	3.1.9 6.1.1 6.2.9	22 28 44	Per CTEP's CTCAE v4.0 to CTCAE v5.0 Conversion Amendment Request, the Common Terminology Criteria for Adverse Event (CTCAE) version number has been updated to 5.0 **Please note:** This CTCAE version change has not been made within the CAEPR(s)
V	7.2	50	**ADVERSE EVENTS CHARACTERISTICS** The statement regarding CTCAE has been updated to reflect the new version 5.0. **The statement now reads:** 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 until March 31, 2018 for AE reporting. CTCAE version 5.0 will be utilized for AE reporting beginning April 1, 2018. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.
	7.3.3	51	**EXPEDITED REPORTING GUIDELINES** Clarified the reporting guidelines for death due to progressive disease. Death due to progressive disease should be reported as **Grade 5 "Disease progression"** in the system organ class (SOC) "General disorders and administration site conditions."…

NCI Protocol #: NCI 9455

Local Protocol #: OSU 13117

TITLE: A SINGLE ARM, PHASE II STUDY OF SINGLE AGENT TRAMETINIB FOLLOWED BY TRAMETINIB IN COMBINATION WITH GSK2141795 IN PATIENTS WITH ADVANCED TRIPLE NEGATIVE BREAST CANCER

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NCI Supplied Agent(s): Trametinib dimethyl sulfoxide (GSK1120212B) NSC 763093 GSK2141795 NSC 767034

IND Sponsor: NCI

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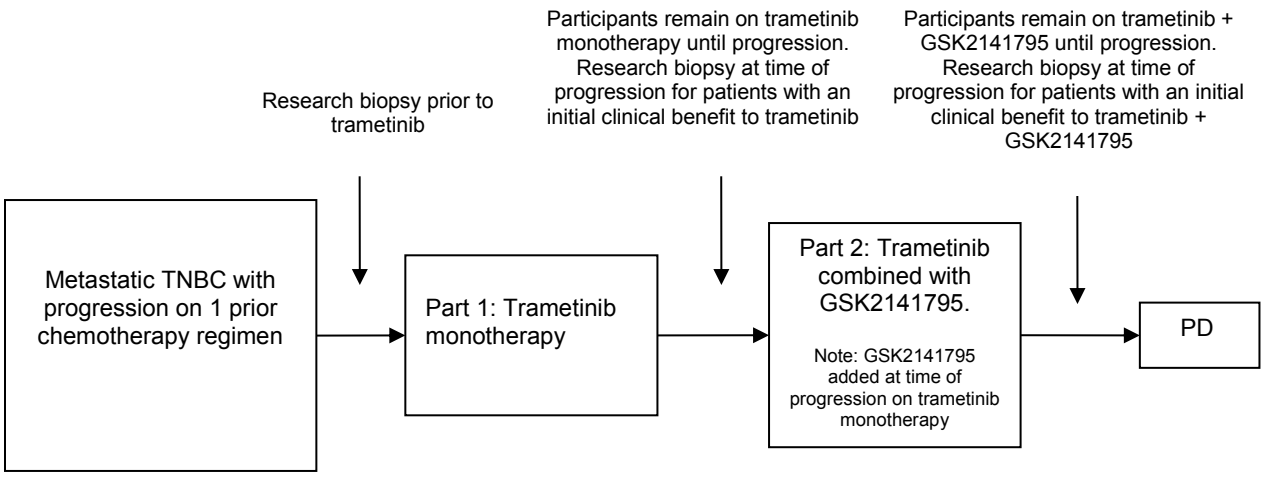
Amendment / Version 1 / November 23, 2016
Amendment / Version 1 / January 27, 2017
Amendment/ Version 1/ July 31, 2017
Amendment/ Version 1/January 30, 2018

FIGURE 1



Abbreviations: TNBC, triple negative breast cancer; PD, progressive disease

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OBJECTIVES

1.1. Primary Objectives

To assess the anti-tumor activity associated with trametinib monotherapy in patients with triple negative breast cancer (TNBC)

1.2. Secondary Objectives

- To assess the anti-tumor activity associated with trametinib in combination with AKT inhibitor GSK2141795 after progression on trametinib in patients with metastatic TNBC.
- To determine the progression-free survival following the initiation of treatment with trametinib monotherapy in patients with metastatic TNBC.
- To determine the progression-free survival following the initiation of treatment with trametinib in combination with GSK2141795 in patients with metastatic TNBC.
- To determine the overall survival following the initiation of treatment with trametinib with GSK214179 in patients with metastatic TNBC.
- To determine the nature and degree of toxicities associated with trametinib monotherapy and trametinib in combination with GSK2141795 in patients with metastatic TNBC.
- To determine the biomarker potential of PTEN to predict response to single agent trametinib
- To determine molecular markers of sensitivity and resistance to trametinib monotherapy and trametinib in combination with GSK2141795 in patients with metastatic TNBC.

2. BACKGROUND

2.1. Study Disease

Breast cancer is heterogeneous disease in terms of presentation, morphology, molecular profile, and response to therapy. Triple negative breast (TNBC) accounts for approximately 20 to 25% of all types of breast cancer and is characterized on standard pathologic evaluation by lack of immunohistochemistry (IHC) expression of estrogen receptor (ER), progesterone receptor (PR) and HER-2 protein.[2] TNBC is clinically the most aggressive phenotype of breast cancer. Women with TNBC have an increased likelihood of distant recurrence and death within 5 years of diagnosis.[2, 3] TNBC is more likely to be poorly differentiated, occur in premenopausal patients and in African American women.[4] No specific systemic therapy is recommended for the treatment of TNBC and only limited data is available to support the selection of appropriate treatment. This is in contrast to ER-positive or HER2-overexpressing breast cancers where the relevant targets are known and specific treatments directed against those targets (i.e. the anti-estrogen tamoxifen, aromatase inhibitors, or trastuzumab and lapatinib, respectively)

favorably affect clinical outcomes. TNBC represents an important challenge due to the lack of responsiveness to available targeted regimens.

2.2. CTEP Agents

2.2.1 Trametinib Dimethyl Sulfoxide (GSK1120212B)

The following information is summarized from the Trameinib Investigator's Brochure 2012.

Mechanism of Action

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

Trametinib is a dimethyl sulfoxide solvate compound 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).[5] Trametinib inhibited MEK1/2 kinase activity and prevented RAF-dependent MEK phosphorylation, producing prolonged pERK1/2 inhibition. BRAF-mutant Colo205, A375P F11s, and HT-29 human tumor xenograft mouse models showed significant mean tumor growth inhibition with multiple complete and partial tumor regressions. Xenograft lines with wild-type RAF/RAS (PC3, BxPC3, and BT474) were much less sensitive, showing only modest tumor growth inhibition with no tumor regressions.

In a phase III trial comparing trametinib with dacarbazine or paclitaxel in patients with BRAF V600E or V600K mutant metastatic melanoma, trametinib demonstrated a significantly better response rate, progression-free survival, and overall survival.[6] However, single agent activities are limited. Extensive research is underway to identify the patient selection markers and develop rational combination strategies. Preclinical studies have provided strong rationale and proof of principle for combination of MEK inhibitors with RTK inhibitors, PI3K/AKT inhibitors and mTOR inhibitors.

Pharmacokinetics

The phase 1 trial of trametinib monotherapy (MEK111054) reported the MTD of trametinib as 3 mg daily, but the recommended phase 2 dose (RP2D) was chosen at 2 mg daily based on tolerability of repeated cycles.[7] PK measurements were conducted under fasting conditions. After a single dose (Day 1), $AUC_{0\text{-}24}$ and C_{max} values were dose-proportional up to 6 mg, lower than dose proportional following 8 mg, and greater than

dose proportional following the 10 mg dose. Median T_{max} was 1.5 hours. After repeat doses (Day 15), trametinib accumulated with a mean accumulation ratio of 6.6 at the RP2D of 2 mg daily. 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.[7] At 2 mg daily 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 Interactions

Trametinib is metabolized predominantly via deacetylation (non-cytochrome P450 [CYP450]-mediated) with secondary oxidation or in combination with glucuronidation biotransformation pathways. The deacetylation is likely mediated by hydrolytic esterases, such as carboxylesterases, or amidases. 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 relative to the *in vitro* inhibition/induction potency suggests an overall low potential for drug-drug interactions.

Pharmacodynamics Markers

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. In general, increasing exposures and/or doses provided greater pharmacodynamic effects. The median change observed at a dose of 2 mg daily was 62% inhibition of pERK, 83% inhibition of Ki67, and a 175% increase in p27.

Antitumor Activity of Trametinib Monotherapy

In the phase 1 trial, 14 patients with BRAF-mutant melanoma received trametinib at 2 mg daily (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 wild type melanoma, 2 patients achieved a partial response (PR), and 3 stable disease (SD).[8] In 26 evaluable pancreatic cancer patients, there were 2 PRs (1 PR was KRAS mutation-positive) and 11 SD (2 achieved ≥20% tumor reduction).[9]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, daily) or chemotherapy (dacarbazine or paclitaxel).[6] 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.

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 daily. Drug-related AEs in 45 patients were similar to that observed in patients with solid tumors, and 2 mg PO daily 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.[10] 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%).

Safety Profile

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

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

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

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

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

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

- *Central serous retinopathy (CRS):* CRS is a class side effect of MEK inhibitors. As of 22 May 2012, 13 cases of CSR have been reported amongst approximately 1,600 patients treated with trametinib, either as monotherapy or in combination with other anti-cancer agents: two cases of grade 1, eight cases of grade 2, and three cases of grade 3. All 13 resolved.
- *Retinal vein occlusion (RVO):* As of 22 May 2012, four cases of RVO have been observed with trametinib. All four cases occurred in one eye only, and study drug was stopped at time of diagnosis in all cases. There was a decrease in visual acuity in two patients with central RVO (CRVO), while the other two patients experienced no meaningful decrease of 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: 20 cases of pneumonitis were reported in subjects treated with trametinib, either as monotherapy or in combination with other anti-cancer agents, in six studies: five cases of grade 1, five cases of grade 2, nine cases of grade 3, and one case of grade 4.

2.2.2 GSK2141795

The following information is summarized from the GSK2141795 Investigator's Brochure 2012.

Mechanisms of Action

AKT is a serine/threonine protein kinase with 3 isoforms (AKT1, AKT2 and AKT3) that participate in multiple pathways regulating several cellular processes, including survival, proliferation, tissue invasion and metabolism.[11] Preclinical data suggest that blocking AKT activity can inhibit the proliferation of tumor cells and either induce an apoptotic response or sensitize tumors to undergo apoptosis in response to other cytotoxic agents. GSK2141795 is a member of the N-alkyl pyrazole class of orally available kinase inhibitors and has been shown to be a potent, pan-AKT inhibitor. GSK2141795 inhibited AKT1, 2 and 3 with IC50 values of 2, 16 and 4 nM, respectively. *In vitro*, GSK2141795 caused a concentration- and time-dependent reduction in phosphorylation of multiple proteins downstream of AKT such as glycogen synthase kinase 3 (GSK-3) β , an insulin-regulated inhibitor of the mammalian target of rapamycin complex 1 (mTORC1) protein kinase (PRAS40), Forkhead gene product (FOXO), and caspase 9.

In vivo studies conducted in immunocompromised mice bearing human tumor cell line xenografts showed GSK2141795 can inhibit multiple AKT substrates downstream of AKT in a dose- and time-dependent manner. A repeat oral administration of GSK2141795 for 3 weeks inhibited the growth of various tumor xenografts, including BT474 (breast), HCC1954 (breast), and SKOV3 (ovarian) in mice.

Pharmacokinetics

The pharmacokinetics, absorption, distribution, metabolism, and elimination of GSK2141795 have been investigated through a series of oral and IV studies in the CD-1 mouse, Sprague Dawley rat, beagle dog, and cynomolgus monkey using unlabeled and [14C]-labeled drug. In these species, oral bioavailability of GSK2141795 was moderate to high (≥40%), blood clearance was low to moderate (20% to 50% of liver blood flow) and volume of distribution was high (>6 times total body water).

In rat and human hepatocytes, the metabolism of GSK2141795 occurred by mono-oxygenation, glucuronidation, oxidative deamination, and N-demethylation; there were no apparent human-specific metabolites, and cytochrome P450 (CYP) 3A4 was the primary enzyme responsible for the oxidative metabolism. *In vitro*, GSK2141795 demonstrated the potential for oxidative bioactivation. No stereo-conversion to the R-enantiomer was observed *in vitro*, and no rearrangement to an acyl migrated isomer of parent was observed either *in vitro* or following oral administration to mice.

In vitro, GSK2141795 was moderately to highly bound (93% to >95%) to plasma proteins, and had minimal to moderate association (0.99 to 1.64) with blood cells.GSK2141795 was a human P-glycoprotein (Pgp) and mouse breast cancer resistance protein (Bcrp 1) substrate and did not inhibit Pgp-mediated digoxin transport. GSK2141795 had moderate passive permeability (60 nm/sec) and low to moderate absorptive membrane permeabilities (3.0 to 11 nm/sec). GSK2141795 inhibited human organic anion transporting polypeptide (OATP) 1B1 (IC50 of 16 μM) and 1B3 (IC50 of 21 μM) and BCRP-mediated transport of cimetidine (up to 50% of control at 3 μM). In a tissue distribution study following single oral administration of [14C] GSK2141795, consistent with its high volume of distribution, radioactivity was widely distributed into tissues, and most tissue concentrations were higher than those observed in blood. Concentrations of radioactivity generally declined over time, and by 7 days post dose, the levels of radioactivity were below the limit of quantification (BLQ) in most tissues. However, radioactivity was still quantifiable at 35 days post dose in the uveal tract, suggesting the potential for the selective association of drug-related material with melanin.

In rats and dogs following single oral administration of [14C] GSK2141795, the major route of elimination of drug-related material was via the feces, while urinary excretion constituted a minor route in both species. In vitro intrinsic clearance for GSK2141795 was low in hepatocytes and microsomes from the mouse, rat, dog, monkey, and human. Biliary and urinary recoveries indicate that approximately 50.6% of the oral dose was absorbed in rats.

PCS112689 an open label, dose-escalation first in human study (FTIH) assessed the safety, pharmacokinetics, and pharmacodynamics of GSK2141795 in subjects with solid tumors or lymphoma. Preliminary data indicated that plasma concentrations for GSK2141795 were measurable for all subjects over the 72 hours after a single dose over the dose range tested (10 mg to 150 mg). In addition, drug concentrations were measurable on Day 8, suggesting that GSK2141795 can still be found in the plasma at least 1 week after a single dose of study drug over the dose range tested (75 mg to 100 mg). While the exposure for the 100 mg and 150 mg doses were similar following a single dose, drug exposure following multiple doses was approximately in proportion to dose. GSK2141795 accumulated 2.5- to 8.4-fold with repeat daily dosing. Mean area under the concentration-time curve [$AUC_{(0-24)}$] and maximum plasma concentration (C_{max}) values generally increased in a dose-proportional manner, although there was variability among subjects. Median time to reach peak concentration (T_{max}) across doses was 3 hours and ranged from 0 to 4 hours. The mean value for the effective half-life of elimination ($t_{½}$, eff), across subjects was 3.0 days and ranged from approximately 1.3 to 5.5 days. The MTD of single-agent GSK2141795 was 75 mg once-daily as determined by the FTIH study.

Drug Interactions

The enzymes responsible for the oxidative metabolism of [14C] GSK2141795 (5 μM)

were investigated in vitro with human liver microsomes and recombinant CYP enzymes. The data from human liver microsomes with selective inhibitors and scaled results from recombinant CYP enzyme studies suggest that CYP3A4 is the primary cytochrome p450 enzyme involved in oxidative metabolism of GSK2141795. GSK2141795 has the potential to have drug-drug interactions upon co-administration with CYP3A4, CYP2C8, Pgp and BCRP inhibitors or potent inducers. Drugs that potently inhibit CYP3A4 could lead to increased GSK2141795 exposure in patients and drugs that are strong inducers of CYP3A and may result in lower exposures of GSK2141795. GSK2141795 also appears to be a moderate in vitro inhibitor of CYP2C8 (IC50 3μM). Therefore drugs that are sensitive substrates of CYP3A4 or CYP2C8 should be used with caution. Moreover, GSK2141795 is a human p-glycoprotein (P-gp, ABCB1) and breast cancer resistant protein (BCRP, ABCG2) substrate. Therefore, drugs that are inhibitors of P-gp and BCRP should be used with caution. Co-administration of drugs that are sensitive BCRP substrates, such as topotecan should be avoided. Combination studies with GSK2141795 have been performed with metformin, trametinib,pazopanib, and lapatinib. Co-administration of GSK2141795 with metformin did not appear to have any impact on the efficacy of GSK2141795 for inhibiting tumor growth and was well tolerated as the treatment with GSK2141795 or metformin alone.

No other pharmacodynamic studies have been performed to evaluate possible interactions of GSK2141795 with other drugs that may be co-administered. Lists of drugs that can possibly interact with GSK2141795 included under **Section 5.2.**

Saftey Profile

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

Due to limited experience in human subjects, there is currently incomplete information available about the relationship of AEs and administration of GSK2141795. Based on available adverse event data from 151 subjects, the most common toxicities of GSK2141795 monotherapy or in combination with trametinib were gastrointestinal (GI)-related (diarrhea, nausea, and vomiting) and fatigue. Hyperglycemia, hypoglycemia, mucositis, and rash are also commonly observed. In addition, three cases of hypothyroidism have been noted. Therefore, clinical monitoring should consider in particular the GI tract, the hematopoietic system, glucose metabolism and thyroid function. Attention should also be given to glandular structures and the possibility of clinical signs due to reduced secretions. In PCS112689 a FTIH study the most common AEs occurring in ≥20% of all subjects in all dose groups were diarrhea (56%), nausea (45%), fatigue (32%), decreased appetite (30%), vomiting (25%), and hyperglycemia (21%). Maximum tolerated dose (MTD) was identified as 75 mg for both once daily dosing as well as for 7 days on/7 days off alternate dosing schedule. Most adverse effects were noted at dose greater than 75 mg.

Diarrhea: This is the most frequent drug-related AE in patients receiving GSK2141795. Most diarrhea events reported were Grade 1 and 2. Based on current data, the majority of cases of diarrhea occur within the first 3 to 4 weeks of starting the drug. In most cases, diarrhea resolves with interruption of GSK2141795 dosing and implementation of supportive treatment. Based on preliminary data, re-challenge with a reduced dose of GSK2141795 is tolerated.

Mucosal inflammation: This may be an on-target effect, i.e. the result of AKT inhibition on mucosal cell renewal or the result of direct irritation of the mucosal surface by the drug substance. Mucositis has been observed as a dose-limiting toxicity (DLT). Early intervention for signs and symptoms of mucosal inflammation is recommended and encouraged. Based on preliminary data, dose interruption followed by dose reduction on rechallenge can ameliorate symptoms.

Rash: Rash may or may not be associated with pruritis. Preliminary data suggest that drug interruption and dose reduction upon re-challenge ameliorate the symptoms. Rash management should focus on symptom relief and maintenance of an intact integument. Dermatology consult is recommended when clinically appropriate. Topical steroid creams have been found to provide some relief from symptoms.

Hyperglycemia: Hyperglycemia occurred in patients receiving ≥75 mg/day with the majority of events occurring at doses exceeding the MTD of 75 mg/day. Treatment-related grade 3 or grade 4 events were observed at 75 mg, 100 mg, and 150 mg daily doses. The frequency and severity of hyperglycemia AEs is reduced at the 75mg/day dose as compared with higher doses. It is not clear if oral anti-hyperglycemic drugs are useful to ameliorate the hyperglycemia, although both intravenous and sliding scale insulin have been helpful.

Hypoglycemia: Asymptomatic hypoglycemia occurred in patients receiving ≥75 mg/day. Treatment-related grade 3 or grade 4 events were observed at 75 mg and 100 mg daily doses. The mechanism of hypoglycemia is currently unknown.

Thyroid Events: Reversible minimal to mild hypertrophy of follicular cells was seen in the thyroid glands of dogs given 5 mg/kg/day for 4 weeks. The relationship to GSK2141795 and clinical significance are unknown, although three cases of drug-related hypothyroidism have been reported

2.2.3 Trametinib in Combination with GSK2141795

GSK2141795 was combined in a 3-day cell proliferation study with trametinib (GSK 1120212) against a panel of 26 colon, 15 lung, and 6 pancreatic cancer cell lines. GSK2141795 and trametinib were synergistic in cancer cell lines from colon (19 of 26), lung (4 of 15), and pancreas (2 of 6). The anti-tumor activity of GSK2141795 alone (10 or 30 mg/kg/day), trametinib alone (0.1, 0.3 or 10 mg/kg/day) or GSK2141795 in

combination with trametinib (10/0.1, 30/0.1, 10/0.3 or 30/0.3 mg/kg/day) was evaluated with once daily oral administration up to 90 days in female SCID mice (n=8/group) bearing established HPAC or Capan-2 human pancreatic tumor xenografts. In general, mice treated with GSK2141795 or trametinib alone had minimal body weight loss, but GSK2141795/trametinib combination doses appeared to be slightly less tolerated than the single agent treatments, particularly the combinations including a 30 mg/kg/day dose of GSK2141795. Administration of trametinib in combination with GSK2141795 appeared to be more effective than either GSK2141795 or trametinib alone in HPAC xenografts. Treatment with both AKT and MEK inhibitors (GSK2141795 at 30 mg/kg/day and trametinib at 0.3 mg/kg/day), given alone or in combination, resulted in a reduction in proliferating cells (Ki67+) following treatment and up to a 200% increase in apoptosis (as measured by cleaved caspase-3).

Twenty-three patients with advanced solid tumors received the combination using a zone-based escalation procedure enabling evaluation of multiple combination doses in parallel cohorts.[12] While the RP2D for single agent for single agent trametinib and GSK2141795 are 2 mg/d and 75 mg/d, dose reductions were required for the combination. DLTs include grade 2 AST and ALT elevation, and grade 3 chest pain with sustained ventricular tachycardia; all DLTs were reversible with drug interruption. The most common AEs (≥10%) included nausea (26%), AST elevation (22%; grade 3/4, 9%), fatigue (22%) and rash (22%). Three MTDs were defined for variable dose ratios: 2 mg trametinib + 25 mg GSK2141795; 0.5 mg trametinib + 75 mg GSK2141795; and 1.5 mg trametinib + 50 mg GSK2141795. Three of 13 evaluable patients (unselected) had tumor shrinkage of 8% (ovarian), 16% (endometrial), and 17% (ovarian) after 8 weeks on study. The dose regimen of 1.5 mg trametinib + 50 mg GSK2141795 would be used in this study. Additional trials to explore alternate schedules (*e.g.*, intermittent) and pharmacodynamic markers are ongoing.

2.3. Rationale

Triple negative breast cancer (TNBC) is often characterized by high expression of epidermal growth factor receptor (EGFR).[13, 14] Hoeflich *et al.* demonstrated that activation of EGFR leads to cell signaling along the RAF/MEK/ERK pathway, and subsequent treatment with a MEK-targeted drug caused reduced tumor growth in basal-like breast cancer models.[15] One proposed mechanism of intrinsic resistance to MEK inhibition is through the ERK-independent PI3K/AKT pathway, as basal-like breast cancer cell lines with loss of PTEN (an inhibitor of the PI3K pathway) or high levels of AKT signaling were less responsive to MEK inhibition.[15] This preclinical finding may be clinically relevant as 30% of women with basal-like breast cancer demonstrate evidence of PTEN loss.[16-18] Additionally, treatment with a selective MEK inhibitor resulted in upregulation of the PI3K/AKT pathway.[15]

To validate these findings, Dr. Ching-Shih Chen (Chair of Cancer Research and Therapy at the Ohio State University, scientific collaborator) performed a series of preclinical *in vitro* experiments. Basal-like breast cancer cell lines were treated with trametinib monotherapy (Unpublished data Chen, Olson). As predicted, treatment of basal-like breast cancer cell lines

with trametinib was associated with greater cell kill in the PTEN wild type MDA-MB-231 cell line compared to PTEN null MDA-MD-468 (Figure 2). Our results are consistent with the findings of Hoeflich *et al.* suggesting that MEK inhibition depends in part on PTEN status.

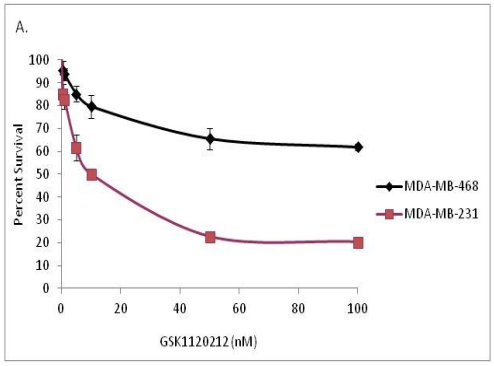

Figure 2. Trametinib (GSK1120212) monotherapy in basal-like breast cancer cells MDA-MD468 (PTEN null) and MDA-MB-231 (PTEN wild type) (Unpublished data, Chen and Olson).

Dual blockade of both PI3K/AKT and MEK signaling synergized to potently impair the growth of basal-like breast cancer models *in vitro* and *in vivo*.[15] Other studies have also shown that inhibiting PI3K signaling in combination with RAS/RAF/MEK/ERK signaling is sufficient to induce apoptosis and suppress proliferation in other cancer models.[19, 20] The RAF/MEK/ERK pathway is also implicated as a mechanism of resistance to chemotherapy in TNBC, providing rationale for targeting this pathway in patients with prior exposure to cytotoxic therapy.[21, 22] Finally, O'Shaughnessy and colleagues recently presented data showing that a TNBC patient with co-activation of the MEK and PI3K/AKT pathways (via whole genome and transcriptome sequencing) demonstrated a near complete response to dual MEK and AKT inhibition.[23, 24]

Given the close interaction of the RAS/RAF/MEK/ERK and PI3K/AKT pathways, biomarkers of response will likely involve proteins within these signaling networks. For example, our work and others have demonstrated that PTEN loss results in upregulation of the PI3K/AKT pathway and is a major predictor of response to agents that target MEK in preclinical studies [Unpublished Data, Chen and Olson].[15] Similarly, DUSP4, a negative regulator of ERK1 and ERK2 activity, has been shown to be a mediator of resistance to chemotherapy and tumor suppressor in TNBC. Loss of DUSP4 results in MAPK pathway activation and pre-clinically was a biomarker of MEK sensitivity.[22] We hypothesize that the anti-tumor activity of single agent MEK inhibition will depend on evidence of MAPK activation (i.e. loss of DUSP4) and down regulation of the PI3K/AKT pathway (i.e. intact PTEN). Furthermore, based on the near complete clinical response seen in the phase I study,[24, 25] we anticipate that patients with biomarkers demonstrating co-activation of MAPK and PI3K signaling to have an enhanced response to MEK and AKT inhibition.

Given the preclinical data showing that 1) RAF/MEK/ERK pathway as a mechanism of resistance to chemotherapy therapy in TNBC,[21, 22] 2) the enhanced effect of MEK inhibition in basal-like breast cancer models with intact PTEN (i.e. reduced PI3K/AKT pathway activity),[15] 3) the mechanism of resistance to MEK inhibition through the PI3K/AKT pathway,[15, 19, 20, 26] 4) the 30% incidence of PTEN loss in basal-like breast cancer[16-18] led to the formulation of two testable hypotheses: that single agent trametinib will demonstrate efficacy in TNBC and the anti-tumor response will depend on the PTEN status of the tumor. Moreover, the addition of AKT inhibitor GSK2141795 to trametinib will overcome intrinsic and acquired resistance to MEK inhibition.

To test these hypotheses, this is a phase II study of single agent trametinib in patients with advanced TNBC, with the addition of AKT inhibitor GSK2141795 to trametinib at time of tumor progression. This is the first trial to test the efficacy of single agent trametinib in this patient population and to determine if PTEN status will predict for response to therapy. Additionally, we will assess the ability of GSK2141795 to overcome MEK inhibitor resistance. Mechanisms of resistance to both single agent trametinib or in combination with GSK2141795 will be assessed via a comprehensive genomic and proteomic approaches on tissues collected after progression on either regimen..[27]

2.4. Correlative Studies Background

Detailed in Section 9

3. PATIENT SELECTION

3.1. Eligibility Criteria

3.1.1 Patients must have histologically or cytologically confirmed metastatic invasive breast cancer that is negative for the estrogen receptor (ER), progesterone receptor (PR) and HER2 by institutional guidelines.

3.1.2 Patients must have measurable disease (RECIST 1.1).

3.1.3 Patients must have had exposure to at least 1 and no more than 3 prior chemotherapy regimens for the treatment of metastatic breast cancer.

3.1.4 Patients must consent to both a pretreatment and a post-treatment mandatory research biopsy prior to enrolling on trial, and therefore, must have tissue (excluding bone or brain) that is amenable to biopsy.[27]

3.1.5 Age ≥18 years. Because no dosing or adverse event data are currently available on the use of trametinib monotherapy or in combination with GSK2141795 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 0-1 (Appendix A).

3.1.7 Life expectancy of greater than 3 months.

3.1.8 Able to swallow and retain orally-administered medication and does not have any clinically significant gastrointestinal abnormalities that may alter absorption such as malabsorption syndrome or major resection of the stomach or bowels.

3.1.9 All prior treatment-related toxicities must be CTCAE v5 grade ≤1 (except alopecia) at the time of enrollment.

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

- Absolute neutrophil count ≥1,500/mcL
- Platelets ≥75,000/mcL
- Total bilirubin ≤1.5 × institutional upper limit of normal
- AST(SGOT)/ALT(SGPT) ≤2.5× institutional upper limit of normal
- LVEF ≥ institutional lower limit of normal by ECHO or MUGA
- Serum creatinine ≤1.5 mg/dL *OR* calculated creatinine clearance (Cockroft-Gault formula) ≥50 mL/min *OR* 24-hour urine creatinine clearance ≥50 mL/min

3.1.11 Patients must have controlled blood pressure with a systolic blood pressure < 140 mmHg and diastolic < 90 mmHg. Anti-hypertensive medications are permitted.

3.1.12 Patients must be at least 4 weeks from last radiation dose. Patients must be at least 4 weeks from last chemotherapy, targeted therapy, or biologic therapy (exception allowed for a 2 week washout for patients who were on chemotherapy at less than a standard of care dose, as long as all other eligibility criteria are met). Patients must be at least 4 weeks from last surgical procedure and recovered from all post-operative complications.

3.1.13 The effects of trametinib monotherapy or trametinib in combination with GSK2141795 on the developing human fetus are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and 4 months after completion of trametinib monotherapy or in combination with GSK2141795 administration.

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

3.2. Exclusion Criteria

3.2.1 History of another malignancy.

Exception: Patients who have been disease-free for 3 years, or patients with a history of completely resected non-melanoma skin cancer and/or patients with indolent secondary malignancies, are eligible. Consult the CTEP Medical Monitor if unsure whether second malignancies meet the requirements specified above.

3.2.2 History of interstitial lung disease or pneumonitis.

3.2.3 History of Type I diabetes mellitus. If a patient has Type II diabetes, they must have a Hemoglobin A1C ≤ 8%. Patients with a screening fasting glucose > 120 mg/dL will be excluded.

3.2.4 Uncontrolled hypothyroidism. Patients must have a normal TSH per institutional standards at baseline.

3.2.5 Patients who are receiving any other investigational agents.

3.2.6 Individuals with symptomatic or progressive brain metastases are ineligible. Subjects with treated brain metastases are eligible if they have no radiographic or other signs of progression in the brain for ≥ 3 weeks after completion of local therapy. Any corticosteroid use for brain metastases must have been discontinued without the subsequent appearance of symptoms for ≥ 3 weeks prior to study enrollment.

3.2.7 History of allergic reactions attributed to compounds of similar chemical or biologic composition to trametinib monotherapy or trametinib in combination with GSK2141795.

3.2.8 Current use of a prohibited medication. The following medications or non-drug therapies are prohibited:

- Other anti-cancer therapy while on study treatment (megestrol if used as an appetite stimulant is allowed).
- 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], gingko biloba, yohimbe, saw palmetto, or ginseng).
- Patients receiving strong inhibitors or inducers of CYP3A4 (See section 5.2) are ineligible.
  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. As part of the enrollment/informed consent procedures, the patient should 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.

3.2.9 History or current evidence/risk of retinal vein occlusion (RVO) or central serous retinopathy (CSR) or predisposing factors to RVO or CSR (*e.g.*, uncontrolled glaucoma or ocular hypertension, uncontrolled hypertension, history of hyperviscosity or hypercoagulability syndromes. Visible retinal pathology as assessed by ophthalmic exam that is considered a risk factor for RVO or CSR such as evidence of new optic disc cupping, evidence of new visual field defects, and intraocular pressure >21 mm Hg.

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

3.2.11 Pregnant women are excluded from this study because trametinib monotherapy or trametinib in combination with GSK2141795 has potential for 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 trametinib monotherapy or trametinib in combination with GSK2141795, breastfeeding should be discontinued if the mother is treated with trametinib monotherapy or trametinib in combination with GSK2141795.

3.2.12 HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with trametinib monotherapy or trametinib in combination with GSK2141795. 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.

3.3. Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial. Because of the relative rarity of male breast cancer, we expect the majority of participants to be women. It is expected that the mix of patients entering this study will reflect the demographics of our clinical population and geographic area. We expect that participants will be racial/ethnic minorities which reflects the catchment area.

4. REGISTRATION PROCEDURES

4.1. General Guidelines

OSU patients will be registered by the OSU research coordinator, as per their standard practice.

Subsite patients will have eligibility verified and will be entered on study centrally at the *The Ohio State University* by the Subsite Coordinator, Jennifer Sexton. All sites should call the Subsite Coordinator at 614-366-5642 to verify enrollment availability. The required forms,

Enrollment Form and Eligibility Screening Worksheet can be found in the Supplemental Forms Document.

Following registration, patients should begin protocol treatment within 5 days. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Subsite Coordinator should be notified of cancellations as soon as possible.

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

4.2. Registration Process

To register a patient, the following documents should be completed by the research nurse or data manager and faxed *(614-366-4721)* or securely e-mailed *(jennifer.sexton@osumc.edu)* to the Subsite Coordinator:

- Copy of all source documents used to verify eligibility
- Signed patient consent form
- Signed HIPAA authorization form
- Eligibility Screening Worksheet, Enrollment Form)

The research nurse or data manager at the participating site will then call *(614-366-5642)* or e-mail (*jennifer.sexton@osumc.edu)* the Stubsite Coordinator to verify receipt of registration request. To complete the registration process, the Coordinator will

- assign a patient study number
- register the patient on the study
- assign the patient a dose
- fax or e-mail the patient study number and dose to the participating site
- call the research nurse or data manager at the participating site and verbally confirm registration.

5. TREATMENT PLAN

5.1. Research Biopsies

Detailed in Section 9

5.2. Agent Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. Details regarding administration of study drugs are detailed in Section 8. 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 will be returned to clinic staff at the end of each course see Appendix C.

5.2.1 Trametinib

Treatment in Part 1 will consist of trametinib at a dose of 2 mg for a 28 day cycle. If a participant had a dose reduction of trametinib during Part 1, the participant will continue on this dose at the commence of Part 2 of the study. If no dose reductions occurred, the patient will start on trametinib at the recommended dose of 1.5 mg oral daily for Part 2. During Part 2 of the study, the participant will be administered trametinib in combination with GSK2141795 per dosing recommendations below for a 28 day cycle.

5.2.2 Trametinib in combination with GSK2141795

. GSK2141795 will not be administered during Part 1. Treatment in Part 2 will consist on GSK2141795 at 50 mg daily in combination with trametinib 1.5 mg daily for a 28 day cycle.

The Trametinib can be taken by mouth on an empty stomach, either 1 hour before or 2 hours after a meal. The GSK2141795 must be taken 1 hour after a meal and two hours before the next meal. It is recommended that the Trametinib be taken in the morning and GSK 2141795 in the evening, however this is not required. The medications will be taken once per day, at the same time each day. If a dose of the medication is missed, it should be taken as soon as it is remembered that day up to 6 hours past the scheduled time. If more than 6 hours has passed since the scheduled time, the missed dose should not be taken.

5.3. General Concomitant Medication and Supportive Care Guidelines

- Nausea/Vomiting – prophylactic pre-medications are not planned as trametinib alone or in combination with GSK2141795 is not highly emetogenic. However, if patients experience nausea during a treatment cycle, anti-emetics may be used per institutional guidelines.

- Cytopenias – prophylactic growth factors (e.g. G-CSF, erythropoietin) are not permitted, however, if patients require admission for fever and neutropenia and the treating physician feels that the administration of growth factors may be beneficial then they can be given according to ASCO guidelines. Use of recombinant erythropoietin is not allowed. Platelets and red cell transfusions can be given as necessitated.

- Short courses (up to a maximum of 14 days) of oral corticosteroids intended to treat study treatment related rash or diarrhea are allowed. Loperamide or lomotil is recommended for supportive care of diarrhea. Oral steroids should be used with caution and subjects monitored for steroid-induced hyperglycemia.

- The use of bisphosphonates for skeletal metastasis is permitted.

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

5.4. 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.

5.5. Duration of Follow Up

Patients will be followed for 52 weeks after removal from study or until death, whichever occurs first. Patients removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

5.6. Criteria for Removal from Study

Patients will be removed from study when any of the criteria listed in Section 5.4 applies. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

6. DOSING DELAYS/DOSE MODIFICATIONS

6.1. Part 1 Dose Modifications for Trametinib Monotherapy

The table below outlines the dose levels to be used for any necessary trametinib dose modifications as a single agent:

Dose Level	Trametinib Dose/Schedule
0	2 mg daily
-1	1.5 mg daily
-2	1 mg daily

A maximum of two trametinib dose level reductions are allowed. If a third dose level reduction is required, treatment will be permanently discontinued.

6.1.1 Trametinib Dose Modification for Toxicities Not Specified in Subsequent Sections

Trametinib Treatment Modification for Clinically Significant Toxicities Deemed Related to Trametinib (This section is not for specific AEs such as hypertension, rash, ejection fraction changes, pneumonitis, diarrhea, liver chemistry, QTc prolongation, or visual changes. Refer to other sections for these specific AEs).		
CTCAE v5 Grade	**Management Guideline**	**Dose Modification**
Grade 1	Monitor as clinically indicated. Provide supportive care according to institutional standards	Continue trametinib at current dose level.
Grade 2		• Interrupt treatment until resolution to grade 1 or baseline. • Upon resolution, restart treatment at current dose level.
Grade 3		• Interrupt treatment until resolution to grade 1 or baseline. • Upon resolution to baseline or grade 1, restart with one level of dose reduction • If the Grade 3 toxicity recurs, interrupt trametinib; When toxicity resolves to Grade 1 or baseline, restart trametinib **reduced by another dose level**
Grade 4		Permanently discontinue trametinib.
Trametinib should be discontinued if treatment delay is ≥21 days due to toxicities. If the investigator concludes that continued trametinib will benefit a patient, the study chair and CTEP Medical Monitor may be consulted for the possibility of resuming trametinib, provided that toxicities have resolved to baseline or grade 1.		

6.1.2 Trametinib Dose Modification for Diarrhea

Episodes of diarrhea have occurred in patients receiving trametinib (Investigator's Brochure, 2012a). Other frequent causes of diarrhea including concomitant medications (*e.g.*, stool softeners, laxatives, antacids, *etc.*), infections by *C. difficile* or other pathogens, or partial bowel obstruction should be excluded.

Guidelines regarding management and dose modification for diarrhea considered related to trametinib are provided in the table below.

Management and Trametinib Dose Modification Guidelines for Diarrhea		
CTCAE Grade	**Adverse Event Management**	**Action and Dose Modification**
Uncomplicated Diarrhea,[1] Grade 1 or 2	• Diet: Stop all lactose containing products; eat small meals, BRAT-diet (banana, rice, apples, toast) recommended. • 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 trametinib. • If diarrhea is grade 2 for > 48 h, interrupt trametinib until diarrhea resolves to grade ≤1. • Restart trametinib at the same dose level • If treatment delay is > 21 days, discontinue trametinib.

Management and Trametinib Dose Modification Guidelines for Diarrhea		
CTCAE Grade	**Adverse Event Management**	**Action and Dose Modification**
Uncomplicated Diarrhea,[1] Grade 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 trametinib until diarrhea resolves to ≤ grade 1. • Restart with trametinib reduced by one dose level.[4] • If 3 dose reductions of study treatment are clinically indicated, **permanently discontinue trametinib**. • If treatment delay is >21 days, discontinue trametinib.
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 trametinib 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.		

6.1.3 Trametinib Dose Modification for Rash

Rash is a frequent AE observed in patients receiving trametinib. Recommendations for supportive care and guidelines for dose modifications for rash are based on experience with other MEK inhibitors and EGFR inhibitors.[28]

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

Guidelines for Supportive Care of Rash	
Type of Care	**Action**
Prevention/Prophylaxis[a]	• Avoid unnecessary exposure to sunlight. • Apply broad-spectrum sunscreen (containing titanium dioxide or zinc oxide) with a skin protection factor (SPF) ≥15 at least twice daily. • Use thick, alcohol-free emollient cream (*e.g.*, glycerine and cetomacrogol cream) on dry areas of the body at least twice daily. • Topical steroids and antibiotics should be applied at least twice daily, starting on Day 1 of study treatment, to body areas such as face, chest, and upper back. • Use mild-strength topical steroid (hydrocortisone 1% cream) or topical antibiotic (*e.g.*, clindamycin) or oral antibiotics (*e.g.*, doxycycline 100 mg BID, minocycline 100 mg BID).
Symptomatic Care[b]	• Pruritic lesions: Cool compresses and oral antihistamine 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 antibiotics; if no improvement, consult dermatologist or surgeon. • Infected lesions: Appropriate bacterial/fungal culture-driven systemic or topical antibiotics.
[a] Rash prophylaxis is recommended for the first 6 weeks of study treatment. [b] Patients who develop rash/skin toxicities should be seen by a qualified physician and should receive evaluation for symptomatic/supportive care management.	

Trametinib Dose Modification Guidelines and Management for Rash		
Rash Severity	**Management Guideline**	**Dose Modification**
Grade 1	• Initiate prophylactic and symptomatic treatment measures.[1] • Use moderate strength topical steroid.[2] • Reassess after 2 weeks.	• Continue trametinib. • If rash does not recover to baseline within 2 weeks despite best supportive care, **reduce trametinib by one dose level**.[3]
Grade 2	• Initiate prophylactic and symptomatic treatment measures.[1] • Use moderate strength topical steroid.[2] • Reassess after 2 weeks.	• **Reduce trametinib 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 trametinib until recovery to ≤ grade 1 then restart trametinib at reduced dose level.[3]
Grade ≥3	• Use moderate strength topical steroids PLUS oral methyl-prednisolone dose pack.[2] • Consider consulting a dermatologist.	• Interrupt trametinib until rash recovers to ≤ grade 1. • **Restart with trametinib reduced by one dose level.**[3,4] • If no recovery to ≤ grade 2 within 4 weeks, **permanently discontinue trametinib**.
1. Rash prophylaxis is recommended for the first 6 weeks of study treatment. 2. Moderate-strength topical steroids: Hydrocortisone 2.5% cream or fluticasone priopionate 0.5% cream. 3. Approval of CTEP Medical Monitor is required to restart study treatment after >4 weeks of interruption. 4. Trametinib may be escalated to previous dose level if no rash is evident 4 weeks after restarting study treatment.		

6.1.4 Trametinib Dose Modifications for Visual Changes

Episodes of visual changes have been observed in patients receiving trametinib, and can be caused by CSR or RVO. Patients are required to have a standard ophthalmic exam performed by an ophthalmologist at baseline and any time patients report visual disturbance. The exam will include indirect fundoscopic examination, visual acuity (corrected), visual field examination, tonometry, and direct fundoscopy with color photos. Special attention should be given to retinal (*e.g.*, CSR) or retinal vein abnormalities (*e.g.*, RVO).
Guidelines regarding event management and dose reduction for visual changes considered to be related to study treatment are provided in the table below.

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

Management and Trametinib Dose Modification for Visual Changes		
Event **CTCAE Grade**	**Management Guideline**	**Dose Modification**
Any grade of CRS or RVO should be reported as SAE to CTEP-AERS		
Grade 2 and 3 Grade 2defined as: Symptomatic with moderate decrease in visual acuity (20/40 or better; limiting instrumental ADL; local or non-invasive intervention indicated. Grade 3 defined as: Symptomatic with marked decrease in visual acuity or marked visual field defect (worse than 20/40 but better than 20/200); severe pain or medically significant; operative intervention indicated.	specialist if available) for CSR and RVO. •	• Interrupt trametinib until signs and symptoms have resolved to baseline. • If CSR and RVO excluded and symptoms resolved to baseline, restart trametinib reduced by one dose level. • <u>If CSR</u>: Interrupt trametinib until symptoms resolve and exam (by retinal specialist if available) shows resolution**.** If CSR resolves restart trametinib **reduced by one dose level**.** • <u>If RVO</u>: **Permanently discontinue study treatment**.
Grade 4 Sight-threatening consequences; urgent intervention indicated; blindness (20/200 or worse).		**Permanently discontinue trametinib.**
Abbreviations: CSR = central serous retinopathy; 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. ** If ocular toxicities do not resolve within 21 days, permanently discontinue trametinib.		

6.1.5 Trametinib Dose Modification for <u>Liver Chemistry Changes</u>

Trametinib Dose Modification for Liver Function Test Abnormalities
Liver chemistry stopping criteria are defined as follows. When any of the liver chemistry stopping criteria are met, immediately discontinue trametinib, perform liver event follow-up assessments, and monitor the patient until liver chemistries resolve, stabilize, or return to baseline values. • ALT ≥3x ULN **and** bilirubin ≥2x ULN (>35% direct bilirubin) (or ALT ≥3x ULN and international normalized ratio [INR] >1.5, if INR measured). **<u>NOTE</u>:** If serum bilirubin fractionation is not immediately available, trametinib should be discontinued if ALT ≥3x ULN **and** bilirubin ≥2x ULN. Serum bilirubin fractionation should be performed if testing is available. If testing is unavailable, **record presence of detectable urinary bilirubin on dipstick**, indicating direct bilirubin elevations and suggesting liver injury. • ALT ≥5x ULN. • ALT ≥3x ULN if associated with the appearance or worsening of symptoms of hepatitis or hypersensitivity such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, or eosinophilia. • ALT ≥3x ULN persisting for ≥4 weeks. • ALT ≥3x ULN and cannot be monitored weekly for 4 weeks.

6.1.6 Trametinib Dose Modification for Pneumonitis

Pneumonitis has been observed in patients receiving trametinib. To reduce the risk of pneumonitis, patients will be monitored closely for symptoms and evaluated with imaging and functional tests. Dose modification and supportive care guidelines for pneumonitis are described in the tables below.

Pneumonitis Guidelines for Trametinib Monotherapy		
CTCAE Grade	**Adverse Event Management**	**Action and Dose Modification**
Grade 1	• CT scan (high-resolution with lung windows) recommended. • Work-up for infection. • Monitoring of oxygenation via pulse-oximetry recommended. • Consultation with pulmonologist recommended.	Continue trametinib at current dose.
Grade 2	• CT scan (high-resolution with lung windows). • 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. • If AE resolved to grade ≤1 and relationship to trametinib is equivocal, restarting trametinib with one dose reduction may be considered, after discussion with the medical monitor. • If treatment delay is > 4 weeks, **permanently discontinue trametinib**.
Grade 3	• CT scan (high-resolution with lung windows). • Work-up for infection. • Consult pulmonologist. • Pulmonary function tests-if < normal, repeat every 8 weeks until ≥ normal. • Bronchoscopy with biopsy and/or BAL if possible. • Symptomatic therapy including corticosteroids as clinically indicated.	• Interrupt trametinib until recovery to grade ≤1. • If AE resolved to grade ≤1 and relationship to trametinib is equivocal, restarting trametinib with one dose reduction may be considered, after discussion with the medical monitor. • If treatment delay is >4 weeks, **permanently discontinue trametinib**.
Grade 4	Same as grade 3.	**Permanently discontinue trametinib.**
Abbreviations: BAL = bronchoalveolar lavage; CT = computed tomography.		

6.1.7 Trametinib Dose Modification for Reduced LVEF

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

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

6.1.8 Trametinib Dose Modification for QTc Prolongation

Trametinib Withholding and Stopping Criteria for QTc Prolongation	
Prolongation*	**Action and Dose Modification**
• QTcB ≥501 msec, or • Uncorrected QT >600 msec, or • QTcB >530 msec for subjects with bundle branch block	• Interrupt study treatment until QTcB prolongation resolves to grade 1 or baseline. • If the QTc prolongation resolves to grade 1 or baseline, trametinib may be resumed if the investigator and CTEP medical monitor agree that the subject will benefit from further treatment. • **If the event does not resolve, permanently discontinue study treatment.** • **If the event recurs, permanently discontinue study treatment.**

Trametinib Withholding and Stopping Criteria for QTc Prolongation	
Prolongation*	**Action and Dose Modification**
Abbreviations: msec = milliseconds; QTcB = QT interval on electrocardiogram corrected using the bazett's formula * 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.	

6.1.9 Trametinib Dose Modification for Hypertension

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

In subjects with an initial BP reading within the hypertensive range, a second reading should be taken at least 1 minute later, with the two readings averaged to obtain a final BP measurement. The averaged value should be recorded in the eCRF.

Management and Trametinib Dose Modification for Hypertension		
Event	**Management Guideline**	**Dose Modification**
Definitions used in the table: - Persistent hypertension: Hypertension detected in two separate readings during up to three subsequent visits. - Well-controlled hypertension: Blood pressure of SBP ≤140 mmHg and DBP ≤90 mmHg in two separate readings during up to three subsequent visits. - Symptomatic hypertension: Hypertension associated with symptoms (*e.g.*, headache, light-headedness, vertigo, tinnitus, episodes of fainting) that resolve after the blood pressure is controlled within the normal range. - Asymptomatic hypertension: SBP >140 mmHg and/or DBP >90 mmHg in the absence of the above symptoms.		
(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 BP. If BP is not well-controlled within 2 weeks, consider referral to a specialist and go to scenario (B).	Continue trametinib at the current dose.

Management and Trametinib Dose Modification for Hypertension		
Event	**Management Guideline**	**Dose Modification**
(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 trametinib. • Once BP is well-controlled, restart trametinib at a reduced dose.
(Scenario C) • Symptomatic[c] 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 trametinib. • Once BP is well-controlled, restart trametinib at a reduced dose.
(Scenario D) Refractory hypertension unresponsive to above interventions or hypertensive crisis.	Continue follow-up per protocol.	Discontinue trametinib.
[a] Trametinib should be discontinued if treatment delay is >21 days due to toxicities. If the investigator concludes that continued trametinib will benefit a patient, the study chair and CTEP sponsor may be consulted for the possibility of resuming trametinib, provided that toxicities have resolved to baseline or grade 1.		

6.2. Dose Modifications for Part 2 Trametinib Combined with GSK 2141795

If a participant had a dose reduction of trametinib during Part 1, the participant will continue on this dose at the commence of Part 2 of the study. If no dose reductions occurred, the patient will start on trametinib at the recommended dose of 1.5 mg oral daily for Part 2. GSK2141795 will be added at time of entry into Part 2 to trametinib, beginning at dose level 0.

Dose Level	Trametinib Dose/Schedule
0	1.5 mg daily
-1	1 mg daily

Dose Level	GSK2141795 Dose/Schedule
0	50 mg daily
-1	25 mg daily

NOTE: AEs occurring in patients treated with GSK2141795 and trametinib may be related to 1) overlapping toxicities between the two agents (*e.g.*, rash and diarrhea); 2) toxicities typically associated with trametinib (*e.g.*, visual disturbance) or GSK2141795 (*e.g.*, hyperglycemia or hypoglycemia). However, toxicities associated with individual agents may be potentiated in the combination, or unanticipated AEs may occur.

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

monitors should be consulted if there are questions about the attribution of AEs and how the doses should be modified.

6.2.1 Trametinib and GSK2141795 Dose Modification for Toxicities Not Specified in Subsequent Sections

Toxicity Grade[a]	Dose Modification
Grade 1	Continue at current dose level. Consider supportive care recommendations.
Grade 2	Consider withholding dose of trametinib and/or GSK2141795 until toxicity resolves to grade 1 or baseline. Upon resolution, then restart at current dose level. Consider supportive care recommendations.
Grade 3	Withhold dose until toxicity resolves to grade 1 or baseline. Upon resolution, resume at the next lower dose level of both trametinib and/or GSK2141795. Consider supportive care recommendations.
Grade 4	Permanently discontinue trametinib and GSK2141795.
a: the guidelines are for AEs thought to be related to GSK2141795 and/or trametinib. However, temporary interruption of the agent should be considered if the patient is critically ill due to AEs of any attribution	

6.2.2 Trametinib and GSK2141795 Dose Modification for Diarrhea

Episodes of diarrhea have occurred in patients receiving trametinib or GSK2141795 (Investigator's Brochure, 2012a). Other frequent causes of diarrhea including concomitant medications (*e.g.*, stool softeners, laxatives, antacids, *etc.*), infections by *Clostridium difficile* or

other pathogens, or partial bowel obstruction should be excluded.

Management and Dose Modification Guidelines for Diarrhea		
CTCAE Grade	**Adverse Event Management**	**Action and Dose Modification**
Uncomplicated Diarrhea,[1] Grade 1 or 2	• Diet: Stop all lactose containing products; eat small meals, BRAT-diet (bananas, 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 hours: Loperamide 2 mg every 2 hours; maximum 16 mg/day. Add budesonide or other second-line therapies (octreotide, or tincture of opium) and oral antibiotics.	• Continue treatment. • If diarrhea is grade 2 for >48 h, interrupt GSK2141795 and trametinib until diarrhea resolves to grade ≤1. • Restart treatment at the same dose level. • If treatment delay is > 21 days, discontinue both agents. (Resumption of trametinib or GSK2141795 alone may be considered based on toxicity-benefit consideration and after consultation with CTEP).

Management and Dose Modification Guidelines for Diarrhea		
CTCAE Grade	**Adverse Event Management**	**Action and Dose Modification**
Uncomplicated Diarrhea,[1] Grade 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 protocol therapy until diarrhea resolves to ≤ grade 1. • Restart with trametinib or trametinib and GSK2141795 reduced by one dose level (for the combination, reduce both agents by one level).[4] • If more than one dose reduction of study treatment is clinically indicated, **permanently discontinue treatment**. • If treatment delay is >21 days, discontinue treatment. (resumption of trametinib or GSK2141795 alone may be considered based on toxicity-benefit consideration and after consultation with CTEP).
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 trametinib or trametinib and GSK2141795 to previous dose level(s) is allowed after consultation with the CTEP monitor and Study Chair and in the absence of another episode of complicated or severe diarrhea in the 4 weeks subsequent to dose reduction.		

6.2.3 Trametinib and GSK2141795 Dose Modification for Rash

Two types of rashes may be seen with the trametinib and GSK2141795 combination:

1. Acneiform rash, typically associated with MEK inhibitor therapy (trametinib).
2. Maculopapular rash, often associated with pruritus (GSK2141795).

If the diagnosis is unclear, a biopsy and photographs should be obtained as well as a dermatology consult. In addition, if the investigator feels the rash is not consistent with a MEK inhibitor-associated acneiform rash and is ≥ Grade 2, a skin punch biopsy should be performed.

In general, topical and oral antibiotics (doxycycline or minocycline) play a larger role in management of the MEK inhibitor acneiform rash, while topical and oral steroids are more relevant to the management of the AKT inhibitor maculopapular rash.

The institutional standards for the management of skin-related AEs can differ from these

guidelines. In this case, best clinical judgment should be applied and a consultation with the study chair or the CTEP Medical Monitor may be required.

Dose Modification Guidelines and Management for Rash		
Rash Severity	**Management Guideline**	**Dose Modification**
Grade 1	• Initiate prophylactic and symptomatic treatment measures.[1] • Use moderate strength topical steroid.[2] • Reassess after 2 weeks.	• Continue treatment. • If rash does not recover to baseline within 2 weeks despite best supportive care, **reduce trametinib or both agents by one dose level**.[3]
Grade 2	• Initiate prophylactic and symptomatic treatment measures.[1] • Use moderate strength topical steroid.[2] • Reassess after 2 weeks.	• Continue treatment • **Reduce trametinib or both agents by one dose level.** • If rash recovers to ≤ grade 1 within 2 weeks, increase dose(s) to previous dose level. • If no recovery to ≤ grade 1 within 2 weeks, interrupt treatment until recovery to ≤ grade 1. • **Restart trametinib or both agents at reduced dose level.**
Grade ≥3	• Use moderate strength topical steroids PLUS oral methyl-prednisolone dose pack.[2] • Consult dermatologist.	• Interrupt trametinib or both agents until rash recovers to ≤ grade 1. • **Restart with trametinib or both agents with one dose level reduction**[3,] • If no recovery to ≤ grade 2 within 3 weeks, **permanently discontinue both agents** (resumption of trametinib or GSK2141795 alone may be considered based on toxicity-benefit consideration and after consultation with CTEP).
1. Rash prophylaxis is recommended for the first 6 weeks of study treatment (refer to guidelines for trametinib). 2. Moderate-strength topical steroids: Hydrocortisone 2.5% cream or fluticasone propionate 0.5% cream. 3. Trametinib or GSK2141795 may be escalated to previous dose level if no rash is evident 4 weeks after restarting study treatment.		

6.2.4 Trametinib and GSK2141795 Dose Modifications for Liver Chemistry Changes

Dose Modification for Liver Function Test Abnormalities
Liver chemistry stopping criteria are defined as follows. When any of the liver chemistry stopping criteria are met, immediately discontinue trametinib and GSK2141795, perform liver event follow-up assessments, and monitor the patient until liver chemistries resolve, stabilize, or return to baseline values. • ALT ≥3x ULN **and** bilirubin ≥2x ULN (>35% direct bilirubin) (or ALT ≥3x ULN and international normalized ratio [INR] >1.5x ULN, if INR measured). **NOTE:** If serum bilirubin fractionation is not immediately available, treatment should be discontinued if ALT ≥3x ULN **and** bilirubin ≥2x ULN. Serum bilirubin fractionation should be performed if testing is available. If testing is unavailable, **record presence of detectable urinary bilirubin on dipstick**, indicating direct bilirubin elevations and suggesting liver injury. • ALT ≥5x ULN. • ALT ≥3x ULN if associated with the appearance or worsening of symptoms of hepatitis or hypersensitivity such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, or eosinophilia. • ALT ≥3x ULN persisting for ≥4 weeks. • ALT ≥3x ULN and cannot be monitored weekly for 4 weeks.

6.2.5 Trametinib and GSK2141795 Dose Modification for Hypertension, Pneumonitis, and Visual Disturbance

- These AEs are typically associated with trametinib. Please follow guidelines for trametinib.
- GSK2141795 may continue when trametinib is on hold if AEs are ≤ grade 2.
- If the above AEs are grade 3-4, GSK2141795 should be held when trametinib is held. Once the AEs have resolved to grade 1 or baseline, GSK2141795 may resume at the same dose.
- If GSK2141795795 has been held for >21 days, a discussion with the CTEP drug monitor is required before resuming treatment with the agent.

6.2.6 Trametinib and GSK2141795 Dose Modifications for Reduced LVEF

Decreases of the left ventricular ejection fraction (LVEF) have been observed in patients receiving trametinib. GSK2141795 dose is to be modified the same as for trametinib. ECHOs must be performed in regular intervals outlined in the Study Calendar. The same procedure (either ECHO or MUGA, although ECHO is preferred) should be performed at baseline and at follow-up visit(s).

Dose Modification Guidelines and Stopping Criteria for LVEF Decrease		
Clinic	**LVEF-drop (%) or CTCAE grade**	**Action and Dose Modification**
Asymptomatic	Absolute decrease of >10% in LVEF compared to baseline and ejection fraction below the institution's LLN.	• Follow the instructions for trametinib. When trametinib is on hold, GSK2141795 should be on hold.

Dose Modification Guidelines and Stopping Criteria for LVEF Decrease		
Clinic	**LVEF-drop (%) or CTCAE grade**	**Action and Dose Modification**
Symptomatic [b]	• Grade 3: resting LVEF 39% or >20% absolute reduction from baseline • Grade 4: Resting LVEF ≤20%.	• **Permanently discontinue trametinib and GSK2141795** • **Report as SAE.** • **Consult with cardiologist.** • Repeat ECHO after 2, 4, 8, 12, and 16 weeks or until resolution.
If ECHO does not show LVEF recovery after 2 weeks, repeat ECHO 2 weeks later. Symptoms may include: dyspnea, orthopnea, and other signs and symptoms of pulmonary congestion and edema.		

6.2.7 Trametinib and GSK2141795 Dose Modification for QTc Prolongation

GSK2141795 is not well known to cause prolongation of the QTc. Therefore, for QTc prolongation, participants should undergo dose modifications of trametinib as listed for Part 1 in **Section 6.1.8** and GSK2141795 should be continued at the same dose. GSK2141795 may be continued as monotherapy if trametinib must be permanently discontinued if otherwise tolerated.

6.2.8 Trametinib and GSK2141795 Dose Modification for Hypertension

GSK2141795 is not well known to cause hypertension. Therefore, for hypertension, participants should undergo dose modifications of trametinib as listed for Part 1 in **Section 6.1.9** and GSK2141795 should be continued at the same dose or held and resumed as detailed in **Section 6.2.5** depending on the grade of the hypertension. GSK2141795 may be continued as monotherapy if trametinib must be permanently discontinued if otherwise tolerated.

6.2.9 Trametinib and GSK2141795 Dose Modifications for Hypo- or Hyperglycemia

GSK2141795 may cause hypo- or hyperglycemia. Trametinib is not known to cause hypo- or hyperglycemia. Subjects will be required to monitor their blood glucose each morning preprandial during Part 2 of the study while receiving GSK2141795and record the results in the provided diary (Appendix F). Subjects should contact their study doctor for readings ≤ 70 or ≥ 250 for physician instructions and management.

Management and Dose Modification Guidelines for Hypo- or Hyperglycemia		
Criteria	**Management Guidelines**	**Study Drug Modification**
(For management purposes, refer to mild, moderate and severe intensity criteria; however for eCRF reporting use NCI-CTCAE version 5.0 Grades 1-5)		
Mild Fasting blood glucose > 150mg/dL	Monitor fasting and preprandial glucose.	**Continue study drug**
Moderate to Severe Fasting blood glucose <70 mg/dL OR any blood glucose > 250mg/dL	• If a blood glucose >250 mg/dL, monitor for ketoacidosis as clinically indicated. • When managing hyperglycemia associated with GSK2141795, be aware that the action of insulin or other antihyperglycemic agents (*e.g.*, sulfonylureas, biguanides, *etc.*) may be substantially blocked by the study agent. However the action of antihyperglycemic agents would be restored as GSK2141795 is cleared. The patient should be observed closely for rebound hypoglycaemia as GSK2141795 is held/or discontinued. • Intravenous insulin treatment is recommended.	Hold drug(s) and notify investigator immediately. The investigator should discuss intervention and possible resumption of study drug(s) with the CTEP monitor.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

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

7.1. Comprehensive Adverse Events and Potential Risks List(s) (CAEPRs)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset of AEs, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with ***bold*** and ***italicized*** text. The SPEER is a list of events that are protocol-specific exceptions to expedited reporting to NCI via CTEP-AERS (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. Frequency provided for trametinib is based on 356 patients. Frequency provided for GSK2141795 is based on 150 patients.

NOTE: The highest grade currently reported is noted in parentheses next to the AE in the SPEER. Report **ONLY** AEs higher than this grade expeditiously viaCTEP-AERS. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

7.1.1 CAEPRs for trametinib and GSK 2141795

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

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

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

Version 2.4, October 7, 2016[1]

Adverse Events with Possible Relationship to Trametinib dimethyl sulfoxide (GSK1120212B) (CTCAE 4.0 Term) [n= 1111]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia (Gr 2)***
CARDIAC DISORDERS			
		Heart failure	
		Left ventricular systolic dysfunction	

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

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

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

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

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

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

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

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

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

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Disseminated intravascular coagulation; Febrile neutropenia; Leukocytosis
CARDIAC DISORDERS - Atrial fibrillation; Cardiac arrest; Myocardial infarction; Restrictive cardiomyopathy; Sinus tachycardia
EYE DISORDERS - Corneal ulcer; Eyelid function disorder; Flashing lights; Floaters; Glaucoma; Papilledema; Photophobia; Retinal detachment
GASTROINTESTINAL DISORDERS - Anal hemorrhage; Ascites; Duodenal ulcer; Enterocolitis; Esophageal necrosis; Esophageal ulcer; Esophagitis; Gastric hemorrhage; Gastric ulcer; Gastritis; Gastrointestinal disorders - Other (intestinal obstruction); Gastrointestinal disorders - Other (oropharyngeal pain); Gastrointestinal disorders - Other (pneumatosis intestinalis); Gastrointestinal fistula; Gingival pain; Hemorrhoidal hemorrhage; Ileus; Lower gastrointestinal hemorrhage; Obstruction gastric; Pancreatitis; Rectal hemorrhage; Small intestinal obstruction; Upper gastrointestinal hemorrhage
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Flu like symptoms; General disorders and administration site conditions - Other (axillary pain); Localized edema; Malaise; Non-cardiac chest pain; Pain
HEPATOBILIARY DISORDERS - Cholecystitis; Hepatic failure; Hepatic pain; Hepatobiliary disorders - Other (hepatic encephalopathy)
INFECTIONS AND INFESTATIONS - Biliary tract infection; Catheter related infection; Device related infection; Endocarditis infective; Enterocolitis infectious; Hepatitis viral; Infections and infestations - Other (abscess limb); Infections and infestations - Other (necrotizing fasciitis); Infections and infestations - Other (oral infection); Pharyngitis; Rash pustular; Sepsis; Upper respiratory infection; Urinary tract infection
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Bruising
INVESTIGATIONS - Blood bilirubin increased; Creatinine increased; Electrocardiogram QT corrected interval prolonged; GGT increased; Investigations - Other (blood lactate dehydrogenase increased); Lipase increased; Lymphocyte count decreased; Platelet count decreased; Serum amylase increased; White blood cell decreased
METABOLISM AND NUTRITION DISORDERS - Hyperglycemia; Hyperkalemia; Hyperuricemia; Hypocalcemia; Hypoglycemia; Hypokalemia; Metabolism and nutrition disorders - Other (hyperphosphatemia)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Generalized muscle weakness; Musculoskeletal and connective tissue disorder - Other (compression fracture); Musculoskeletal and connective tissue disorder - Other (muscle spasm); Myalgia; Neck pain
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (tumor hemorrhage); Tumor pain
NERVOUS SYSTEM DISORDERS - Dysgeusia; Encephalopathy; Intracranial hemorrhage; Lethargy; Nervous system disorders - Other (diplopia); Seizure; Somnolence; Stroke; Syncope; Transient ischemic attacks
PSYCHIATRIC DISORDERS - Anxiety; Confusion; Delirium; Depression; Hallucinations; Insomnia; Personality change
RENAL AND URINARY DISORDERS - Acute kidney injury; Cystitis noninfective; Hematuria; Proteinuria; Renal and urinary disorders - Other (dysuria); Urinary incontinence
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Vaginal fistula; Vaginal hemorrhage
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Bronchopulmonary hemorrhage; Epistaxis; Hypoxia; Laryngeal edema; Pleural effusion; Pneumothorax; Productive cough; Pulmonary hypertension; Respiratory failure; Sinus disorder
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Bullous dermatitis; Photosensitivity; Purpura; Skin and subcutaneous tissue disorders - Other (erythema nodosum); Skin and subcutaneous tissue disorders - Other (nail

disorder); Skin and subcutaneous tissue disorders - Other (skin fissures); Skin ulceration; Urticaria
VASCULAR DISORDERS - Hematoma; Hot flashes; Hypotension; Thromboembolic event (venous)

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

7.1.1.2. CAEPR for GSK2141795

Comprehensive Adverse Events and Potential Risks list (CAEPR)
for
GSK2141795 (NSC 767034)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI via CTEP-AERS (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 150 patients.* Below is the CAEPR for GSK2141795.

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

Version 2.1, July 26, 2013[1]

Adverse Events with Possible Relationship to GSK2141795 (CTCAE 4.0 Term) [n= 150]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
GASTROINTESTINAL DISORDERS			
Diarrhea			***Diarrhea (Gr 2)***
	Esophagitis		
	Gastrointestinal mucositis[2]		
Nausea			***Nausea (Gr 2)***
Vomiting			***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Fatigue			***Fatigue (Gr 2)***
METABOLISM AND NUTRITION DISORDERS			
Anorexia			***Anorexia (Gr 2)***
	Hyperglycemia		***Hyperglycemia (Gr 2)***
	Hypoglycemia		
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Respiratory mucositis[3]		
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Rash maculo-papular		

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

[2]Gastrointestinal mucositis may include Anal mucositis, Mucositis oral, Rectal mucositis, or Small intestinal mucositis under the GASTROINTESTINAL DISORDERS SOC.

[3]Respiratory mucositis may include Laryngeal mucositis, Pharyngeal mucositis, or Tracheal mucositis under the RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS SOC

Also reported on GSK2141795 trials but with the relationship to GSK2141795 still undetermined:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Leukocytosis
CARDIAC DISORDERS - Cardiac arrest, Left ventricular systolic dysfunction, Ventricular tachycardia
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Non-cardiac chest pain
HEPATOBILIARY DISORDERS - Hepatic failure
INFECTIONS AND INFESTATIONS - Wound infection
INVESTIGATIONS - Alanine aminotransferase increased; Alkaline phosphatase increased; Aspartate aminotransferase increased; Ejection fraction decreased; GGT increased
METABOLISM AND NUTRITION DISORDERS - Hypokalemia; Hyponatremia, Hypophosphatemia
NERVOUS SYSTEM DISORDERS - Dysgeusia; Dysphasia
RENAL AND URINARY DISORDERS - Acute kidney injury
VASCULAR DISORDERS - Thromboembolic event

Note: GSK2141795 in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.2. Adverse Event Characteristics

-
- CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized until March 31, 2018 for AE reporting. CTCAE version 5.0 will be utilized for AE reporting beginning April 1, 2018. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.
- .**For expedited reporting purposes only:**
  - AEs for the agent that are ***bold and italicized*** in the CAEPR (*i.e.*, those listed in the SPEER column, **Section 7.1.1**) should be reported through CTEP-AERS only if the grade is above the grade provided in the SPEER.
  - Other AEs for the protocol that do not require expedited reporting are outlined in section 7.3.4.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.

- Unlikely – The AE *is doubtfully related* to the study treatment.
- Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3. Expedited Adverse Event Reporting

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

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

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

7.3.3 Expedited Reporting Guidelines

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

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

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

Submitted CTEP-AERS reports must be forwarded to the Subsite Coordinator via fax or secure email.

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

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

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

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

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

Expedited AE reporting timelines are defined as:

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

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 3, 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

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

Effective Date: May 5, 2011

7.3.4 Additional Protocol – Specific Expeditied Adverse Event Reporting Exclusions

N/A

7.4. Routine Adverse Event Reporting

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

7.5. Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.
CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported viaCTEP-AERS. Three options are available to describe the event:

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

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

7.6. Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine reporting via CDUS unless otherwise specified.

8. PHARMACEUTICAL AGENT INFORMATION

A list of the adverse events and potential risks associated with the investigational agents, trametinib and GSK2141795 administered in this study can be found in Section 7.1.

8.1. CTEP Agent(s)

8.1.1 Trametinib dimethyl sulfoxide (GSK1120212B) (NSC 763093)

Chemical Name (IUPAC): equimolecular combination of acetamide, N-[3-[3-cyclopropyl-5-[(2-fluoro-4-iodophenyl)amino]-3,4,6,7-tetrahydro6,8-dimethyl- 2,4,7-trioxopyrido[4,3-d]pyrimidin-1(2H)-yl]phenyl] with 1,1'-sulfinylbis[methane]

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

CAS Registry Number: 1187431-43-1

Classification: MEK inhibitor

Molecular Formula: $C_{26}H_{23}FIN_5O_4 \cdot C_2H_6OS$ **M.W.:** 693.53

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

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

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

How Supplied: Novartis supplies and CTEP, NCI, DCTD distributes 0.5 mg and 2 mg (as free base) tablets. Each commercially-labeled bottle contains 30 tablets with a desiccant.

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

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

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

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

for drug-drug interaction. Trametinib is a substrate for P-gp and BSEP, but this is not expected to be clinically relevant due to trametinib's high permeability.

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

Patient Care Implications: Advise women study participants of reproductive potential to use effective contraception while receiving study treatment and for 4 months after the last dose of trametinib. Refer to the protocol document for specific guidance

8.1.2 GSK 2141795 (NSC 767034)

Chemical Name: N-[(1S)-2-amino-1-[(3,4-difluorophenyl)methyl]ethyl]-5-chloro-4-(4-chloro-1-methyl-1H-pyrazol-5-yl)-2-furancarboxamide

Other Names: GSK2141795C

Classification: pan-AKT inhibitor

CAS Registry Number: 1047634-65-0

Molecular Formula: $C_{18}H_{16}Cl_2F_2N_4O_2$

M.W.: 429.25 g/mol

Approximate Solubility: Very slightly soluble in water at room temperature (0.18 mg/mL). Solubility decreases as pH increases; for example solubility in gastric fluid at 37° C is >11 mg/mL.

Mode of Action: GSK2141795 is an ATP competitive pan-AKT inhibitor. AKT, a serine/threonine protein kinase with three isoforms, is active in several pathways that regulate survival, proliferation, tissue invasion and metabolism. Since AKT-mediated pathways are important in tumor proliferation and survival, AKT kinases are promising targets for therapeutic intervention. Hyperactivation of the AKT pathway can also correlate with chemotherapy resistance and poorer prognosis.

Description: white to off-white powder

How Supplied: GSK2141795 capsules are supplied by GlaxoSmithKline and distributed by the DCTD, NCI. The 25 mg capsule is a size 2 Swedish orange opaque body and Swedish orange opaque cap with no markings. The capsule contains active pharmaceutical ingredient, microcrystalline cellulose, and magnesium stearate. The capsules are packaged in white high density polyethylene (HDPE) bottles with white plastic, induction-seal, child-resistant caps. Each bottle contains 35 capsules.

GSK does not have stability data to support repackaging GSK2141795 capsules. Capsules must be dispensed in the original container.

Storage: Store bottles at 2-8° C (36-46° F).

Stability: Shelf life studies of GSK2141795 are on-going.

Route of Administration: Oral administration. GSK2141795 must be taken fasting 1 hour following a meal and 2 hours before the next meal. If possible take each capsule about 5 minutes

apart. Water is allowed during fasting.

Potential Drug Interactions: *In vitro* data suggest GSK2141795 is a substrate of CYP450 3A4. Potent inhibitors and inducers of 3A4 are prohibited. GSK2141795 appears to be a moderate inhibitor of CYP 2C8 and 3A4 by *in vitro* testing. Drugs that are substrates of these isoenzymes should be used with caution and ones with a narrow therapeutic index should be avoided.

GSK2141795 is a substrate of p-glycoprotein (P-gp) and breast cancer resistant protein (BCRP). It is also an inhibitor of BCRP and OATP1B1. Administration of sensitive BCRP substrates should be prohibited, such as topotecan.

Availability

GSK 2141795 is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI. GSK 2141795 is provided to the NCI under a Collaborative Agreement between GlaxoSmithKline and the DCTD, NCI (see Section 12.3).

8.1.3 Agent Ordering and Agent Accountability

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

Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees can submit agent requests through the PMB Online Agent Order Processing (OAOP) application (https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx). Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account (https://eapps-ctep.nci.nih.gov/iam/) and the maintenance of an "active" account status and a "current" password. For questions about drug orders, transfers, returns, or accountability, call 240-276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET) or email PMBAfterHours@mail.nih.gov anytime.

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

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1. Collection of Specimen(s)

The primary hypothesis of the correlative component to this trial is that biomarkers of sensitivity and resistance to MEK inhibition can occur through ERK-dependent and ERK-independent pathways. To test this hypothesis we propose correlative studies on research biopsies acquired at these time points 1) in all patients prior to trametinib monotherapy (mandatory) 2)all patients after progression on Part 1 with trametinib (mandatory) 3) all patients at time of progression on trametinib combined with GSK2141795 (optional). In addition, archival FFPE sections, plasma and a whole blood samples will be collected..

9.1.1 Blood and Tissue Requirements:

A list and detailed preparation procedures for all required specimens is included in the Specimen Laboratory Manual, see Appendix E.

- A whole blood sample should be collected prior to treatment for subsequent DNA extraction and use as a germline for subsequent sequencing analyses. Whole blood (6 mL) should be collected in an EDTA tube and stored at -70^{o}C or below until shipping. The tube should be labeled with the protocol number, subject number, date and institution.

- Biopsies will be performed preferably under image-guidance, although biopsy method will be chosen based on feasibility for each individual patient and tumor location. Collection will be per the Standard Operating Procedure included in the laboratory manual. Briefly however, five to six core needle biopsies (preferably using an 18 gauge needle, except when contraindicated [i.e. 20G for lung biopsies is acceptable]) will be taken depending on the size of the tumor. Two of the cores will be placed within 5 minutes in 10% neutral buffered formalin, processed using standard operating procedures and embedded in paraffin. At least 2 cores will be frozen within 5 minutes in Optimal Cutting Temperature (OCT) compound. The fifth and 6th core (if possible), should be flash frozen in liquid nitrogen. All frozen specimens should be stored in liquid nitrogen or at -80°C until shipping.). Blocks should be labeled with the protocol number, subject number, date and institution.

- An additional 10mL whole blood samples will be collected to obtain a plasma sample for all patients prior to treatment on Cycle 1 Day 1, Cycle 2 Day 1, at time of progression on trametinib, then prior to dosing on Day 1 of 2nd cycle of combination

therapy and at time of progression on combination. Whole blood should be collected in EDTA tubes and centrifuged at 1900 x g for 10 minutes at +4°C within 1 hour of collection to limit degradation and lysis. Plasma supernatant should be carefully aspirated without disturbing the buffy coat layer. About 4-5 mL plasma can be obtained. Aliquot plasma into 4 properly labeled 1.5 mL cryovials (1 to 1.25 mL per tube). Store at - 70°C or below until shipping.

- If available, an archival tissue block containing tumor or a minimum of ten slides cut at 4-5 microns (unstained and placed onto poly-l-lysine-coated or plus (+) slides) will be required for IHC and genomic analyses.

9.1.2 Shipping of Blood and Tissue Specimen(s):

Samples to be collected and prepared in this study include whole blood, plasma, FFPE core biopsies, fresh frozen and OCT embedded frozen core biopsies and archival FFPE tumor blocks or sections. Samples should be shipped in batches according to requirements along with the specimen submission form (included in the laboratory manual).

All frozen specimens should be packed in dry ice in styrofoam shippers with enough dry ice to ensure that the samples will not thaw within a 48-hour period. A minimum 5 kg of dry ice should be used. Dry ice should be placed along the bottom of the styrofoam container. Each set of frozen specimens should be placed in a plastic resealable bag, grouped according to the subject. A paper towel should be wrapped around the samples. Each plastic bag for each patient should be identified with the following information: protocol number, institution name, patient study ID number. Before plastic bags are placed into the styrofoam box a layer of paper towels should be placed on top of the dry ice. The plastic bag should then be placed into the box, and additional dry ice should be placed along its sides. The styrofoam container should be sealed with strapping tape and placed into a sturdy cardboard mailing box. Copies of all completed "RESEARCH SPECIMEN REQUEST FORM" (included in laboratory manual) should be included in a plastic resealable bag and placed on top of the Styrofoam container. The box should subsequently be sealed but in a way that will permit carbon dioxide to escape as the dry ice sublimates. Frozen specimens should be shipped overnight by carrier of choice on Monday, Tuesday, or Wednesday. Shipments should not be made on Thursday or Friday. Deliveries on Weekends and holidays will not be accepted.

FFPE core biopsies should be placed in a resealable plastic bag (separate bags per patient) and can be shipped with the frozen samples but on the outside of the styrofoam container. If not shipping with the frozen samples, place bags in a cardboard box and place in a padded envelope. These specimens with the specimen submission form can be sent by regular US mail or UPS ground.

FFPE slides should be packaged in a slide holder and shipped in a well cushioned box. These specimens with the relevant pathological report/s can be sent by regular US mail

or UPS ground.

Blood and Tissue Specimens should be shipped to the following address:

Cynthia Timmers, Ph.D.
Room 460B Biomedical Research Tower
460 West 12th Avenue
Columbus, Ohio 43210
Tel. (614)-366-9041
Cynthia.timmers@osumc.edu

9.2. Laboratory Correlative Studies

To perform the correlative studies in this trial, we are request 5-6 tumor core biopsies from patients. Two will be fixed in formalin for IHC assays, 2 will be embedded in OCT for the RPPA assay and DNA/RNA preparations and 1-2 flash frozen for the kinome assay. Because it may not be possible to obtain this many cores or because the cores may have limited tumor content, we will prioritize the following planned studies accordingly. Each core collected should be the largest size that can be obtained safely and will depend on the site from which it is collected. 18 gauge cores will be preferred; however, it will be per institutional standard practice.

1) Whole exome sequencing
2) RPPA assay
3) IHC for PTEN
4) Whole transcriptome sequencing and DUSP4 expression
5) QTAP/Kinome Assay
6) IHC for other biomarkers

9.2.1 Pathway Activation Evaluation

9.2.1.1. Immunohistochemistry (IHC)

To evaluate the status of several proteins in the PI3K and RAS/RAF/MEK pathways before and after trametinib, immunohistochemistry (IHC) will be performed on pretreatment and post-treatment biopsies by the OSU Solid Tumor Translational Science Resource using antibodies that have been validated by the laboratory. Formalin-fixed paraffin embedded (FFPE) tissue sections of biopsies will be analyzed for expression of PTEN and other relevant protein identified in the RPPA assay. Sections from archival material may also be used in cases where insufficient material was obtained from the fresh biopsies. An H&E stained section will be reviewed by a pathologist to verify the presence of tumor. Negative control slides without primary antibody will be included for each staining as well as a positive control. Following processing with appropriate secondary antibodies, slides will be analyzed and scored by two independent

pathologists. Staining intensity will be rated on a semi-quantitative scale: -, negative with no staining; +, weak staining intensity in >10% of the tumor cells; ++, moderate staining intensity in >10% of the tumor cells' +++, strong staining intensity in >10% of the tumor cells.

9.2.1.2. Reverse Phase Protein Assay (RPPA)

In addition to IHC, we will plan to perform other methods of assessing protein levels such as RPPA. This assay will be run by MD Anderson Cancer Center RPPA-Functional Proteomics Core Facility, website below:

http://www.mdanderson.org/education-and-research/resources-for-professionals/scientific-resources/core-facilities-and-services/functional-proteomics-rppa-core/index.html

Sections of the OCT embedded core biopsies will be evaluated by a pathologist to verify tumor content. Tissue will then be cut out of the OCT and protein will be extracted using standard methods. The protein will be denatured, diluted and arrayed on nitrocellulose coated slides. The slides will be probed with a set of 150 validated antibodies (including antibodies to proteins being analyzed by IHC) and spot density determined by MiroVigene software. Samples will be normalized for protein loading and concentrations will be determined using the Super Curve Fitting program. Supervised and un-supervised hierarchical clustering analysis will then be performed. This data will be compared to results derived by IHC and used to determine differences in protein expression between patients that respond to treatment and those that do not. Proteins of interest that are included in this assay are; PTEN, PI3KCA, Cyclin D1 and total and phosphorylated forms of AKT, 4E-BP1, p70-S6, ERK and MEK.

9.2.1.3. Quantitative Targeted Absolute Proteomics (QTAP)

If sufficient biopsy material is available, we will also evaluate changes in expression or phosphorylation levels of key protein kinases using QTAP. This assay will be performed in the laboratory of Dr. Gary Johnson at the University of North Carolina School of Medicine. The QTAP/MRM technique has been previously used to quantitatively define TNBC oncogenic kinome signatures in human cell lines, mouse xenograft models and human tumor biopsy samples[31]. When comparing non-treated samples to those treated with a targeted MEK inhibitor, this assay was also able to mechanistically define kinome reprogramming and potentially predict combination therapies to overcome resistance.

QTAP is an LC/MS technology that uses multiplexed Multiple Reaction Monitoring (MRM) for the absolute quantitation of targeted peptides. Protein

lysates will be made from the flash frozen core biopsies collected pre-treatment and at the time of progression and analyzed as previously described.[32] In brief, lysates will be passed through columns of kinase inhibitor-conjugated beads to isolate protein kinases. After several washes, bound proteins will be eluted and digested with trypsin. Digested peptides will then be labeled with iTRAQ and separated by a Tempo LC MALDI Spotting System. Data obtained from the MALDI TOF/TOF will be processed with the ProteinPilot software to identify proteins from databases searches and the Paragon Algorithm software will be used to quantify changes in bound kinase levels. Human cell lines and mouse xenograft tumors were used in pre-clinical studies to define kinome activation state and response to targeted kinase inhibition. Synthetic peptides with incorporated heavy isotopes (^{13}C, ^{15}N-labeled arginine and lysine) serve as quantitative internal standards and can be made in phosphorylated and non-phosphorylated forms. The method will be multiplexed so many kinases (~25-50) can be monitored simultaneously. Main proteins to be included in kinome analysis include: EGFR, PDGFR, RAF, RAS, RSK, MEK, ERK, PTEN, P13K, AKT and MYC. Other proteins in the MAPK and PI3K pathways, cell cycle regulators, stress response, and receptor tyrosine kinases will also be included.

9.2.2 Genome and Transciptome Analysis

In conjunction with the Garraway Laboratory (Levi Garraway, MD and Nikhil Wagle, MD; scientific collaborators, Boston, MA) we will perform massively parallel exome and transcriptome sequencing on tumor samples collected pretreatment. They have previously reported the development of a targeted, massively parallel sequencing approach to detect genomic alterations in 137 cancer genes from FFPE tumor samples.[29] They used this approach to identify a novel genetic mechanism of resistance to the BRAF-inhibitor vemurafenib in a patient with metastatic melanoma who developed resistance after an initial dramatic response.[30] More recently, they have performed whole exome sequencing on FFPE tumor and normal DNA from 15 patients with a wide variety of cancer types, including lung adenocarcinoma, prostate adenocarcinoma, leiomyosarcoma, and urothelial carcinoma.[31]

In this project, they will employ unbiased, comprehensive studies of patient-derived breast tumor tissue to identify clinical mechanisms of response and resistance to trametinib and/or GSK2141795 in triple-negative breast cancer. To accomplish this, they will perform whole exome analysis using massively parallel sequencing, analyzing "sets" consisting of pre-treatment and post-progression samples (when applicable) together with corresponding matched normal DNA from blood samples on an estimated 18 patients with an initial clinical response (CR, PR) to trametinib monotherapy (n=9) or to trametinib combined with GSK2141795 (n=9). They will also sequence all of the mRNA transcripts (the transcriptome) in the frozen breast cancer samples from these patients. The processes for whole exome sequencing and whole transcriptome sequencing have been thoroughly validated by Dr. Nikhil Wagle in the laboratory of Dr. Levi Garraway of the Broad Institute.[29, 32]

ABL1	CTNNB1	IDH1	MYCL1	RET
ABL2	EGFR	IDH2	MYCN	RICTOR
AKT1	EPHA3	IGF1R	NF1	RUNX1
AKT2	EPHA5	IKBKE	NF2	RUNX1T1
AKT3	EPHB6	IKZF1	NKX2-1	SMAD2
ALK	ERBB2	JAK2	NOTCH1	SMAD3
APC	ERBB3	JAK3	NOTCH2	SMAD4
ATM	ERBB4	KDR	NOTCH3	SMARCA4
AURKA	FAM123B	KEAP1	NOTCH4	SMARCB1
BCL2	FBXW7	KIAA0774	NPM1	SMO
BRAF	FGFR1	KIAA1303	NRAS	SOCS1
BRCA1	FGFR2	KIT	NTRK1	SRC
BRCA2	FGFR3	KRAS	NTRK2	STK11
CCND1	FGFR4	MAP2K1	NTRK3	SUFU
CCNE1	FHIT	MAP2K2	PAX5	TCF4
CDC73	FKBP9	MAP2K4	PDGFRA	TERT
CDH1	FLT1	MCL1	PDGFRB	TET2
CDK4	FLT3	MDM2	PDPK1	TGFBR2
CDK6	FLT4	MDM4	PIK3CA	TNFAIP3
CDK8	FRAP1	MEN1	PIK3R1	TOP1
CDKN1A	GATA1	MET	PTCH1	**TP53**
CDKN2A	GNAQ	MITF	PTEN	TSC1
CEBPA	GNAS	MLH1	PTK2B	TSC2
CHEK1	GUCY1A2	MLL MPL	PTPN11	TSHR
CHEK2	HNF1A	MRE11A	PTPRD	VHL
CREBBP	HRAS	MSH2	RAF1	WT1
CRKL	HSP90AA1	MSH6	RB1	ZNF668
CSF1R		MYC	REL	

Table 3. Example of cancer genes to be included by massively parallel sequencing.[1]

All of the sequencing data will be combined to generate a catalogue of candidate genomic alterations that cause resistance to these targeted therapies. High priority genetic lesions will be introduced into cell lines to confirm that they cause resistance to these therapies. In addition, recurrent alterations in the pre-treatment samples can be correlated with response, with emphasis on exquisite sensitivity/durable responses.

Whole exome and whole transcriptome sequencing will be performed at the Broad Institute Sequencing platform. Genomic DNA will be extracted from OCT embedded tissue after verification of tumor content. Cores that contain the highest amount of tumor tissue (>50%) will be used for the purification of DNA using the Promega Maxwell 16 System and Tissue DNA purification kit. DNA derived from archival sections may be used in cases where insufficient material was obtained in the pre-treatment biopsy. The resulting DNA will be quantitated by PicoGreen fluorescence and 100 ng of DNA will be used for library construction, although as little as 50 ng can be used if needed. The exome will be selected using the solution-phase hybrid capture method and sequencing will be performed using the Illumina HiSeq instrument, yielding an average depth of coverage of 150-fold. Data analysis will be conducted in collaboration with computational biologists in the Garraway lab / Broad Cancer Program computational group. Whole exome sequencing data will be analyzed for base substitutions, small insertions and deletions, copy number alterations, and loss of heterozygosity. Selected alterations of interest will be validated by Sequenom genotyping or orthogonal technologies in use in the lab.

In parallel to exome sequencing, we will also sequence all of the mRNA transcripts (the transcriptome) from frozen OCT biopsy samples that contain >50% tumor. Total RNA will be extracted using the Promega Maxwell 16 System and simplyRNA purification

kit. Quality of RNA will be evaluated using an Agilent Bioanalyzer and quantitated using a Nanodrop. Approximately 200 ng of RNA from each patient samples will be used to prepare a library using the Illumina TruSeq RNA-seq library preparation kit following the manufacturer's directions. Each sample will be sequenced with an Illumina HiSeq instrument. Transcriptome data will be analyzed for gene fusions, chimeric read-through transcripts, point mutations, and instances of alternative splicing. RNA-seq data will also be used to determine gene expression levels for the entire breast cancer transcriptome.

9.2.3 DUSP4 Expression

Quantitative gene expression levels for DUSP4 and several control genes (defined in Balko et al.[22]) will be detected using real-time PCR with the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) and TaqMan MGB probes (FAM dye labeled). Primers and probes for all genes analyzed will be purchased from Applied Biosystems Assays-on-Demand Gene Expression products. Total RNA will be purified from tissue as previously described and will be used to synthesize cDNA for RT-PCR reactions. Relative quantification will be applied using a standard curve method and normalization to control gene expression.

9.2.4 Additional Studies

It is anticipated that future studies will include evaluation of genetic, epigenetic, immunologic, hormonal, and/or other issues related to breast cancer. In particular, we will be collecting and storing plasma samples to test future potential circulating biomarkers. We will inform participants in the informed consent document that we may use their specimens for research related to breast cancer and other cancers.

9.3. Site(s) Performing Correlative Studies

- IHC assay and DUSP4 expression analysis will be performed at the OSU Solid Tumor Translational Science Shared Resource.

- Whole exome and transcriptome sequencing will be performed at the Broad Institute.

- The RPPA assay will be performed by MD Anderson Cancer Center RPPA-Functional Proteomics Core Facility.

- The kinome assay will be performed in the laboratory of Dr. Gary Johnson at the University of North Carolina at Chapel Hill.

9.4 Potential risks and procedures for minimizing risks

9.4.1 Risks of blood draws

All blood draws will be performed by trained personnel that use standard sterile techniques. Infection is unlikely since stringent aseptic techniques are followed during all venipuncture procedures.

9.4.2 Risks of biopsy

The risks of this study relate primarily to the risk of a biopsy. In general, these procedures are associated with a small risk of pain, bleeding, infection, and damage to adjacent organs. The magnitude of this risk depends somewhat upon the site of the procedure.

Potential risks according to site are:

Risks of core breast biopsy:

- Likely: local discomfort and minor bleeding.
- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization due to bleeding or other complications, infection, pneumothorax, damage to adjacent organs.

Risks of skin/chest wall punch biopsy:

- Likely: local discomfort and minor bleeding.
- Less likely: moderate or major bleeding, or infection

Risks of lymph node or soft tissue core needle biopsy:

- Likely: local discomfort and minor bleeding.
- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization due to bleeding or other complications, infection, pneumothorax, damage to adjacent organs.
- Additional risks may be present if i.v. conscious sedation is required.

Risks of liver core needle biopsy:

- Likely: local discomfort and minor bleeding
- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization due to bleeding or other complications, infection, bowel perforation or damage to adjacent organs
- Additional risks may be present if i.v. conscious sedation is required.

Lung biopsy core needle biopsy

- Likely: local discomfort and minor bleeding
- Less Likely: moderate or major bleeding, need for blood transfusion, lung collapse, hospitalization due to bleeding or other complications, infection,

damage to nearby organs, allergic reaction to the numbing medicine

In order to minimize the risk of a biopsy, only qualified personnel will perform these procedures. Prior to the procedure, the physician performing the procedure will discuss the risks with each study participant, answer any questions, and obtain separate procedure consent. For biopsies of lesions that are not superficial and clearly palpable, imaging studies such as CT, ultrasound, or MRI will be used to guide the biopsy in order to minimize the risk of damage to adjacent structures. After lymph node biopsies, patients will be observed for approximately 2 hours (range 2-4 hours) after the procedure, or per institutional standard guidelines. After liver biopsies, patients will be observed for approximately 4 hours (range 4-6 hours) after the procedure, or per institutional standard guidelines. Less than the goal quantity of tissue is acceptable for each type of biopsy, and will be left to the clinical judgment of the physician performing the procedure.

All grade 3 or 4 events attributable to any of the study procedures will be reported to the Principal Investigator within 24 hours of occurrence. Adverse events will be reported by the PI to the IRB consistent with standard procedures.

9.4.3 Anesthesia Risk

Risks of local anesthesia

All biopsy procedures require local anesthesia using lidocaine, xylocaine, or related compounds. There is a small risk of an allergic reaction associated with these drugs.

In order to minimize the risk of local anesthesia, only qualified personnel will perform the biopsy procedure. Patients will be queried if they have had previous allergic reactions to local anesthetics.

Risks of intravenous conscious sedation

Certain biopsy procedures, such as lymph node or liver biopsies, may require intravenous conscious sedation (IVCS). IVCS is a minimally depressed level of consciousness that retains the patient's ability to maintain a patent airway independently and continuously and respond appropriately to physical stimulation and verbal commands.

The risks of intravenous conscious sedation include: inhibition of the gag reflex and concomitant risk of aspiration, cardiopulmonary complications (myocardial infarction, cardiac arrhythmias, hypoxemia), and allergic reactions to the sedative or analgesic medications. These risks are small but real; for example, in a prospective study of 14,149 patients undergoing IVCS during upper gastrointestinal endoscopies, the rate of immediate cardiopulmonary events was 2 in 1000. The 30-

day mortality was 1 per 2,000 cases. In this study, there was a strong association between lack of monitoring and use of high-dose benzodiazepines with adverse outcomes. There was also an association between the use of local anesthetic sprays to the oropharynx and the development of pneumonia.

In order to minimize the risk of intravenous conscious sedation, only qualified personnel will be responsible for conscious sedation as per institutional protocols. A minimum of two individuals will be involved in the care of patients undergoing conscious sedation—the physician performing the biopsy procedure, and the individual (M.D. or R.N.) who monitors the patients and his/her response to both the sedation and the procedure, and who is capable of assisting with any supportive or resuscitative measures. The room where the procedure utilizing IVCS takes place will have adequate equipment to provide supplemental oxygen, monitor vital signs, and maintain an airway should this be necessary. An emergency cart will also be immediately accessible to the room where the procedure is to take place, and emergency support services will be available on page. Patients will be screened and evaluated for their fitness to undergo conscious sedation by a trained physician. Following the procedure, patients will be observed closely in the recovery room according to standard institutional guidelines.

9.4.4 Risk of imaging studies

Some biopsy procedures require imaging studies, either to plan or guide the procedure. Imaging studies that may be used in obtaining tissue samples include CT scans and ultrasound. CT scans will expose study participants to controlled amounts of radiation. The total dose of radiation from these tests is not anticipated to cause any adverse effects. There is also a risk of an allergic reaction to the intravenous contrast dye used during CT imaging, as well as a risk of experiencing feelings of anxiety or claustrophobia while undergoing a CT scan. There are no anticipated risks with the use of ultrasound.
In order to minimize these risks, patients will be queried, as per standard institutional practice, regarding their history of reactions to intravenous contrast dye. If a patient has had such a reaction, she/he will be premedicated, or dye will not be used, as per standard institutional practice. If a patient has previously experienced anxiety or claustrophobia while undergoing a CT scan, anxiolytics may be considered as indicated.

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 2 weeks prior to start of protocol therapy. Scans and x-rays must be done ≤4 weeks prior to the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy. Appropriate delays in study treatment or procedures are allowed to adjust for holidays or weekends.

Study Calendar

Study Assessments[1]	Screen	Part 1 Trametinib monotherapy	Part 2 Trametinib in combination with GSK2141795	Final Study Visit[3]	Post Treatment Follow Up[3]
Informed Consent	X				
Baseline Demographics	X				
Medical History / Interim History	X	Day 1 (± 3 days) of each cycle	Day 1 (± 3 days) of each cycle	X	
Concomitant Medications	X	Continuous			
Physical Exam	X	Day 1 (± 3 days) of each cycle	Day 1 (± 3 days) of each cycle	X	
ECOG Performance Status	X	Day 1 (± 3 days) of each cycle	Day 1 (± 3 days) of each cycle	X	
Vital Signs (BP, HR, Temperature, weight)	X	Day 1 (± 3 days) of each cycle	Day 1 (± 3 days) of each cycle	X	
Hematology, Clinical Chemistry[4]	X	Day 1 (± 3 days) of each cycle	Day 1 (± 3 days) of each cycle	X	
Pregnancy Test[5]	X				
Hemoglobin A1C, TSH, fasting glucose	X	Repeated every 3 cycles ± 7 days or as Clinically Indicated			
Fingerstick blood glucose			At home daily monitoring for weeks 1 -4, then as clinically indicated	As Clinically Indicated	
12-Lead ECG	X		Prior to dosing on Week 1 day 1 and day 15, and then every 4 weeks (Week 5, Week 9, etc.) ± 7 days or as clinically indicated		

Study Assessments[1]	**Screen**	**Part 1 Trametinib monotherapy**	**Part 2 Trametinib in combination with GSK2141795**	**Final Study Visit[3]**	**Post Treatment Follow Up[3]**
LVEF (ECHO)	X	Repeated every 3 cycles ± 7 days during Part 1 or as Clinically Indicated	Week 5, Day 1 of Part 2 and then at the start of every 12 weeks ± 7 days or as clinically indicated	As Clinically Indicated	
Ophthalmic Exam[6]	X	As Clinically Indicated			
Blood sample[7]		Day 1 (± 3 days) of cycle 1			
Plasma[8]	X	Day 1 cycle 2 and at progression	Day 1 of cycle 2 (prior to dosing)	X	
Research Biopsy(s) [9]	Mandatory research biopsies will be attempted on all patients prior to the initiation of study treatment on Part 1	Mandatory research biopsies will be attempted on all patients at time of progression on Part 1	Optional research biopsies will be attempted on patients at time of progression on Part 2		
Study Agents		Trametinib will be administered daily in Part 1. Trametinib will be administered in combination with GSK2141795 in Part 2. Please see Section 6 for Dose Modifications.			
Tumor measurements[2]	X	Staging will occur after every 2 cycles ± 7 days or as Clinically Indicated			
Adverse Events		Continuous			
Follow Up Data[3]					X

1. Assessments scheduled on days of dosing should be done prior to administration of study drug(s), unless otherwise specified.
2. Tumor measurements within 4 weeks of first dose. Documentation (radiologic) must be provided for patients removed from study for progressive disease. If subject was withdrawn due to progression of disease (PD), disease assessments do not need to be repeated at the Final Study Visit. CT chest/abdomen/pelvis preferred. Bone scan, MRI, PET scan may be used at the discretion of the treating physician.
3. Final Study Visit should occur 21 days (±7 days) after last dose of study drug. Follow up will continue for 52 weeks after removal from study or until death, whichever occurs first. Follow up will be every 3 months to confirm patient's living status and whether the patient has progressed (if patient did not progress on protocol therapy) gathered from clinic notes or telephone calls (no patient visit required).
4. Should include alkaline phosphatase, total bilirubin, creatinine, glucose, potassium, SGOT, SGPT, sodium.
5. Perform only in women of child-bearing potential.
6. Ophthalmic exam will include indirect and direct fundoscopy, visual acuity, visual field examination, tonometry and color fundus photos. Additional ophthalmic exams will be performed if symptomatically warranted.

Study Assessments[1]	Screen	Part 1 Trametinib monotherapy	Part 2 Trametinib in combination with GSK2141795	Final Study Visit[3]	Post Treatment Follow Up[3]

7. Whole blood (6 mL) should be collected in an EDTA tube and stored at -70°C or below until shipping.
8. Plasma should be purified from 10 mL of blood collected in an EDTA tube. Aliquots should be stored at -70°C or below until shipping.
9. Research biopsies may include breast core biopsy, skin or chest wall biopsy, lymph node biopsy, liver biopsy, or lung biopsy.

11. MEASUREMENT OF EFFECT

11.1. Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every 8 weeks. In addition to a baseline scan, confirmatory scans should also be obtained every 8 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1).[33] 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 and GSK 214179.

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 may be considered measurable.

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

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, 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.

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.

11.1.7 Response Review

All responses will be reviewed by an expert(s) independent of the study at the study's completion. Simultaneous review of the patients' files and radiological images will occur.

11.2. Other Response Parameters

N/A

12. DATA REPORTING / REGULATORY REQUIREMENTS

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

12.1. Data Reporting

12.1.1 Method

This study will be monitored by the Clinical Data Update System (CDUS) Version 3.0. Cumulative protocol- and patient-specific CDUS data will be submitted electronically to CTEP on a quarterly basis, either by FTP burst of data or via the CDS web application. Reports are due January 31, April 30, July 31, and October 31. Instructions for submitting data using the CDUS can be found on the CTEP Web site (http://ctep.cancer.gov/reporting/cdus.html).

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

12.1.2 Responsibility for Data Submission

Study participants are responsible for submitting CDUS data and/or data forms to either the Coordinating Center or to the Lead Organization on the study quarterly. The date for submission to the Coordinating Center or to the Lead Organization will be set by them. CDUS does not accept data submissions from the participants on the study. When setting the dates, allow time for Coordinating Center compilation, Principal Investigator review, and timely submission to CTEP by the quarterly deadlines (see **Section 12.1.1**). For trials monitored by CTMS, a quarterly report of data will be provided by Theradex to the Coordinating Center.

Either the Coordinating Center or the Lead Organization is responsible for compiling and submitting CDUS data to CTEP for all participants and for providing the data to the Principal Investigator for review.

12.2. CTEP Multicenter Guidelines

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

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

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

12.3. Collaborative Agreements Language

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

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

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

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

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

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

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

Identifiable Health Information set forth in 45 C.F.R. Part 164.

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

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

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

Email: ncicteppubs@mail.nih.gov

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

13 STATISTICAL CONSIDERATIONS

13.1. Study Overview

This is an open label, two-stage phase II study designed to assess the proportion of patients who have a partial or complete response to treatment with trametinib in advanced TNBC patients, followed by trametinib in combination with AKT inhibitor GSK2141795 at time of tumor progression (Figure 1, Schema). Preclinical data from the Ohio State University and others suggest that a subset of triple negative breast cancer (TNBC) patients that are PTEN wild type may demonstrate sensitivity to single agent MEK inhibition (Unpublished Olson, Chen),[15] and dual targeting of the RAS/RAF/MEK/ERK pathway and PI3K/AKT pathways may result in durable response after progression on MEK inhibitor monotherapy.[15] Therefore, we have two parts to this study, where the primary driving hypotheses are two-fold: (1) that the anti-tumor response to the single agent trametinib will depend on the PTEN status of the tumor, with wild type PTEN having a higher likelihood of response than PTEN

null tumors; and (2) that simultaneous inhibition of the RAS/RAF/MEK and PI3K/AKT pathway with trametinib and GSK2141795 will overcome both intrinsic and acquired resistance to trametinib monotherapy. In addition to evaluating clinical efficacy of this regimen, we will also assess correlative markers in relation to clinical outcomes of interest. Part 1 focuses on evaluation of efficacy after treatment with single agent trametinib, and Part 2 is focused on evaluating salvage efficacy of treatment with the combination of trametinib and GSK2141795 in patients who progress on trametinib alone.

13.1.1 **Primary Endpoint**

The primary endpoint for this trial is the objective response rate, defined as the proportion of patients who have had a partial response (PR) or complete response (CR) within the first 6 months after initiation of therapy with trametinib. All eligible patients who have received at least one dose of the study regimen will be evaluable for response. Participants will be re-evaluated for response every 2 cycles (8 weeks). Response, stable disease and progression will be evaluated using the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 guideline.[33] In addition to a baseline scan, confirmatory scans will be obtained after approximately 8 weeks following initial documentation of a clinical benefit.

In determining this rate, the number of patients with RECIST 1.1 based PR or CR will be divided by the number of evaluable patients. All evaluable patients will be used for this analysis. Evaluable patients will be defined as only those participants who have received at least one cycle of therapy, and have had their disease re-evaluated. Exact binomial 95% confidence intervals for the true PR+CR response rate will be calculated.

13.2. Sample Size, Accrual, and Study Duration:

This study design requires a minimum of 12 and a maximum of 37 evaluable patients. In addition, we expected to accrue an additional 10% to accommodate the potential for problem cases (i.e. patients who are not evaluable for some reason). The first 45 evaluable patients will be used in the decision analysis for this part of the trial, and estimates will also be generated using all evaluable patients. We expect approximately 3 breast cancer patients to be enrolled per month. This will translate to a total of 26 months to accrue a maximum of 50 patients and allowing for the 6 months interim analysis in both Part 1 and Part 2.
The number of patients inevaluable due to screen failure was higher than expected. As such, a total of 50 patients were consented across all sites in order to enroll 37 evaluable patients

13.3. Study design, primary endpoint analysis plans and power:

To test these hypotheses, we propose a single arm, two-stage phase II trial of single agent trametinib in advanced TNBC patients, followed by trametinib in combination

with AKT inhibitor GSK2141795 at time of tumor progression (Figure 1, Schema). This design was chosen because trametinib monotherapy is more tolerable than trametinib in combination with GSK2141795, and therefore, it is vital to first assess if there is a subpopulation of TNBC patients (such as those that are PTEN wild type) that will derive benefit from anti-MEK therapy alone before exposing patients to dual MEK and AKT inhibition. GSK2141795 will be added at time of tumor progression to determine if the addition of AKT inhibition will overcome the primary proposed mechanism of resistance to trametinib through blockade of the PI3K/AKT pathway.

The goals of this study are to evaluate whether or not trametinib alone provides sufficient clinical response in this patient population (Part 1), and to evaluate if the combination of trametinib plus GSK2141795 in patients who progress will improve the objective response in this patient population (Part 2). To minimize the expected number of subjects treated if the trametinib monotherapy is not sufficiently active in this setting, a Simon two-stage Optimum Design will be used for Part 1 that allows for early termination of accrual if insufficient activity with monotherapy is observed. In addition, upon completion of Part 1 of this study we will further evaluate the combination of trametinib plus GSK2141795 in those patients in Part 1 who progress (Part 2). Similarly, to limit the number of patients accrued if this combination regimen proves to be insufficiently active, we will utilize a Fleming Two-Stage Design. As in any two-stage design, the 2nd stage will only commence and accrue additional patients if the specified decision criteria are met for the 1st stage. Even though Part 2 is directly linked to Part 1 (in the fact that those who progress on Part 1 are able to go on to Part 2) each part will have its own study design and decision criteria associated with them. The primary evaluation and goal of interest is Part 1, where the Part 2 evaluation is a secondary goal of this trial.

For Part 1: We hypothesize that the true ORR with monotherapy is at least 20% versus a null hypothesis that the ORR is at most 5%, reflecting that any treatment success (clinical response) is due to chance alone. Under this Simon Optimal two-stage design, we aim to test the null hypothesis H0: ORR $\leq$ 5% vs. H1: ORR $\geq$ 20% to evaluate the efficacy of trametinib monotherapy. With 90% power and a Type I error rate of 10%, the interim analysis for this design requires that at least one patient of the first 12 evaluable patients enrolled have observed objective response (CR+PR); otherwise, if no patients have an objective response to treatment, then we will consider this sufficient early evidence that this treatment regimen is not worth pursuing further as monotherapy. At this point, we will evaluate the combination regimen in Part 2 in any patients subsequently enrolled on the trial and not just those who fail monotherapy in Part 1. If at least 1 patient has clinical response out of the first 12 enrolled patients treated with combination therapy, we will continue accrual to the second stage and accrue an additional 25 patients for a total of 37 patients. In the final analysis, we would need to see at least 4 patients with clinical response as defined above out of the first 37 evaluable patients accrued to determine that this monotherapy has promising associated activity and thus is worth pursuing further. Otherwise, if 3 or fewer have clinical benefit, then we will consider this monotherapy to not be sufficiently active on its own.

For Part 2: In this part of the trial, we will focus on evaluating the ability to obtain clinical response in those patients in Part 1 who progress on monotherapy; we expect that about 80% of patients will progress and remain free of limiting toxicity to go on to Part 2. With our planned over-accrual and if all are evaluable, we will have 32 patients available for this portion of the trial. If we observe issues with patients going off study during Part 1, the study team will discuss with CTEP potential solutions and if patients who cannot tolerate the MEK inhibitor as single agent therapy will be replaced in the overall sample size to facilitate subsequent evaluation of the combination regimen in Part 2. However, in evaluation of the primary outcome of the MEK inhibitor as single agent therapy, any such patient who is unable to tolerate therapy and/or goes off study during Part 1 will still be included in the analyses and decision rule evaluations.

Since this is also a relapsed/refractory setting, our goals are the same as those in Part 1. Therefore, we hypothesize that the objective response rate of this combination therapy is at least 20% versus the null hypothesis that it is at most 5%. With 90% power and a Type I error rate of 10%, this Fleming two-stage design has an interim analysis after the first 16 evaluable patients are accrued. If no patients have documented objective response (CR+PR) out of the first 16 enrolled patients the trial cohort then we will consider this sufficient early evidence that this is not a promising regimen and accrual will be terminated. If at least 1 patient has clinical response out of the first 16 enrolled patients, then accrual will continue and an additional 16 patients will be accrued for a total 32 evaluable patients. In the final analysis, if at least 4 patients have clinical response as defined above, then we will consider this a promising regimen and worthy of further study.

Assuming that the number of responses is binomially distributed, we will estimate objective response rates independently for parts 1 and 2, and generate corresponding 95% binomial confidence intervals.

13.4. Secondary Endpoints

The Kaplan-Meier method will be used to estimate overall survival and progression-free survival distributions. Each of these variables will be measured from the start date of the treatment to the date of the event (i.e., death or disease progression) or the date of last follow-up to evaluate that event. Similarly, we will also evaluate duration of objective response in the subset of patients who achieve a response to treatment, where we will define this from the date of documentation of response to the date of progression and/or death. This will be more exploratory and descriptive given the expected response rate, but we will use the methods of Kaplan and Meier to evaluate this. Overall survival will only be evaluated for Part 2, where for Part 1 patients who progress and go on to Part 2 will be censored at that time making the estimation not as informative. Progression-free survival will not be confounded due to the fact that Part 1 patients need to progress first before going to the Part 2 treatment regimen. The clinical benefit rate (CR+PR+SD) will be reported for patients after Part 1 and after Part 2.

Toxicity and tolerability: As per NCI CTCAE v4.0, the term toxicity is defined as adverse events that are classified as either possibly, probably, or definitely related to study treatment. The maximum grade for each type of toxicity will be recorded for each patient, and frequency tables will be reviewed to determine toxicity patterns. In addition, we will review all adverse event data that is graded as 3, 4, or 5 and classified as either "unrelated" or "unlikely to be related" to study treatment in the event of an actual relationship developing. The incidence of severe (grade 3+) adverse events or toxicities will be described. We will also assess tolerability of the regimens for part 1 and part 2 through assessing the number of patients who required dose modifications and/or dose delays. In addition, we will also capture the proportion of patients who go off treatment due to adverse reactions or even those who refuse further treatment for lesser toxicities that inhibit their willingness to continue participation on the trial. These tolerability measures will be assessed within each of the treatment parts independently. All patients who have received at least one dose of any of the therapeutic agents will be evaluable for toxicity and tolerability.

Correlative endpoints:

The statistical analysis corresponding to the correlative studies will be descriptive and exploratory in nature.

RPPA:
For each patient the protein intensity fold-change ratios will be calculated from the RPPA intensity values. Ratios between tumors sampled prior to initiating trametinib single agent treatment and at time of progression on either single agent trametinib or the combination (TOP/pre-treat ratio), will be calculated. These ratios will be median-centered and clustered using Cluster 2.0 software (http://rana.lbl.gov/EisenSoftware.htm). Student's t-test for differences between average protein intensity ratios at these intervals will be calculated using Microsoft Excel by grouping all the ratios for individual clusters.

Table 4	Biomarker Prevalence				
	10%	20%	30%	40%	50%

HR	0.43	0.51	0.55	0.56	0.57

Table 1: Statistical Considerations for Biomarker Analysis. Maximum detectable hazard ratios (HR) using one-sided log-rank test with 80% power and alpha=0.2, assuming a sample size of 37, 26 months accrual and 12 months follow-up.

These results and exploratory analyses will be used in planning future studies of this combination treatment regimen in this patient population. Biomarker analyses on required research biopsies at study entry will be conducted to assess the predictive value of PTEN and other biomarkers on patient outcome. For example, we will assess impact of PTEN status and other markers on survival outcomes as well as ORR and CBR. For survival-based outcomes, Table 4 shows detectable hazard ratios between marker groups for varying prevalence rates and at least 80% power. The Type I error constraint is quite lax in this setting, reflecting a phase II approach and relaxation of constraints but also in the univariate setting can be used to cast a wider net to capture potential markers of interest that may provide insights for the development of future biomarker-driven therapeutic strategies. Fisher exact tests will also be used to identify potential differences in ORR and CBR rates between biomarker groups as well, although depending on prevalence rates we will likely have sufficient power to only detect large differences (e.g. 35%) if we constrain Type I error to 0.05. Again, these will be largely exploratory, and lack of significant results will be viewed with caution given these limitations but will provide insights into potential relationships and mechanisms. To assess mechanisms of acquired resistance, research biopsies will be conducted at time of progression on trametinib monotherapy and at time of progression to the combination of trametinib and GSK2141795. Restaging will occur after every 2 treatment cycles (8 weeks).

Whole exome and transcriptome sequencing:
Genetic alterations from whole exome sequencing data would be tabulated. Data analysis will be conducted in collaboration with computational biologists in the Garraway lab / Broad Cancer Program computational group. Whole exome sequencing data will be analyzed for base substitutions, small insertions and deletions, copy number alterations, and loss of heterozygosity. Selected alterations of interest will be validated by Sanger sequencing or related orthogonal technologies in use in the Garraway lab. Data will be compiled to generate a catalogue of candidate genomic alterations that cause resistance to MEK and ATK inhibition. Genes listed above in Section 9, Table 3 will be the first to be examined as well-known genes related to cancer growth and survival. Molecular alterations of known significance will be reported. Molecular alterations of unknown significance will also be presented; however, assessment of the clinical significance will be investigated by *in vitro* studies (i.e. site-directed mutagenesis, growth inhibition analysis, immunoblot studies, and kinase assays).. Identified markers of interest will be explored in relation to several clinical outcomes of interest for each treatment component. Specifically, we will look at incidence of mutations and marker expression levels similar to that described above, where we will first look graphically at associations with objective response as well as with progression-free survival. Model-based evaluations may also be used in an exploratory manner to better assess these relationships in a quantitative manner, but will be limited given the sample size available for these analyses.

QTAP/Kinome assay:

Table 5. Power calculation to detect kinase difference				
D=Difference in log2 scale	1.00	1.58	2.00	2.32
Fold change (2^D)	2	3	4	5
Power (%)	8.93	81.69	97.39	99.61

Table 5 summarizes the power to detect a true difference of D with a sample size of 9 patients in each group (pre and post treatment)

For the QTAP/Kinome assay: Paragon Algorithm software will be used to quantify changes in bound kinase levels. As seen in Table 5, a two-sided paired sample t-test will have at least 80% power to detect a 3-fold change in bound kinase levels before and after treatment with 9 patients in each group (assuming Type I error of 0.05). Based on preliminary data,[34] we assume that 100 kinases are captured, of which 10% of them are truly differentially expressed between pre- and post- treatment and a common standard deviation of 0.95 (in log2 scale, Table 5). Given the limited paired sample size, nonparametric tests will be used (i.e. Wilcoxon signed rank test) if approximate normality is not achievable through transformation.

13.5. Reporting and Exclusions

13.5.1 Evaluation of Toxicity

All patients would be evaluable for toxicity from the time of their first treatment with trametinib monotherapy or trametinib in combination with GSK2141795

13.5.2 Evaluation of Response

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

All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) would be included in the main analysis of the response rate. Patients in response categories 4-9 would be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration would not result in exclusion from the analysis of the response rate.

All conclusions would be based on all eligible patients. Sub-analyses would then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (*e.g.*, early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these sub-analyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals would also be provided.

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APPENDIX A PERFORMANCE STATUS CRITERIA

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

APPENDIX B CTEP MULTICENTER GUIDELINES

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

Responsibility of the Protocol Chair

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

Responsibilities of the Coordinating Center

- Each participating institution will have an appropriate assurance on file with the Office for Human Research Protection (OHRP), NIH. The Coordinating Center is responsible for assuring that each participating institution has an OHRP assurance and must maintain copies of IRB approvals from each participating site.
- Prior to the activation of the protocol at each participating institution, an OHRP form 310 (documentation of IRB approval) must be submitted to the CTEP PIO.
- The Coordinating Center is responsible for central patient registration. The Coordinating Center is responsible for assuring that IRB approval has been obtained at each participating site prior to the first patient registration from that site.
- The Coordinating Center is responsible for the preparation of all submitted data for review by the Protocol Chair.
- The Coordinating Center will maintain documentation of AE reports. There are two options for AE reporting: (1) participating institutions may report directly to CTEP with a copy to the Coordinating Center, or (2) participating institutions report to the Coordinating Center who in turn report to CTEP. The Coordinating Center will submit AE reports to the Protocol Chair for timely review.
    - Routine AE reporting: Participating institutions report to the Coordinating Center who in turn report to CTEP.
    - Expedited AE reporting: Participating institutions will report directly to CTEP via CTEP-AERS and will copy the Coordinating Center. The CTEP-AERS-generated email with the study#, subject#, and ticket# must be forwarded by the participating institution to the Subsite Coordinator either via fax (614-366-4721) or secure email (jennifer.sexton@osumc.edu)
- Audits may be accomplished in one of two ways: (1) source documents and research

records for selected patients are brought from participating sites to the Coordinating Center for audit, or (2) selected patient records may be audited on-site at participating sites. If the NCI chooses to have an audit at the Coordinating Center, then the Coordinating Center is responsible for having all source documents, research records, all IRB approval documents, NCI Drug Accountability Record forms, patient registration lists, response assessments scans, x-rays, etc. available for the audit.

- Data from participating institutions must be sent to the Coordinating Center within 2 weeks of each patient visit.
- Safety Reports and Action Letters from CTEP will be distributed by the Subsite Coordinator to the participating institutions.

Agent Ordering

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

APPENDIX C MEDICATION DIARY – PART 1

PATIENT ID: _____			**PATIENT INITIALS (F M L): _____ _____ _____**		
PART _____ CYCLE#: _____			**Dose of trametinib _____ daily.**		
Date	**Day of Treatment**	**Day of the Week**	**Time**	**Check Box if Dose Missed**	**Patient Comments**
Example 1: 5/21/12	1	Monday	3 : 15 ☐ AM ☒ PM	☐	
Example 2: 5/22/12	2	Tuesday	_____:_____ ☐ AM ☐ PM	☒	Forgot to take my pills and didn't remember until the next day.
	1		_____:_____ ☐ **AM** ☐ **PM**	☐	
	2		_____:_____ ☐ **AM** ☐ **PM**	☐	
	3		_____:_____ ☐ **AM** ☐ **PM**	☐	
	4		_____:_____ ☐ **AM** ☐ **PM**	☐	
	5		_____:_____ ☐ **AM** ☐ **PM**	☐	
	6		_____:_____ ☐ **AM** ☐ **PM**	☐	
	7		_____:_____ ☐ **AM** ☐ **PM**	☐	
	8		_____:_____ ☐ **AM** ☐ **PM**	☐	
	9		_____:_____ ☐ **AM** ☐ **PM**	☐	
	10		_____:_____ ☐ **AM** ☐ **PM**	☐	
	11		_____:_____ ☐ **AM** ☐ **PM**	☐	
	12		_____:_____ ☐ **AM** ☐ **PM**	☐	
	13		_____:_____ ☐ **AM** ☐ **PM**	☐	
	14		_____:_____ ☐ **AM** ☐ **PM**	☐	
	15		_____:_____ ☐ **AM** ☐ **PM**	☐	
	16		_____:_____ ☐ **AM** ☐ **PM**	☐	

Date	Day of Treatment	Day of the Week	Time	Check Box if Dose Missed	Patient Comments
	17		**_____:_____ ☐ AM ☐ PM**	☐	
	18		**_____:_____ ☐ AM ☐ PM**	☐	
	19		**_____:_____ ☐ AM ☐ PM**	☐	
	20		**_____:_____ ☐ AM ☐ PM**	☐	
	21		**_____:_____ ☐ AM ☐ PM**	☐	
	22		**_____:_____ ☐ AM ☐ PM**	☐	
	23		**_____:_____ ☐ AM ☐ PM**	☐	
	24		**_____:_____ ☐ AM ☐ PM**	☐	
	25		**_____:_____ ☐ AM ☐ PM**	☐	
	26		**_____:_____ ☐ AM ☐ PM**	☐	
	27		**_____:_____ ☐ AM ☐ PM**	☐	
	28		**_____:_____ ☐ AM ☐ PM**	☐	

DIRECTIONS: Take by mouth on an empty stomach, either 1 hour before or 2 hours after a meal. You will take the mediciation once per day, at the same time each day. If you miss a dose of your mediciation, you should take it as soon as you remember that day up to 6 hours past the scheduled time. If more than 6 hours has passed since the scheduled time, do NOT take the missed dose. Please inform your study doctor of any new medications you are taking.

Please be sure to bring this calendar and all pill bottles with you when you return to the clinic at the end of this cycle. Please remember to give everything to the research staff member that gave them to you. Please sign and date below. Thank you!

Patient's Signature: _____
Date: _____

For Official Use Only

Medication Amt Dispensed _____

Date Dispensed _____

Medication Amt Returned _____

Date Returned _____

Official Use Section Completed by:
Name: _____

Date: _____

APPENDIX D MEDICATION DIARY – PART 2

PATIENT ID: _____			**PATIENT INITIALS (F M L): _____ _____ _____**		
PART _____ CYCLE#: _____			**Dose of trametinib _____ daily. Dose of GSK2141795 _____ daily.**		
Date	**Day of Treatment**	**Day of the Week**	**Time**	**Check Box if Dose Missed**	**Patient Comments (please indicate if both pills were taken)**
Example 1: 5/21/12	1	Monday	**Trametinib** 7 : 15 ☒ AM ☐ PM **GSK2141795** 6 : 30 ☐ AM ☒PM	☐ ☐	
Example 2: 5/22/12	2	Tuesday	**Trametinib** 7 : 00 ☒ AM ☐ PM **GSK2141795** _____:_____ ☐ AM ☐ PM	☐ ☒	Forgot to take my pills and didn't remember until the next day.
	1		**Trametinib** _____:_____ ☐ AM ☐ PM **GSK2141795** _____:_____ ☐ AM ☐ PM	☐ ☐	
	2		**Trametinib** _____:_____ ☐ AM ☐ PM **GSK2141795** _____:_____ ☐ AM ☐ PM	☐ ☐	
	3		**Trametinib** _____:_____ ☐ AM ☐ PM **GSK2141795** _____:_____ ☐ AM ☐ PM	☐ ☐	
	4		**Trametinib** _____:_____ ☐ AM ☐ PM **GSK2141795** _____:_____ ☐ AM ☐ PM	☐ ☐	
	5		**Trametinib** _____:_____ ☐ AM ☐ PM **GSK2141795**	☐ ☐	

			_____:_____ □ AM □ PM		
	6		**Trametinib** _____:_____ □ AM □ PM **GSK2141795** _____:_____ □ AM □ PM	□ □	
Date	**Day of Treatment**	**Day of the Week**	**Time**	**Check Box if Dose Missed**	**Patient Comments**
	7		**Trametinib** _____:_____ □ AM □ PM **GSK2141795** _____:_____ □ AM □ PM	□ □	
	8		**Trametinib** _____:_____ □ AM □ PM **GSK2141795** _____:_____ □ AM □ PM	□ □	
	9		**Trametinib** _____:_____ □ AM □ PM **GSK2141795** _____:_____ □ AM □ PM	□ □	
	10		**Trametinib** _____:_____ □ AM □ PM **GSK2141795** _____:_____ □ AM □ PM	□ □	
	11		**Trametinib** _____:_____ □ AM □ PM **GSK2141795** _____:_____ □ AM □ PM	□ □	
	12		**Trametinib** _____:_____ □ AM □ PM **GSK2141795** _____:_____ □ AM □ PM	□ □	
	13		**Trametinib** _____:_____ □ AM □ PM **GSK2141795** _____:_____ □ AM □ PM	□ □	

	14		**Trametinib** _____:_____ □ AM □ PM **GSK2141795** _____:_____ □ AM □ PM	□ □	
	15		**Trametinib** _____:_____ □ AM □ PM **GSK2141795** _____:_____ □ AM □ PM	□ □	
	16		**Trametinib** _____:_____ □ AM □ PM **GSK2141795** _____:_____ □ AM □ PM	□ □	
Date	**Day of Treatment**	**Day of the Week**	**Time**	**Check Box if Dose Missed**	**Patient Comments**
	17		**Trametinib** _____:_____ □ AM □ PM **GSK2141795** _____:_____ □ AM □ PM	□ □	
	18		**Trametinib** _____:_____ □ AM □ PM **GSK2141795** _____:_____ □ AM □ PM	□ □	
	19		**Trametinib** _____:_____ □ AM □ PM **GSK2141795** _____:_____ □ AM □ PM	□ □	
	20		**Trametinib** _____:_____ □ AM □ PM **GSK2141795** _____:_____ □ AM □ PM	□ □	
	21		**Trametinib** _____:_____ □ AM □ PM **GSK2141795** _____:_____ □ AM □ PM	□ □	
	22		**Trametinib**	□	

			_____:_____ □ AM □ PM **GSK2141795** **_____:_____** □ AM □ PM	□	
	23		**Trametinib** _____:_____ □ AM □ PM **GSK2141795** **_____:_____** □ AM □ PM	□ □	
	24		**Trametinib** _____:_____ □ AM □ PM **GSK2141795** **_____:_____** □ AM □ PM	□ □	
	25		**Trametinib** _____:_____ □ AM □ PM **GSK2141795** **_____:_____** □ AM □ PM	□ □	
	26		**Trametinib** _____:_____ □ AM □ PM **GSK2141795** **_____:_____** □ AM □ PM	□ □	
Date	**Day of Treatment**	**Day of the Week**	**Time**	**Check Box if Dose Missed**	**Patient Comments**
	27		**Trametinib** _____:_____ □ AM □ PM **GSK2141795** **_____:_____** □ AM □ PM	□ □	
	28		**Trametinib** _____:_____ □ AM □ PM **GSK2141795** **_____:_____** □ AM □ PM	□ □	
DIRECTIONS: Take trametinib by mouth on an empty stomach, either 1 hour before or 2 hours after a meal. GSK2141795 must be taken 1 hour after a meal and two hours before the next meal. It is recommended that you take trametinib in the morning and GSK2141795 in the evening, however this is not required. You will take the medication once per day, at the same time each day. If you miss a dose of your medication, you should take it as soon as you remember that day up to 6 hours past the scheduled time. If more than 6 hours has passed since the scheduled time, do NOT take the missed dose. Please inform your study doctor of any new medications you are taking.					

Please be sure to bring this calendar and all pill bottles with you when you return to the clinic at the end of this cycle. Please remember to give everything to the research staff member that gave them to you. Please sign and date below. Thank you!	
Patient's Signature: _____ Date: _____	
For Official Use Only Medication Amt Dispensed _____ Medication Amt Returned _____	Date Dispensed _____ Date Returned _____
Official Use Section Completed by: Name: _____ Date: _____	

APPENDIX E LABORATORY MANUAL

The laboratory manual from the Solid Tumor Shared Resource of the Ohio State University is available upon request from the Director Dr. Cynthia Timmers

Cynthia Timmers, Ph.D.
Director, Solid Tumor Translational Science Resource
The Ohio State University Comprehensive Cancer Center-
Arthur G. James Cancer Hospital and Richard J. Solove Research Institute
460 12th Avenue, BRT Rm 486
Columbus, OH 43210
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cynthia.timmers@osumc.edu

APPENDIX F: BLOOD GLUCOSE TESTING DIARY – OSU-13117 (NCI-9455)

Patient ID: _____	**Patient Initials (F M L): ____ ____ ____**

Directions: Take your blood sugar each morning before you eat or drink anything. Record your blood sugar readings, date, time and comments in this diary. Call your doctor if your blood sugar reading is less than 70 or greater than 250.

Date MM/DD/YY	Time ____:____ AM/PM	Blood Glucose Reading	Patient Comments
Example 1: 11/15/13	8:00 AM	120	
Example 2: 11/16/13	7:30 AM	64	Called doctor to report low blood sugar. Drank orange juice and repeated blood sugar per doctor instructions. Repeat blood sugar at 8:15 AM = 106

Please be sure to bring this diary with you when you return to the clinic for every visit.

Patient's Signature:_____________________________________ Date: ____________________

For Official Use Only:

Diary Received By: _____________________________________ Date: ____________________
(Study Staff Name)

APPENDIX G: INFORMATION ON POSSIBLE DRUG INTERACTIONS

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

[Note to investigators: This appendix consists of an "information sheet" to be handed to the patient at the time of enrollment. Use or modify the text as appropriate for the study agent, so that the patient is aware of the risks and can communicate with their regular prescriber(s) and pharmacist. A convenient wallet-sized information card is also included for the patient to clip out and retain at all times.]

The patient _____ is enrolled on a clinical trial using the experimental agent **trametinib DMSO and GSK2141795.** *This clinical trial is sponsored by the National Cancer Institute. This form is addressed to the patient, but includes important information for others who care for this patient.*

GSK2141795 interacts 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:

GSK2141795 interacts with certain specific enzymes in your liver.

- The enzymes in question are ***CYP450 3A4 and 2C8.*** GSK2141795 levels are affected by some of these enzymes and can lower the levels of other medicines you take.
- GSK2141795 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. GSK2141795 should not be taken with any other drugs that are strong inducers or inhibitors of CYP 3A4. 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. Mild/moderate inhibitors/inducers should be used with caution.
    - GSK2141795 is considered an inhibitor of CYP 3A4 and 2C8, meaning that it can increase 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***."
- 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 drink grapefruit juice or eat grapefruit, Seville oranges, pummelos, exotic citrus fruits or grapefruit hybrids: Avoid these until the study is over.
    - If you take herbal medicine regularly: You should not take St. John's wort while you are taking GSK2141795.

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

Bhuvaneswari Ramaswamy, MD and she can be contacted at :

Phone number: 614-293-6401

INFORMATION ON POSSIBLE DRUG INTERACTIONS

You are enrolled on a clinical trial using the experimental agents **trametinib DMSO and GSK2141795.** This clinical trial is sponsored by the NCI. GSK2141795 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.

GSK2141795 interacts with specific liver enzymes called **CYP 3A4 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**."
- 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 _____.