To: CTEP Protocol and Information Office
From: Rachel Layman, M.D.
Date: December 13, 2022
Re: Response to Biobank Comments of Protocol #10480: "Phase Ib/II Study of EPA-based EphA2 Targeted Therapy for Patients with Metastatic Triple-Negative Inflammatory Breast Cancer"

I. Protocol Revision 5 Summary of Change:

#	Section	Comments
1.	Header	Updated version date from April 27, 2022 to December 13, 2022.
2.	Protocol Title Page	Local protocol number is added, Principle investigator is changed into Rachel Layman, add Study Coordinator Angela Alexander, protocol version is updated. **Rationale**: Dr. Naoto Ueno has left institution and Dr. Layman is the new PI assigned for this trial. Meanwhile, the local protocol number, study coordinator and protocol version are updated accordingly.
3.	Protocol Content	There is no change to protocol content in this amendment except personnel change.

NCI Protocol #: 10480

Local Protocol #: 2022-0246

ClinicalTrials.gov Identifier: NCT05198843

TITLE: Phase Ib/II Study of EPA-based EphA2 Targeted Therapy for Patients with Metastatic Triple-Negative Inflammatory Breast Cancer

Corresponding Organization: LAO-TX035 / University of Texas MD Anderson Cancer Center LAO

Principal Investigator: Rachel Layman
The University of Texas MD Anderson Cancer Center
Dan L. Duncan Building (CPB5.3450)
Houston, TX 77030-3721
Phone: (713) 745-8401
Fax: (713) 794-4385
RLayman@mdanderson.org

Co-Principal Investigator: Toshiaki Iwase
The University of Texas MD Anderson Cancer Center
Dan L. Duncan Building (CPB5.1354)
Houston, TX 77030-3721
Phone: (713) 294-1726
Fax: (713) 794-4385
tiwase@mdanderson.org

Translational PI: Jangsoon Lee
Zayed Research Building (Z12.5036)
6565 MD Anderson Blvd.
Houston, TX 77030-4009
Phone: (713) 563-9221
Fax: (713) 794-4385
jslee@mdanderson.org

Participating Organizations

LAO-11030 / University Health Network Princess Margaret Cancer Center LAO
LAO-CA043 / City of Hope Comprehensive Cancer Center LAO
LAO-CT018 / Yale University Cancer Center LAO
LAO-MA036 / Dana-Farber - Harvard Cancer Center LAO
LAO-MD017 / JHU Sidney Kimmel Comprehensive Cancer Center LAO

LAO-OH007 / Ohio State University Comprehensive Cancer Center LAO
LAO-PA015 / UPMC Hillman Cancer Center LAO
LAO-TX035 / University of Texas MD Anderson Cancer Center LAO
LAO-NCI / National Cancer Institute LAO

Interventional Radiologist:
Sanjay Gupta
University of Texas MD Anderson Cancer Center
T. Boone Pickens Academic Tower
(FCT14.6032)
1400 Pressler St.
Houston, TX 77030-4008
Phone: (713) 794-4855
Fax: (713) 792-4098
sgupta@mdanderson.org

Statistician:
Ying Yuan
University of Texas MD Anderson Cancer Center
T. Boone Pickens Academic Tower
(FTC14.6034)
1400 Pressler St.
Houston, TX 77030-4008
Phone: (713) 563-4271
Fax: (713) 563-4243
yyuan@mdanderson.org

Study Coordinator:
Angela Alexander
The University of Texas MD Anderson Cancer Center
1155 Pressler Street
Houston, TX 77030
(CPB5.3443)
Phone: (713) 792-9137
Fax: (713) 563-0905
AAlexand@mdanderson.org

Responsible Research Nurse:
Vivian Chiv
University of Texas MD Anderson Cancer Center
1155 Pressler St.
Houston TX 77030
(CPB5.3443)
Phone: (713) 792-4157
Fax: (713) 563-0905
vychiv@mdanderson.org

Responsible Data Manager:
Megumi Kai
University of Texas MD Anderson Cancer Center
1155 Pressler St.
Houston TX 77030
(CPB5.3443)

Phone: (713)792-3628
Fax: (713)563-0905
mkai@mdanderson.org

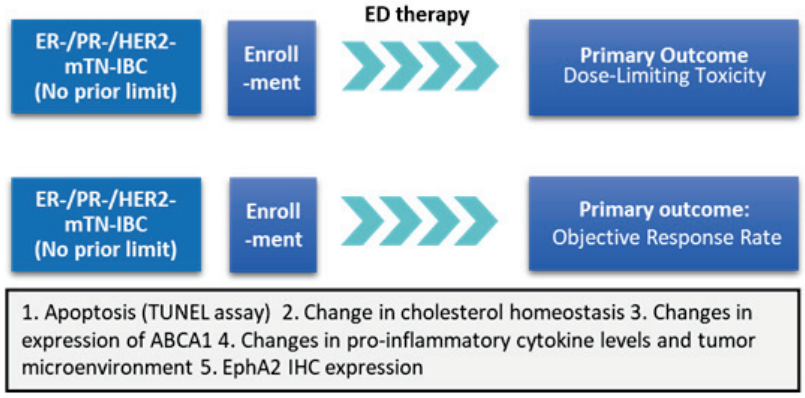
NCI-Supplied Agent: Dasatinib (BMS-354825, Sprycel) (NSC#732517)
Commercial Agent: Icosapent ethyl (EPA) (NSC#759597)

IND Sponsor: DCTD, NCI

Protocol Type / Version # / Version Date:
Original / July 21, 2021
Revision 1 / October 13, 2021
Revision 2 / December 14, 2021
Revision 3a / January 28, 2022
Revision 4 / March 03, 2022
Amendment 1 / April 27, 2022
Amendment 2/December 13, 2022

SCHEMA

This is a multicenter, single-arm, non-randomized phase 1b/2 trial of dasatinib (BMS-354825, Sprycel) and icosapent ethyl (EPA) combination therapy for patients with metastatic triple-negative inflammatory breast cancer (mTN-IBC). All eligible patients will receive dasatinib and EPA combination therapy without randomization or stratification. The study design is summarized below.



Overview of phase 1b/2 trial of EPA-dasatinib combination therapy. 1 Cycle= 28 days.

Phase 1b Dose Escalation Schedule			
Dose Level	**Dose***		
	Dasatinib (mg)	**Icosapent ethyl (EPA) (mg)**	**Cycle Length**
Level 1*	100 mg, PO, QD	2000 mg, PO, BID	28 days
Level 2	100 mg, PO, QD	3000 mg, PO, BID	
Level 3	100 mg, PO, QD	4500 mg, PO, BID	
*Starting Dose Level. PO = Orally, QD = Once daily, BID = Twice a day			

Phase 2				
Agent	**Dose**	**Route**	**Schedule**	**Cycle Length**
Dasatinib	100 mg	PO	QD	28 days
Icosapent ethyl (EPA)	MTD/RP2D	PO	BID	28 days
MTD = Maximum tolerated dose, RP2D = Recommended phase 2 dose, PO = Orally, QD = Once daily, BID = Twice a day				

TABLE OF CONTENTS

1. OBJECTIVES

1.1 Primary Objectives

1.1.1 Primary Objective (Phase 1b): To determine MTD/RP2D of EPA and dasatinib in patients with mTN-IBC.

1.1.2 Primary Objective (Phase 2): To determine the overall response rate (ORR) of EPA and dasatinib therapy in patients with mTN-IBC.

1.2 Secondary Objectives

1.2.1 To determine the clinical benefit rate (CBR) of EPA and dasatinib therapy in patients with mTN-IBC.

1.2.2 To determine progression-free survival (PFS) at 1 year for patients with mTN-IBC who were enrolled in the study and received EPA and dasatinib therapy.

1.2.3 To determine overall survival (OS) at 2 years for patients with mTN-IBC who were enrolled in the study and received EPA and dasatinib therapy

1.2.4 To determine the induction of apoptosis by EPA and dasatinib therapy.

1.3 Exploratory Objectives

1.3.1 To determine the effect of EPA and dasatinib therapy on the expression of cholesterol transporter.

1.3.2 To determine the relationship between the expression of EphA2 and the treatment response to EPA and dasatinib therapy.

1.3.3 To determine the change in Ki67 by EPA and dasatinib therapy.

1.3.4 To evaluate the change in cholesterol homeostasis and tumor membrane rigidity after EPA and dasatinib therapy.

1.3.5 To investigate the effect of EPA and dasatinib therapy on the systemic inflammation.

2. BACKGROUND

2.1 Study Disease

Triple-negative inflammatory breast cancer (TN-IBC) is the most aggressive subtype of breast cancer, which has poor outcomes (Masuda *et al*., 2013). It is also associated with higher rates of recurrence and metastasis and a lower survival rate than non-IBC (Hance *et al*, 2005). The

incidence of IBC in the United States is 1.6–3.1 per 100,000 women with a higher incidence among black women and younger women, but owing to its aggressive nature, IBC represents approximately 8–10% of breast cancer deaths (Hance *et al*., 2005; Goldner *et al*., 2014).

The inflammatory process contributes to the aggressiveness of IBC (Lim *et al*., 2018). Recent studies have suggested that immune cells in the tumor microenvironment play a major role in regulating the malignant phenotype of IBC (Allen *et al*., 2016; Wolfe *et al*., 2016). IBC is a rare tumor without specific treatment (Lim *et al*., 2018). Further, IBC with a triple-negative molecular subtype lacks specific targeted therapy; chemotherapy is the main treatment choice and results in major toxicities. These are compelling reasons for developing a novel treatment for TN-IBC.

2.2 CTEP IND Agent

2.2.1 Dasatinib (BMS-354825, Sprycel)

Dasatinib (BMS-354825, Sprycel), an aminothiazole analogue, is an orally administered (PO) protein tyrosine kinase (PTK) inhibitor with specificity for five kinases/kinase families that have been strongly linked to multiple forms of human malignancies (Hunter, 1997; Investigator's Brochure, 2020; Lombardo *et al.*, 2004). These targets include: BCR-ABL, c-SRc, c-KIT, PDGFβ receptor, and EPHA2. *In vivo* and *in vitro* studies have established that dasatinib demonstrates potent antiproliferative activity in a wide spectrum of cancer cell lines/types, and clinical results suggest anticancer activity of dasatinib in chronic myelogenous leukemia (CML) and solid tumor patients (Demetri *et al.,* 2009; Li *et al.*, 2004; Schittenhelm *et al.*, 2006; Wu *et al.*, 2004).

2.2.1.1 Mechanism of Action

Dasatinib potently and selectively inhibits the five oncogenic PTKs/kinase families by competing with ATP for the ATP-binding sites in the kinases: SRC family kinases (IC_{50}: SRC = 0.55 nM, LCK = 1.1 nM, YES = 0.41 nM, FYN = 0.2 nM); BCR-ABL (<3 nM); c-KIT (13 nM); EPHA2 (17 nM) and PDGFβ receptor (28 nM) (Investigator's Brochure, 2020). The agent was found to be less potent against unrelated PTKs and several serine/threonine kinases. Dasatinib also demonstrates potent inhibition of VEGF- and bFGF-driven proliferation of human umbilical vein endothelial cells (HUVECs), with IC_{50} values of 43 and 248 nM, respectively.

BCR-ABL, a constitutively active cytoplasmic tyrosine kinase, is present in >90% of all patients with CML and in 15-30% of adult patients with acute lymphoblastic leukemia (ALL). The inhibition of BCR-ABL by imatinib, another PTK inhibitor, is effective in the management of CML thus providing proof-of-concept for targeting PTKs. However, resistance to imatinib therapy associated with BCR-ABL gene mutation/over-expression and activation of selected SRC kinases has been increasingly encountered (Hochhaus and Hughes, 2004). Dasatinib has activity in a number of imatinib-resistant tumors (Donato *et al*., 2004; Lee *et al.*, 2004; Shah, 2005; Shah *et al.*, 2004; Talpaz *et al.*, 2006) in addition to being 500-fold more potent than imatinib in inhibiting BCR-ABL. The ability of dasatinib to inhibit imatinib-resistant forms of BCR-ABL is presumed to be due to its relaxed binding requirements because, unlike imatinib

which binds only to the inactive conformation of the BCR-ABL kinase, dasatinib binds to both the active and inactive conformations (Tokarski *et al.*, 2004).

2.2.1.2 Nonclinical Studies

2.2.1.2.1 Efficacy

Dasatinib inhibits growth of multiple BCR-ABL-dependent leukemic cell lines and also shows activity against 14 of 15 imatinib-resistant BCR-ABL kinase mutants (Shah *et al.*, 2004). Inhibition of CML cell lines established from patients who were resistant to imatinib therapy has also been reported (Wu *et al.*, 2004). Dasatinib potently inhibits wild-type (IC_{50}: 1-10 nM) and mutant (IC_{50}: 10-100 nM) KIT kinases in M07E cells and human mast cell leukemia cell lines, respectively (Schittenhelm *et al.*, 2006). Also of note, dasatinib selectively killed primary neoplastic bone marrow mast cells from patients with systemic mastocytosis while sparing other hematopoietic cells (Shah *et al.*, 2005).

Dasatinib demonstrated antiproliferative activity in a wide spectrum of solid tumor types, including mastocytoma, prostate, and breast cell lines with IC_{50} values ranging from 5.4-103 nM (Investigator's Brochure, 2020). The agent also inhibited stem cell factor-driven proliferation of three small cell lung cancer (SCLC) cell lines with IC_{50} values in the range of 114-220 nM and showed activity in head and neck squamous cell carcinoma and non-small cell lung cancer cell lines (Johnson *et al.*, 2005).

When dasatinib was administered twice daily (BID) on a 5-days-on/2-days-off schedule for a total of 14 to 25 days at doses of 10-50 mg/kg/dose, *in vivo* antitumor activity of dasatinib was seen in prostate, colon, breast, and pancreatic xenograft models (Investigator's Brochure, 2020). Similarly, dasatinib was effective against K562 and imatinib-resistant K562-R human CML xenografts in SCID mice at doses as low as 2.5-5 mg/kg/day (Lee *et al*., 2004). In combination with docetaxel, dasatinib produced antitumor effects against PC3 human prostate carcinoma xenografts that were substantially better than the effects of either single agent alone (Investigator's Brochure, 2020).

Dasatinib at 20 or 50 mg/kg inhibited the T-cell proliferation response in mice following the transfer of lymphocytes from allogeneic donor mice (Investigator's Brochure, 2020). In addition, treatment of mice with dasatinib 25 mg/kg BID inhibited the graft-versus-host response in a non-vascularized model of murine heart transplant. The 5-days-on/2-days-off regimen almost completely eliminated immunosuppressive activity in this model.

SRC kinase is known to play a major role in osteoclast function. In short-term studies, dasatinib acted as a potent inhibitor of bone resorption as measured by its ability to reduce the release of ^{45}calcium into the culture medium by fetal rat long bones *in vitro* (IC_{50} = 2 nM). Dasatinib also inhibited parathyroid hormone (PTH)-stimulated release of ^{45}calcium in a dose-dependent manner with an apparent IC_{50} of 2 nM. At 5 nM, dasatinib completely blocked PTH-stimulated bone resorption in thyro-parathyroidectomized rats. The therapeutic utility of dasatinib in the treatment of cancer-related hypercalcemic syndromes has not been fully explored, and the long-term effects of dasatinib on bone physiology are also unknown.

2.2.1.2.2 Nonclinical Pharmacokinetic and Pharmacodynamic Studies

Nonclinical metabolic and pharmacokinetic (PK) studies were conducted with dasatinib in several species including mouse, rat, dog, and monkeys to assess the absorption, distribution, metabolism, and excretion of the compound in animals. These studies showed that dasatinib has varying degrees of oral bioavailability, ranging from 15% in monkeys to 34% in dogs. The permeability of dasatinib in the Caco-2 cell model is 102 nm/sec at pH 7.4, suggesting that it has the potential for good (>50%) oral absorption in humans. The agent is highly bound to serum proteins (>91%) and has extensive extravascular distribution. Dasatinib is principally eliminated by hepatic metabolism and excreted in feces. The agent is primarily metabolized by the CYP3A4 enzyme to produce multiple metabolites.

The value of phospho-SRC as a biomarker of dasatinib efficacy has been explored in nonclinical studies (Luo *et al.*, 2005). In nude mice bearing subcutaneous PC-3 tumors (human prostate), measurement of phospho-SRC by western blot in tumor and peripheral blood mononuclear cells (PBMCs) following treatment with a single dose of dasatinib (15 or 50 mg/kg) produced similar results in both tissues. Levels of phospho-SRC were maximally inhibited at 3 hours post dose, then recovered partially between 7 and 17 hours and returned to the basal level by 24 hours after agent administration. These results were quantitated by image scanning and compared to efficacy results when the agent was administered PO BID at 15-50 mg/kg/dose for 14 days on a 5-days-on/2-days-off schedule. Efficacy and phospho-SRC inhibition appeared to correlate, and this pharmacodynamic model permitted the authors to predict that the plasma concentration of dasatinib required to produce 90% inhibition of phospho-SRC would be 164 nM and 91 nM in PC-3 tumor and PBMCs, respectively. Studies to evaluate the clinical utility of phospho-SRC as a biomarker are ongoing.

2.2.1.2.3 Toxicology

A range of toxicology studies have been conducted to support the oral administration of dasatinib in humans. The oral studies indicated that dasatinib induced reversible toxicities of the gastrointestinal (GI) and lymphoid systems in rats and monkeys, and of the hematopoietic system in rats. Embryofetal development studies in rats and rabbits indicated that dasatinib caused embryo lethality or skeletal malformations at doses that did not cause maternal toxicity, suggesting that it is a selective developmental toxicant. An *in vitro* cytogenetics study in CHO cells indicated that it was clastogenic at concentrations >5 μg/mL, a level not achievable in vivo. The agent is nongenotoxic and did not show significant potential for undesirable functional activity in in vitro receptor/ion channel binding and enzyme assays. *In vitro* potassium channel current (HERG/IKr) and Purkinje fiber assays suggested that dasatinib could potentially prolong cardiac ventricular repolarization (QT interval), and a single-dose cardiovascular study in monkeys demonstrated that the agent at a dose of 10 mg/kg caused a minimal increase in blood pressure for approximately 2 hours post dose. There were no drug-related neurologic observations in rats or monkeys. Dasatinib was found to be phototoxic in an *in vitro* assay in mouse fibroblasts.

2.2.1.3 Clinical Experience

Over 2000 subjects have received dasatinib, the majority with CML refractory or intolerant to imatinib (Investigator's Brochure, 2020). Studies conducted in healthy volunteers include the following: PK; formulation comparisons; the effect of food; drug interactions; and supportive care. Data are available from 11 phase 1 and phase 2 studies in patients with CML, Philadelphia chromosome-positive (Ph+) ALL, or solid tumors using different dosage regimens and designed to determine PK, pharmacodynamics, safety, and efficacy in these populations.

2.2.1.3.1 Pharmacokinetics

Pharmacokinetic (PK) studies were conducted using a single 100 mg dose of dasatinib administered to healthy volunteers in four different formulations: 50 mg clinical tablets x 2, 5 mg clinical tablets x 20, 20 mg commercial tablets x 5, and 50 mg commercial tablets x 2. The PK profile of the agent was similar in all four formulations. The PK profile of dasatinib was also assessed in CML and Ph+ ALL patients providing data which showed that the PK parameters in the patient population appear to be similar to that in the healthy volunteers. The agent was absorbed rapidly following oral administration; peak plasma concentrations were achieved in 0.5-3 hours and dose-related increases in plasma concentrations were observed. The mean terminal half-life ($t_{1/2}$) of dasatinib was 4 hours. Dosing interval exposures and $t_{1/2}$ values were comparable regardless of whether the agent was administered on a once daily or twice daily (BID) 5-day-on/2-day-off schedule, or BID continuously.

A phase 1 study has been initiated in solid tumor patients to determine the effect of the CYP3A4 inhibitor ketoconazole on dasatinib PK. In a study of 18 patients with solid tumors, 20 mg of dasatinib once daily coadministered with 200 mg of ketoconazole twice daily increased the dasatinib C_{max} and AUC by four- and five-fold, respectively.

2.2.1.3.2 Efficacy

A phase I study treated patients with CML in chronic phase (CP) or advanced disease (accelerated phase or blast crisis) or Ph+ ALL who were intolerant or resistant to imatinib (Sawyers *et al*., 2005; Talpaz *et al.*, 2006). Dasatinib was administered once daily at doses ranging from 15 to180 mg/day or BID at doses ranging from 25 to 50 mg for 5-7 consecutive days each week. Complete hematologic response was documented in 37 of 40 CP patients (92%) and the rate was similar with both schedules (once daily or BID). Fourteen CP patients (35%) achieved a complete cytogenetic response and four (10%) experienced partial responses. In 44 patients with advanced CML or Ph+ ALL, 31 major hematologic responses were documented (70%). Cytogenetic responses were documented in 25 advanced CML or ALL patients, including complete responses in 11 patients.

A phase 1 study has been conducted in patients with refractory solid tumors in order to evaluate the safety, tolerability, and the pharmacologic profile of dasatinib (Demetri *et al.*, 2009). Patients received escalating doses (25 to 120 mg) of dasatinib without food administered BID for 5 consecutive days every week followed by 2 days of rest (5D2 schedule), or on a continuous daily dosing (CDD) schedule. There were no objective responses on CT scans, but stable disease

(SD) was observed in 11 patients (16%), including three gastrointestinal stromal tumor (GIST) patients. These 11 were comprised of seven (21%) of 33 patients on the 5D2 schedule and four (12%) of 34 patients on the CDD schedule. The median duration of SD was 3.6 months (range 1.7 – 23.6 months). The investigators noted that the clinical benefits of the agent in a subset of imatinib-resistant GIST patients have been encouraging.

In solid tumors, the recommended phase 2 dose for dasatinib was found to be 120 mg BID on the 5D2 schedule, or 70 mg BID on the CDD regimen (Demetri *et al.,* 2009). In October 2010, dasatinib was approved by the FDA for treatment of chronic, accelerated, and blast phase CML and Ph+ ALL with resistance or intolerance to imatinib. Results from two phase 3 dose-optimization studies supported starting doses of 100 mg once/day for CP CML and 140 mg once/day for imatinib-resistant accelerated phase CML, myeloid or lymphoid blast phase CML, or Ph+ ALL. A randomized phase 3 trial comparing dasatinib to imatinib led to FDA approval of dasatinib (100 mg once/day) for first-line therapy of newly diagnosed CML.

2.2.1.3.3 Safety

Myelosuppression, probably attributable to suppression of the Ph+ clone, was the most frequent adverse event (AE) in the phase 1 study in CML or Ph+ ALL, while the most significant AE was grade 3/4 thrombocytopenia (28%) (Investigator's Brochure, 2020). Severe myelosuppression was reversible and easily managed with a short dose interruption; about 60% of patients required interruption of treatment, and the myelosuppression generally resolved within 3 months, often in association with a cytogenetic response (Talpaz *et al.,* 2006). Twenty-five percent of leukemia patients required a dose reduction. In the phase 1 study in solid tumor patients, hematologic AEs were uncommon: two patients on the 5D2 schedule with grade 1 or 2 anemia developed grade 3 anemia while on study drug, whereas on the CDD schedule one patient developed grade 4 neutropenia and another grade 3 anemia (Demetri *et al.,* 2009).

Non-hematologic AEs from the two phase 1 trials include GI intolerance (primarily diarrhea, nausea, and vomiting), GI hemorrhage, fatigue, dyspnea, anorexia, dehydration, fluid retention, pleural and pericardial effusion, a moderate increase in QTcF (with no QTcF >500 msec), elevated creatinine, depression, and tumor lysis syndrome. In addition to these AEs, dasatinib treatment has the potential to produce skin rashes, other respiratory events, and CNS hemorrhage. While neither immunosuppression nor osteoclast function abnormalities (*e.g.*, osteoporosis) were observed in these short-term studies, SRC kinase inhibitors have the potential to cause these types of events.

As of September 2010 in the overall population of 2840 subjects, a total of 740 (26%) deaths were reported in adult dasatinib-treated subjects with CML or Ph+ ALL. Of these 740 deaths, 296 occurred within 30 days of the last dose of study therapy. Of the 740 deaths, 380 (51%) were due to disease progression and 11 (1.5%) have been positively attributed to study drug toxicity.

2.2.1.4 Potential Drug Interactions

Dasatinib is primarily metabolized by CYP3A4 and therefore, potent inhibitors of this enzyme

are contraindicated (Investigator's Brochure, 2020). Dasatinib is also a significant inhibitor of this hepatic enzyme but a weak inhibitor of other cytochrome enzymes, and the agent does not induce CYP3A4. Thus, dasatinib may decrease the clearance of drugs that are significantly metabolized by the CYP3A4 enzyme, and caution should be used with concurrent use of such drugs or substances. In a study in cancer patients, concomitant use of a potent CYP3A4 inhibitor (ketoconazole) produced >5-fold increase in exposure to dasatinib, while healthy subjects treated concurrently with dasatinib and a potent CYP3A4 inducer experienced a 5-fold decrease in dasatinib exposure. When the CYP3A4 substrate simvastatin was studied in combination with dasatinib, increased simvastatin exposure resulted, indicating the necessity of caution when dasatinib is administered with CYP3A4 substrates with a narrow therapeutic margin (*e.g.,* cyclosporine).

2.3 Icosapent ethyl (EPA)

Icosapent ethyl is an ethyl ester of the omega-3 fatty acid eicosapentaenoic acid (EPA), and acts as a lipid-regulating agent (VASCEPA™ package insert, 2019). Studies suggest that EPA reduces hepatic very low-density lipoprotein triglycerides (VLDL-TG) synthesis and/or secretion, and enhances TG clearance from circulating VLDL particles. The peak plasma concentrations of EPA are reached approximately 5 hours following oral doses of icosapent ethyl. EPA is mainly metabolized by the liver via beta-oxidation similar to dietary fatty acids. Beta oxidation splits the long carbon chain of EPA into acetyl Coenzyme A, which is converted into energy via the Krebs cycle. Cytochrome P450-mediated metabolism is a minor pathway of EPA elimination. The total plasma clearance of EPA at steady state is 684 mL/hr and the plasma elimination half-life ($t_{1/2}$) of EPA is approximately 89 hours. Furthermore, icosapent ethyl does not undergo renal excretion.

The potential mechanisms of action of icosapent ethyl include increased β-oxidation; inhibition of acyl-CoA:1,2-diacylglycerol acyltransferase (DGAT); decreased lipogenesis in the liver; and increased plasma lipoprotein lipase activity (VASCEPA™ package insert, 2019). The mechanisms of action contributing to reduction of cardiovascular events with icosapent ethyl are not completely understood but are likely multi-factorial. Increased EPA lipid composition from carotid plaque specimens and increased circulating EPA/arachidonic acid ratio have been observed following EPA treatment. EPA inhibits platelet aggregation under some *ex vivo* conditions. However, the direct clinical meaning of these individual findings is not clear.

Icosapent ethyl is indicated as an adjunct to maximally tolerated statin therapy to reduce the risk of myocardial infarction, stroke, coronary revascularization, and unstable angina requiring hospitalization in adult patients with elevated TG levels (≥150 mg/dL) as well as (1) established cardiovascular disease or (2) diabetes mellitus and 2 or more additional risk factors for cardiovascular disease (VASCEPA™ package insert, 2019). Icosapent ethyl is also indicated as an adjunct to diet to reduce TG levels in adult patients with severe (≥500 mg/dL) hypertriglyceridemia. However, the effect of icosapent ethyl on the risk for pancreatitis in patients with severe hypertriglyceridemia has not yet been determined and remains a limitation of its use. For more information, see the package insert for VASCEPA™ (2019).

2.4 Rationale

Icosapent ethyl (EPA), a natural omega-3 polyunsaturated fatty acid found in fish oil and available as a dietary supplement, is a U.S. Food and Drug Administration (FDA)-approved agent (Vascepa, Amarin Pharmaceuticals) that has anti-inflammatory properties and the capacity to modulate lipid metabolism with a safe toxicological profile (mild diarrhea occurs in <2.5% of patients) (Bays *et al*., 2011). EPA is an inhibitor of the delta-5-desaturase that produces arachidonic acid, which is necessary for the production of pro-inflammatory eicosanoids (Calder, 2010). Although most cancer prevention studies of EPA have not shown an improved outcome, EPA has demonstrated effects supportive of therapeutic activity. In a randomized clinical trial in recurrent colorectal cancer, EPA treatment reduced plasma CCL2 levels, which predicted improved disease-free survival (DFS; hazard ratio [HR] = 0.32; $P = 0.003$) after surgery for resectable liver metastases (Volpato *et al*., 2016). Also, in patients with newly diagnosed breast cancer, supplementation with EPA and docosahexaenoic acid maintained the serum levels of $CD4^+$ T cells and high-sensitivity C-reactive protein, suggesting a beneficial effect on the immune system (Paixao *et al*., 2017). Preliminary studies revealed the therapeutic potential of EPA in the preclinical setting; EPA reduced *in vitro* proliferation and *in vivo* tumor growth of TN-IBC cells by modulating cholesterol homeostasis (Torres-Adorno *et al*., 2019). A single agent however is not sufficient to obtain a clinically relevant anti-tumor effect in TN-IBC.

To identify a panel of kinase targets whose inhibition bolstered the anti-tumor activity of EPA in TN-IBC, a non-biased functional genomics screen was conducted. This screen led to the identification of EphA2 (ephrin type-A receptor 2) as a potential therapeutic target in TN-IBC. A combination of EPA and inhibition of EphA2 showed the most effective anti-tumor activity among the targets identified. Several EphA2-targeting agents, including multi-tyrosine kinase inhibitor dasatinib (Bristol-Myers Squibb) (Huang *et al*., 2014) and liposomal formulations of EphA2 siRNA, have demonstrated anti-tumor efficacy in preclinical studies (Landen *et al*. 2005). Although dasatinib as a single agent had a low tumor response rate in metastatic breast cancer and was not effective in a preclinical model (Pusztai *et al*., 2014), preclinical data for a response to EPA and dasatinib combination in TN-IBC mouse models is very compelling. This study proposes to develop an EPA and dasatinib combination therapy for metastatic TN-IBC (mTN-IBC) and test its safety and efficacy in a phase 1b/2 trial. As EphA2 is overexpressed in 60% of TN-IBC xenografts (data not shown), successful translation of EPA and dasatinib combination therapy would hold significant clinical benefit for patients with $EphA2^+$ mTN-IBC.

A functional genomics screen identified EPA plus EphA2 inhibition as an enhanced combination therapy for TN-IBC.

With the goal of developing a combination therapy, which includes EPA, for TN-IBC, a high-throughput siRNA screen was conducted (Lee *et al*., 2015) to identify molecular targets that can improve EPA's therapeutic activity without introducing bias. By screening SUM149 TN-IBC cells using a siRNA "druggable gene" library that targets 939 genes, 20 targets were identified that, when inhibited, significantly enhanced the therapeutic potential of EPA; these targets included EphA2, FZD4, FZD8, GSK3B, and Src (Torres-Adorno *et al*., 2019). Gene Ontology analysis of these 20 identified targets revealed enrichment in targets associated with the plasma membrane ($P < 0.05$), suggesting that functional changes within the cell that make EPA more

effective involve membrane biology. Of these targets, 14 genes are associated with plasma membranes (Torres-Adorno *et al*., 2019). EphA2 was selected for further evaluation on the basis of the following findings: 1) EphA2 mRNA levels in TNBC patient tumors (Fig. 1A) and EphA2 protein levels in TNBC cell lines (Fig. 2A) were higher than in non-TNBC compartments; 2) EphA2 mRNA levels were significantly associated with short DFS in patients with TNBC (Fig. 1B). EphA2, a cell surface receptor tyrosine kinase, controls multiple signaling pathways regulating cell differentiation, migration, and proliferation (Ireton and Chen, 2005).

EphA2 expression is elevated in patient samples and TNBC cell lines.

The prevalence of EphA2 in breast cancer cell lines and patient samples was assessed. mRNA (data not shown) and protein (Fig. 2A) levels of EphA2 were higher in TNBC cells than in non-TNBC cells. Analysis of EphA2 mRNA levels in primary breast tumors in 3 datasets of more than 600 patients consistently showed elevated levels in TNBC tumors (Fig. 1A). Importantly, EphA2 mRNA levels in untreated primary tumors were inversely correlated with poor DFS in breast cancer patients (Fig. 1B). These data provide a solid rationale for targeting EphA2 in TNBC.

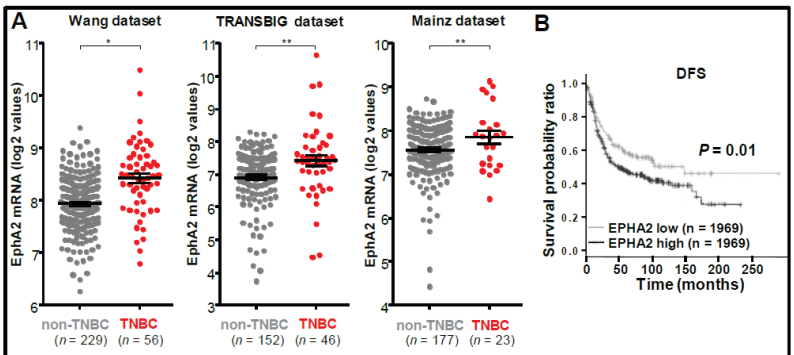


Figure 1. EPHA2 is a clinically significant target in TNBC. A) EphA2 mRNA expression in human breast tumors derived from three datasets. *, $P < 0.05$; **, $P < 0.005$; unpaired *t*-test. **B)** Kaplan-Meier survival curves were generated for disease-free survival (DFS) of patients with TNBC/basal-like breast cancer classified by ***EPHA2*** mRNA levels in tumors (log-rank test comparing 'Lowest' and 'Highest' EphA2 quartiles).

EphA2 expression in subtypes of breast cancer

TNBC is characterized by the distinct pathological feature that it lacks receptor targets (e.g., ER, PR, HER2 receptor). Thus, identification and evaluation of new biomarkers and therapeutic agents constitute a highly mandated unmet medical need. There are 3 major classification methods established for TNBC: the Vanderbilt, Baylor, and French subtypes (Masuda *et al*., 2013). These studies were performed using the Vanderbilt subtypes, which are based on gene expression analysis from 587 TNBC tumors. According to the original Vanderbilt classification, TNBC cells are classified into 7 subtypes by their genetic context: basal-like 1 (BL1), BL2, immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like (MSL), luminal androgen receptor (LAR), and unstable (UNS)(12). In 2016, these subtypes were refined to 4 subtypes (BL1, BL2, M, and LAR) (Lehmann *et al*., 2016).

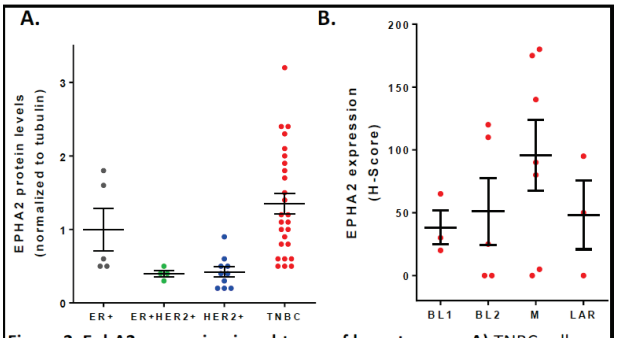


Figure 2. EphA2 expression in subtypes of breast cancer. A) TNBC cell lines express higher levels of EphA2 than non-TNBC cell lines. **B)** BL2 and M subtypes of TNBC tumors relatively express higher levels of EphA2 than BL1 and LAR subtypes.

EphA2 protein expression was analyzed in

different molecular subtypes of breast cancer cell lines and 4 molecular subtypes of TNBC cell-derived xenograft (CDX) tumors. TNBC cells expressed higher levels of EphA2 than other subtypes of breast cancer cells (Fig. 2A). Basal-like 2 (BL2) and mesenchymal (M) subtypes of TNBC CDX tumors had elevated EphA2 expression (Fig. 2B). These results suggest that different TNBC molecular subtypes may have different responses to treatment with EPA and dasatinib combination therapy.

EPA in combination with EphA2 inhibition drastically inhibits the growth of TN-IBC xenografts *in vivo*.

To validate that EphA2 is an important target in the setting of EPA treatment, functional gene silencing using siRNA libraries was performed (Fig. 3A). siEphA2-treated SUM149 and TN-IBC cells were more sensitive to EPA treatment compared to the control siRNA treatment group (Fig. 3B). For the further biological assay, dasatinib was selected, an SRC-family tyrosine kinase, and EphA2 inhibitor, due to a lack of FDA-approved drug to targeting EphA2. EphA2 inhibition by dasatinib enhanced EPA's therapeutic activity in 2 EphA2+ TN-IBC cell lines, SUM149 and BCX010 (Fig. 3C). BCX010 was derived from a TN-IBC patient-derived xenograft (PDX) model that is chemotherapy resistant (McAuliffe *et al*., 2015). When these cells were treated with increasing doses of dasatinib and EPA at an IC_{50} of 50µM, high synergism was observed (combination index < 1 by the Chou-Talalay method [Chou 2010]). Importantly, dasatinib did not show efficacy as a monotherapy or in combination with EPA in HCC38 TN-IBC cells, which express very low levels of EphA2 (Fig. 3C). Moreover, restoration of Src gene expression did not rescue TN-IBC cells from the enhanced killing by EPA and dasatinib combination therapy (Fig. 4), suggesting that the mechanism of action requires EphA2 inhibition.

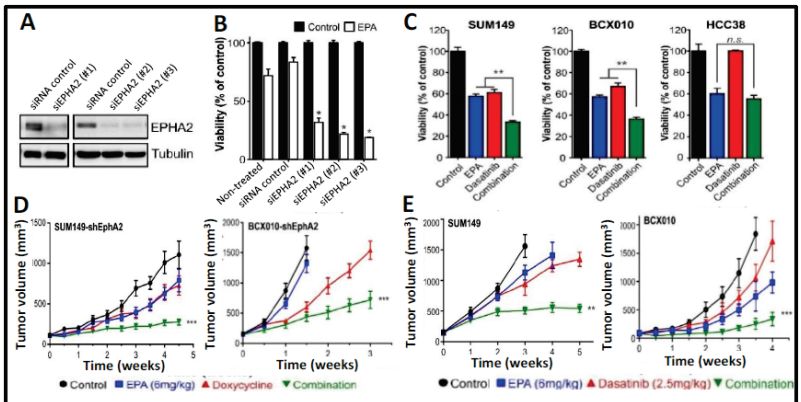


Figure 3. Identification of EphA2, a potential therapeutic target for enhanced EPA-based combination therapy. A) Western blot showing EphA2 knockdown in SUM149 cells using 3 different EphA2 siRNAs. **B)** Enhanced growth inhibition of SUM149 cells following siRNA transfection and EPA treatment, assayed using CellTiter-Glo assay. **C)** EPA/dasatinib combination is effective only in EphA2-expressing TNBC cells. **D)** *In vivo* growth of SUM149-shEphA2 and BCX010-shEphA2 tumor xenografts in mice (n=10/group) treated with EPA, doxycycline, or EPA/doxycycline combination. **E)** *In vivo* growth of SUM149 and BCX010 tumor xenografts in mice (n=10/group) treated with EPA, dasatinib, or EPA/dasatinib combination. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; unpaired *t*-test compared controls and individual monotherapies. *n.s.*, not significant.

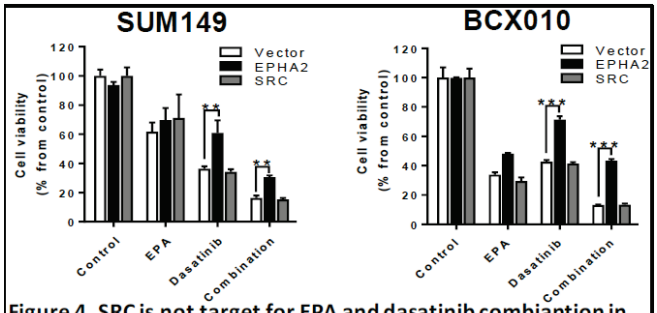


Figure 4. SRC is not target for EPA and dasatinib combiantion in IBC-TNBC. IBC-TNBC cells were transfected with EphA2 or SRC expression vector and then treated with EPA and/or Dox/dasatinib. **, $P < 0.01$; ***, $P < 0.001$; unpaired *t*-test compared to vector control or therapies.

To evaluate whether EPA-based EphA2-targeted combination therapy was effective in animal

models of TN-IBC, EphA2 activity was inhibited *in vivo* by 1) silencing EphA2 gene expression using shRNA or 2) treatment with EphA2 inhibitor dasatinib. Silencing EphA2 gene expression was critical for assessing the effect of specifically targeting EphA2 because dasatinib can target multiple kinases besides Src. Dasatinib was utilized, even though it can target multiple kinases, to demonstrate the translatability of this research to combination therapy in the clinic. Two TN-IBC cell lines were generated with doxycycline (Dox)-inducible shRNA (SUM149- shEphA2 and BCX010-shEphA2) or non-silencing controls (Vector) using pTRIPZ lentiviral vectors. SUM149-shEphA2 or BCX010-shEphA2 cells were implanted into NSG mice (n=10/group). When tumors reached 100 mm^3, the mice were switched to 1) normal diet, normal water (Control); 2) EPA diet (6 mg/kg), normal water; 3) normal diet, water containing Dox (to induce EphA2 knockdown); or 4) EPA diet and water containing Dox. While both monotherapies (EPA treatment or Dox-induced EphA2 knockdown) slowed tumor growth, EPA and dasatinib combination therapy markedly prevented tumor growth (Fig. 3D). Dox treatment had no effect on the growth of tumors derived from non-silencing controls, suggesting that the enhanced therapeutic effect was a result of the loss of EphA2.

EPA and dasatinib combination therapy was further evaluated using SUM149 (chemo-sensitive) and BCX-010 (chemo- resistant) xenograft models. When tumors reached 100 mm^3, the mice were divided into 1) control; 2) EPA diet (6 mg/kg); 3) dasatinib (2.5 mg/kg); and 4) EPA and dasatinib groups. Similarly, compared to treatment with monotherapies (EPA or dasatinib) or vehicle control, EPA and dasatinib combination therapy drastically reduced the growth of tumor xenografts of both cell lines (Fig. 3E). Interestingly, EPA and dasatinib combination reduced tumor growth in both chemo-sensitive (SUM149) and chemo-resistant (BCX-010) TNBC-IBC xenografts. This data indicated that EPA and dasatinib combination therapy could be considered as a novel treatment option for metastatic TNBC patients who received have previously received chemotherapeutic regimens. Collectively, these results provide a rational basis for the development of EPA and dasatinib combination therapy for $EphA2^+$ TN-IBC and demonstrate, by two independent methods, that inhibition of EphA2 drastically boosts the anti-tumor efficacy of EPA.

EPA in combination with EphA2 inhibition increases TN-IBC cell membrane rigidity.

Because EPA is incorporated into the cell membrane in TN-IBC (data not shown), the fluidity of TN-IBC cell membranes was assessed following treatment. Membrane fluidity was assessed by measuring general polarization (GP), whereby higher GP indicates less fluidity (Fig. 5A). EphA2 silencing by Dox (Fig. 5B) or EphA2 activity inhibition by dasatinib (Fig. 5C) did not alter the fluidity of SUM149 cell membranes, whereas when these treatments were combined with EPA, the cell membranes become rigid (Fig.

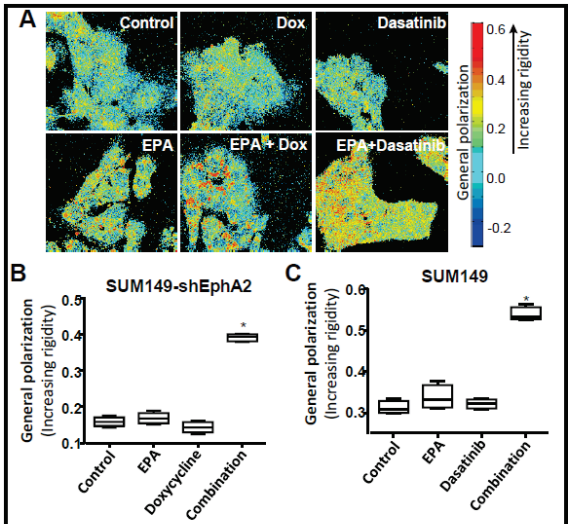


Figure 5. EPA-based combination therapy increases membrane rigidity. A) Fluorescence microscopy showing increased general polarization (GP) of SUM149 cells following EPA-based combination treatment. **B** & **C**, Quantification of GP in **(B)** SUM149-shEphA2 or **(C)** SUM149 cells following treatment. Dox, doxycycline. *, $P < 0.01$; unpaired *t*-test, compared to control and monotherapies.

5B, 5C). The same was observed in BCX010 cells (data not shown). Collectively, these data suggest that EPA and dasatinib combination therapy increases cell membrane rigidity, leading to cell death.

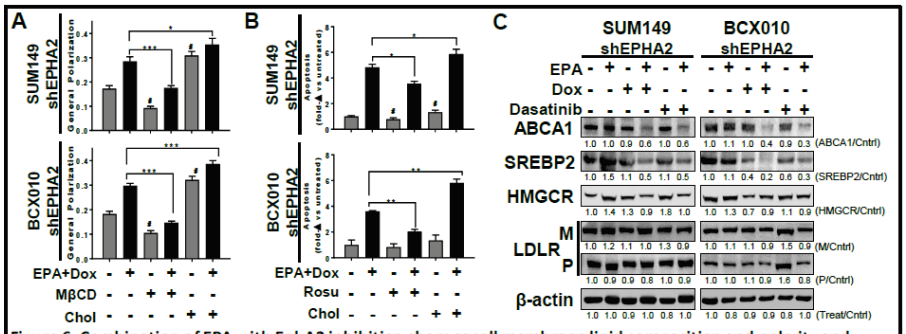


Figure 6. Combination of EPA with EphA2 inhibition changes cell membrane lipid composition and polarity and induces apoptosis via ABCA1 inhibition in TNBC cells. A & B, Dox-inducible SUM149-shEPHA2 and BCX010-shEPHA2 cells were treated with Dox and EPA, with or without cellular cholesterol removal (by methyl-β-cyclodextrin [MβCD] or rosuvastatin [Rosu]) or supplementation (Chol), and then **(A)** general polarization and **(B)** apoptosis were quantified. For all graphs, differences between groups were compared by unpaired *t*-test: *, $P < 0.05$; **, $P < 0.001$; ***, $P < 0.0001$; ‡, $P < 0.05$ compared to EPA; §, $P < 0.0001$ compared to dasatinib or Dox; #, $P < 0.05$ compared to untreated control. **C)** Dox-inducible shEPHA2 cells were treated with EPA, Dox, dasatinib, or combinations and subjected to immunoblotting analysis of key cholesterol homeostasis-regulating proteins: ABCA1, SREBP2, HMGCR, and LDLR mature (M) and precursor (P) forms.

Cholesterol is a major modulator of cell membrane fluidity, and the intercalation of cholesterol into the phospholipid bilayer leads to increased rigidity of the cell membrane (Jaureguiberry *et al*., 2014). Because the level of cholesterol within the membrane in TN-IBC is higher than in other breast cancers (data not shown), it was hypothesized that a change in cholesterol homeostasis was the mechanism of EPA therapy's efficacy. Changes in GP of the plasma membrane in TN-IBC cells following exogenous cholesterol supplementation or cyclodextrin-based cholesterol depletion were assessed. As expected, in TN-IBC cells treated with a combination of EPA with Dox or dasatinib, cholesterol supplementation elevated GP, whereas cholesterol depletion lowered GP (Fig. 6A). Furthermore, the addition of exogenous reducing cholesterol content in these cells with rosuvastatin (a cholesterol biosynthesis inhibitor) partially blocked the effectiveness of dasatinib and EPA combination therapy (Fig. 6B). Conversely, reducing cholesterol content in these cells with rosuvastatin (a cholesterol biosynthesis inhibitor) partially blocked the effectiveness of EPA and dasatinib combination therapy (Fig. 6B). These data suggest that EPA in combination with EphA2 inhibition increases membrane rigidity through cholesterol accumulation in TN-IBC cell membranes, leading to cell apoptosis.

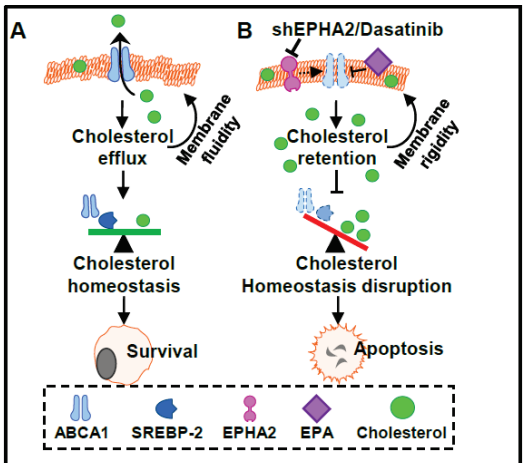


Figure 7. Schematics show the proposed mechanism of action. **A)** Cellular cholesterol homeostasis is normally maintained by the balancing effects of the cholesterol efflux channel protein ABCA1 and cholesterol biosynthesis inducer SREBP2. These proteins maintain cell membrane polarity and promote cell survival. **B)** Treatment with EPA and EphA2 inhibition inhibits the cholesterol-exporting functions of ABCA1, which increases cell membrane accumulation of cholesterol and cell membrane polarity. This aberrant accumulation of intracellular/ membrane cholesterol prevents the activity of cholesterol biosynthesis inducer protein SREPB2 and disrupts cellular cholesterol homeostasis, resulting in apoptosis.

ABCA1 is a critical mediator of cholesterol homeostasis.

Cholesterol levels within the cell can be modulated through increased import, reduced export, and/or increased biosynthesis (Nandi *et al*., 2009; Miserez *et al*., 2002). The expression levels of 4 major proteins (ABCA1, SREBP2, LDLR [low-density lipoprotein receptor], and HMGCR [3-hydroxy-3-methylglutaryl-CoA reductase]) involved in cholesterol regulation was examined in both SUM149 and BCX010 cells.

ABCA1 is the membrane-bound cholesterol efflux protein that transports cholesterol from the cell cytosol, and SREBP2 is a transcription factor that regulates cholesterol and fatty acid biosynthesis for cellular cholesterol homeostasis (Fig. 7A). If the cholesterol level is increased in the cells, SREBP2 loses transcriptional activity through inhibition of the formation of a complex with a transcription activator (Horton *et al*., 2002). Compared to monotherapies (EPA or Dox/dasatinib) and untreated controls, EPA combined with Dox or dasatinib therapy diminished the expression of ABCA1 and mature SREBP2 but did not have any effect on the expression of LDLR and HMGCR (Fig. 7B).

The reduced levels of ABCA1 may trigger the accumulation of cholesterol within cells, which could result in increased cell membrane polarity and cell death (Fig.7B). To determine whether ABCA1 is critical to maintaining cell membrane polarity, gain/loss-of-expression studies were performed to assess the response of SUM149 and BCX010 cells to combination treatment. Overexpression of ABCA1 significantly blocked GP induced by EPA and Dox combination therapy ($P < 0.001$) and subsequently prevented apoptosis ($P < 0.05$) when compared to controls (Fig. 8). Furthermore, EPA and EphA2 inhibition treatment dramatically reduced ABCA1 expression in the shEphA2-treated SUM149 and BCX010 tumor xenografts described above (Fig. 9). These results provide proof of concept and efficacy that dasatinib and EPA combination therapy induces apoptosis in TN-IBC cells by impairing cholesterol export from the cell membrane by reducing ABCA1 expression.

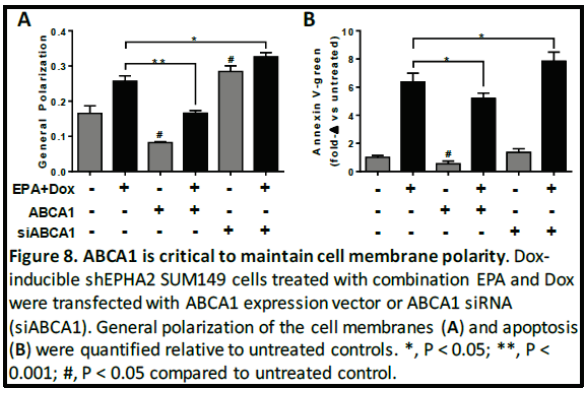


Figure 8. ABCA1 is critical to maintain cell membrane polarity. Dox-inducible shEPHA2 SUM149 cells treated with combination EPA and Dox were transfected with ABCA1 expression vector or ABCA1 siRNA (siABCA1). General polarization of the cell membranes (**A**) and apoptosis (**B**) were quantified relative to untreated controls. *, $P < 0.05$; **, $P < 0.001$; #, $P < 0.05$ compared to untreated control.

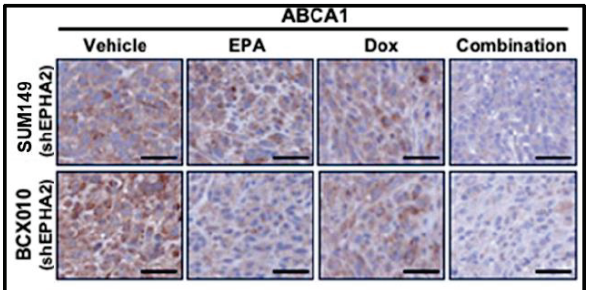


Figure 9. Combination of EPA and EphA2 inhibition suppresses ABCA1 expression in TNBC tumor xenografts. Scale bars = 50 µm.

Taken together, it is hypothesized that the combination of EPA and dasatinib, as EphA2 inhibitor, synergistically inhibits the growth of mTN-IBC, by changing the membrane rigidity. Given previously shown the safety of both agents, the MTD for the combination will be determined and efficacy induced by MTD combination will be studied via this phase 1b/2 trail.

2.5 Correlative Studies Background

2.5.1 Integrated Studies

2.5.1.1 Apoptosis Multiplex Immunoassay, Luminex

Biological rationale: In SUM149PT and BCX010 tumor xenograft models, the combination of EPA and dasatinib significantly reduced Ki67 expression and increased cleaved caspase-3 apoptosis marker by IHC. Cleaved caspase-3 expression will be the main focus in the multiplex

immunoassay panel; however, the comprehensive immunoassay will assess the general change in apoptosis markers by EPA and dasatinib combination therapy.

Hypothesis: Patients who respond to EPA and dasatinib combination therapy will show a significant increase in the expression of apoptotic markers.

2.5.2 Exploratory Studies

2.5.2.1 DNA fragmentation

Biological rationale: Our preclinical results showed that the combination of EPA and dasatinib induced cancer cell apoptosis by altering cholesterol homeostasis.

Preclinical and clinical data: In the shEphA2 experiment described in Section 2.4, treatment with EPA and dasatinib in combination drastically reduced the growth of tumor xenografts of two TN-IBC cell lines by induction of apoptosis compared to treatment with monotherapies (EPA or dasatinib) or vehicle control.

Hypothesis: EPA and dasatinib combination therapy induces cancer cell apoptosis by altering cholesterol homeostasis.

2.5.2.2 ABCA1

Biological rationale: Preclinical results showed that the cholesterol transporter, ABCA1, has an important role in regulating cholesterol homeostasis in the tumor cell membrane.

Preclinical and clinical data: Overexpression of ABCA1 significantly blocked the membrane GP induced by EPA and EphA2 shRNA in combination and subsequently prevented apoptosis ($P < 0.05$) when compared to controls (Fig. 6). Furthermore, EPA and EphA2 inhibition dramatically reduced ABCA1 expression in the shEphA2-treated SUM149 and BCX010 tumor xenografts.

Hypothesis: EPA and dasatinib combination therapy induces breast cancer cell apoptosis via inhibiting ABCA1 function.

2.5.2.3 EphA2

Biological rationale: The public gene dataset shows TNBC subtype has significantly higher gene expression of EphA2 compared with other subtypes, and the patients with EphA2 positive TNBC demonstrates poor survival outcome than those with EphA2 negative TNBC (Figures 1, 3). Thus, the expression of EphA2 is the critical indicator of the efficacy of the proposed treatment.

Preclinical and clinical data: For TN-IBC, our preclinical data strongly suggested that EPA in combination with dasatinib induced apoptosis by accumulating cholesterol in the cancer cell membrane. Thus, expression of EphA2 may be a critical indicator of the efficacy of the

proposed treatment.

Hypothesis: EphA2 inhibition by EPA and dasatinib combination therapy induces apoptosis by accumulating cholesterol in the cancer cell membrane.

2.5.2.4 Ki67

Biological rationale: In SUM149PT and BCX010 tumor xenograft models, the combination of EPA and dasatinib significantly reduced Ki67 expression and increased cleaved caspase-3 apoptosis marker by IHC.

Hypothesis: Patients who respond to EPA and dasatinib combination therapy will show a significantly reduced Ki67 by IHC.

2.5.2.5 Membrane rigidity

Biological rationale: Cholesterol is a major modulator of cell membrane fluidity, and the intercalation of cholesterol into the phospholipid bilayer leads to membrane stiffening (Jaureguiberry *et al*., 2014). A preliminary preclinical study showed that EPA and dasatinib combination therapy enhanced anti-tumor activity and that this activity involved increased membrane rigidity through induction of cholesterol accumulation in tumor cell membranes (Torres-Adorno *et al*., 2019). To examine whether EPA and dasatinib combination therapy inhibits tumor growth in patients through the same mechanism, its effects on cholesterol homeostasis will be assesses in snap-frozen tumor samples.

Preclinical and clinical data: Our data suggest that the combination of EPA and EphA2 inhibition increased membrane rigidity through cholesterol accumulation in TN-IBC cell membranes, leading to apoptosis.

Hypothesis: EPA and dasatinib combination therapy increases cell membrane rigidity by altering cholesterol homeostasis.

2.5.2.6 Cholesterol homeostasis in cell membrane

Biological rationale: Cholesterol is a major modulator of cell membrane fluidity as intercalation of cholesterol into the phospholipid bilayer leads to membrane stiffening.

Preclinical and clinical data: The likely mechanism of action of EPA-dasatinib combination therapy is inducing apoptosis by reduction of ABCA1 expression that increases cholesterol accumulation and membrane rigidity in cancer cells (Figures 5-9).

Hypothesis: EPA and dasatinib combination therapy alters cholesterol homeostasis.

2.5.2.7 Multiplex cytokine panel

Biological rationale: IBC has numerous inflammatory processes that drives its aggressiveness

(Lim *et al*., 2018; Reddy *et al*., 2019). The multiplex cytokine assay will be performed to determine the effect of EPA and dasatinib combination therapy on systemic inflammation.

Preclinical and clinical data: A previous preclinical study reported that EPA treatment decreased the tumoral expression of COX-2 (Ford *et al*., 2015). In addition, membrane cholesterol efflux by IL-4–mediated reprogramming in tumor-associated macrophages (TAMs) was associated with tumor progression in a xenograft model (Goossens *et al*., 2019). Further, inhibition of IL-4 and IL-13 decreased M2 polarization of macrophages by inhibition of STAT phosphorylation and resulted in increased efficacy of radiation in co-culture conditions with IBC cell lines (Rahal *et al*., 2018). In a small randomized double-blind controlled trial, breast cancer patients who received EPA had a significantly reduced percentage of $CD4^+$ T lymphocytes in peripheral blood (Paixao *et al*., 2017). Recent studies have indicated that TAMs are a potential target for the treatment of solid tumors. M1 macrophage cytokine IL-1β induces PD-L1 expression in cancer cells (Zong *et al*., 2019), and inhibition of TAMs enhances the response to immune checkpoint inhibitors such as anti-PD-1, anti-PD-L1, or anti-CTLA-4 (Cassetta *et al*., 2018). We expect that EPA and dastinib combination therapy will inhibit TAMs by altering cholesterol homeostasis. Overall, these results imply that EPA has an immunomodulatory effect on the tumor microenvironment (TME) and systemic anti-inflammatory effect.

Hypothesis: EPA and dasatinib combination therapy decreases M2 polarization of the macrophages represented by decreased M2 markers CD163 and CD206. Dasatinib and EPA combination therapy decreases expression level of TAM-associated cytokines (IL-1β, IL-6, IL-8, IL-12, IL-23, IP-10, reactive oxygen intermediates, TNF-α, IL-10, TGF-β, CCL18, CCL22, and TNF-α) in the blood.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Patients must have histologically confirmed mTN-IBC. TNBC is defined as:
   a. <10% estrogen receptor and progesterone receptor expression by immunohistochemistry (IHC)
   b. Negative or 1+ for HER2 by IHC or negative by fluorescent *in situ* hybridization based on ASCO/CAP guideline)

3.1.2 Patients must have had or currently have a clinical diagnosis of IBC according to the IBC-specific clinical manifestation as determined by a multidisciplinary team.

3.1.3 Patients must have measurable disease by RECIST 1.1 for the phase 2 component of the study. See Section 12 (Measurement of Effect) for the evaluation of measurable disease. Measurable disease is not a criterion for eligibility for the phase 1 component of the study.

3.1.4 Patients must have minimum of one standard regimen exposure in a metastatic setting.

3.1.5 Age ≥18 years. Because no dosing or adverse event data are currently available on the

use of dasatinib in combination with EPA in patients <18 years of age, children are excluded from this study.

3.1.6 ECOG performance status: 0-2.

3.1.7 Patients must have adequate organ and marrow function as defined below:

- absolute neutrophil count ≥ 1,500/mcL
- platelets ≥ 100,000/mcL
- total bilirubin ≤ 1.5 × institutional upper limit of normal (ULN)
- AST(SGOT)/ALT(SGPT) ≤ 2.5 × institutional ULN
- creatinine ≤ 1.5 × institutional ULN
- glomerular filtration rate (GFR) ≥ 60 mL/min/1.73 m^2 (see Appendix B)

3.1.8 Patients with distant metastasis and/or local recurrence accessible for biopsy who are willing to undergo two mandatory biopsies. Metastasis to brain, lung, and bone will be considered not accessible for safety reasons.

3.1.9 Negative serum or urine pregnancy test for subjects with childbearing potential.

3.1.10 Human immunodeficiency virus (HIV)-infected patients on effective anti-retroviral therapy with undetectable viral load within 6 months are eligible for this trial.

3.1.11 For patients with evidence of chronic hepatitis B virus (HBV) infection, the HBV viral load must be undetectable on suppressive therapy, if indicated.

3.1.12 Patients with a history of hepatitis C virus (HCV) infection must have been treated and cured. For patients with HCV infection who are currently on treatment, they are eligible if they have an undetectable HCV viral load.

3.1.13 Patients with a prior or concurrent malignancy whose natural history or treatment does not have the potential to interfere with the safety or efficacy assessment of the investigational regimen are eligible for this trial.

3.1.14 Patients with known history or current symptoms of cardiac disease, or history of treatment with cardiotoxic agents, should have a clinical risk assessment of cardiac function using the New York Heart Association Functional Classification. To be eligible for this trial, patients should be of class 2B or better.

3.1.15 The effects of dasatinib on the developing human fetus are unknown. For this reason and because other therapeutic agents used in this trial are known to be teratogenic, 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 3 months after completion of dasatinib administration.

3.1.16 Patients who are currently on bisphosphonate therapy should be able to temporarily stop bisphosphonate therapy for the duration of the study pending assessment of the need for calcium supplementation.

3.1.17 Ability to understand and the willingness to sign a written informed consent document. Participants with impaired decision-making capacity (IDMC) who have a legally-authorized representative (LAR) and/or family member available will also be eligible.

3.2 Exclusion Criteria

3.2.1 Patients who have had chemotherapy or radiotherapy within 2 weeks (3 weeks for nitrosoureas or mitomycin C) prior to entering the study.

3.2.2 Patients who are receiving any other investigational agents.

3.2.3 History of allergic reactions attributed to compounds of similar chemical or biologic composition to dasatinib or icosapent ethyl or any of its components (tocopherol, gelatin, glycerin, maltitol, and sorbitol) or other agents used in study.

3.2.4 Patients who have not recovered from adverse events due to prior anti-cancer therapies, (*i.e.*, have residual toxicities > Grade 1) with the exception of alopecia.

3.2.5 Patients with known active central nervous system metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate if they are stable, and have no evidence of new or enlarging brain metastases for at least 3 months, and are not using steroids for at least 7 days prior to trial treatment.

3.2.6 Patients with an active autoimmune disease requiring systemic treatment within the past 3 months or a documented history of clinically severe autoimmune disease, or a syndrome that requires systemic steroids or immunosuppressive agents. Subjects with vitiligo or resolved childhood asthma/atopy would be an exception to this rule. Subjects that require intermittent use of bronchodilators or local steroid injections would not be excluded from the study. Subjects with hypothyroidism stable on hormone replacement or Sjorgen's syndrome will not be excluded from the study.

3.2.7 Patients with a history of (non-infectious) pneumonitis that required steroids or has a current diagnosis of pneumonitis.

3.2.8 Patients with an active infection requiring systemic therapy.

3.2.9 Patients with an allergy to fish, shellfish, or omega-3 unsaturated fatty acid.

3.2.10 Patients who received a live vaccine within 30 days prior to the first dose of trial

treatment.

3.2.11 Patients receiving concurrent anti-cancer therapy for metastatic disease.

3.2.12 Patients with uncontrolled intercurrent illness judged by the investigator to be unsafe for trial participation.

3.2.13 Pregnant women are excluded from this study because Dasatinib is a protein tyrosine kinase (PTK) inhibitor agent with the 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 Dasatinib, breastfeeding should be discontinued if the mother is treated Dasatinib. These potential risks may also apply to other agents used in this study.

3.2.14 Patients with severe hypertriglyceridemia (>300 mg/dL -500 mg/dL) are at an unknown risk of developing pancreatitis following icosapent ethyl treatment.

3.2.15 Patients with diabetes who are being treated with insulin. Patients with oral medication and showing stable HbA1c <7% for the last three months will be eligible.

3.2.16 Patients who require concurrent treatment with any medications or substances that are potent inhibitors or inducers of CYP3A4 are ineligible. (See Appendix G for lists of specifically prohibited medications or substances.) Efforts should be made to switch patients with gliomas or brain metastases who are taking enzyme-inducing anticonvulsant agents to other medications. Patients who are on H2 blockers and proton pump inhibitors 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 will be counseled on the risk of interactions with other agents, and what to do if new medications need to be prescribed or if the patient is considering a new over-the-counter medicine or herbal product.

3.2.17 Use of antithrombotic and/or anti-platelet agents (*e.g.*, warfarin, heparin, low molecular weight heparin, aspirin, and/or ibuprofen).

3.2.18 Patients with any condition (*e.g.*, gastrointestinal tract disease resulting in an inability to take oral medication or a requirement for IV alimentation, prior surgical procedures affecting absorption, or active peptic ulcer disease) that impairs their ability to swallow and retain dasatinib and icosapent ethyl tablets are excluded.

3.2.19 Patients may not have any clinically significant cardiovascular disease including the

following:

- myocardial infarction or ventricular tachyarrhythmia within 6 months
- prolonged QTc ≥480 msec (Fridericia correction)
- ejection fraction less than institutional normal
- major conduction abnormality (unless a cardiac pacemaker is present).

Patients with any cardiopulmonary symptoms of unknown cause (*e.g.*, shortness of breath, chest pain, etc.) should be evaluated by a baseline echocardiogram with or without stress test as needed in addition to electrocardiogram (EKG) to rule out QTc prolongation. The patient may be referred to a cardiologist at the discretion of the principal investigator. Patients with underlying cardiopulmonary dysfunction should be excluded from the study.

3.3 Inclusion of Women and Minorities

NIH policy requires that women and members of minority groups and their subpopulations be included in all NIH-supported biomedical and behavioral research projects involving NIH-defined clinical research unless a clear and compelling rationale and justification establishes to the satisfaction of the funding Institute & Center (IC) Director that inclusion is inappropriate with respect to the health of the subjects or the purpose of the research. Exclusion under other circumstances must be designated by the Director, NIH, upon the recommendation of an IC Director based on a compelling rationale and justification. Cost is not an acceptable reason for exclusion except when the study would duplicate data from other sources. Women of childbearing potential should not be routinely excluded from participation in clinical research. Please see http://grants.nih.gov/grants/funding/phs398/phs398.pdf.

4. REGISTRATION PROCEDURES

4.1 Investigator and Research Associate Registration with CTEP

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all individuals contributing to NCI-sponsored trials to register and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account at https://ctepcore.nci.nih.gov/iam. In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) at https://ctepcore.nci.nih.gov/rcr.

RCR utilizes five person registration types.

- IVR: MD, DO, or international equivalent,
- NPIVR: advanced practice providers (*e.g.*, NP or PA) or graduate level researchers (*e.g.*, PhD),
- AP: clinical site staff (*e.g.*, RN or CRA) with data entry access to CTSU applications such as the Roster Update Management System (RUMS), OPEN, Rave, acting as a primary site contact, or with consenting privileges,

- Associate (A): other clinical site staff involved in the conduct of NCI-sponsored trials, and
- Associate Basic (AB): individuals (*e.g.*, pharmaceutical company employees) with limited access to NCI-supported systems.

RCR requires the following registration documents:

Documentation Required	IVR	NPIVR	AP	A	AB
FDA Form 1572	✓	✓			
Financial Disclosure Form	✓	✓	✓		
NCI Biosketch (education, training, employment, license, and certification)	✓	✓	✓		
GCP training	✓	✓	✓		
Agent Shipment Form (if applicable)	✓				
CV (optional)	✓	✓	✓		

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and Cancer Trials Support Unit (CTSU) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and Institutional Review Boards (IRBs) covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Addition to a site roster,
- Assign the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN,
- Act as the site-protocol Principal Investigator (PI) on the IRB approval, and
- Assign the Clinical Investigator (CI) role on the Delegation of Tasks Log (DTL).

In addition, all investigators acting as the Site-Protocol PI (Investigator listed on the IRB approval), consenting/treating/drug shipment investigator in OPEN, or as the Clinical Investigator (CI) on the DTL must be rostered at the enrolling site with a participating organization.

Additional information is located on the CTEP website at https://ctep.cancer.gov/investigatorResources/default.htm. For questions, please contact the **RCR Help Desk** by email at RCRHelpDesk@nih.gov.

4.2 Site Registration

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

IRB Approval

Sites participating with the NCI Central Institutional Review Board (NCI CIRB) must submit the Study Specific Worksheet (SSW) for Local Context to the CIRB using IRBManager to indicate their intent to open the study locally. The NCI CIRB's approval of the SSW is automatically communicated to the CTSU Regulatory Office, but sites are required to contact the CTSU

Regulatory Office at CTSURegPref@ctsu.coccg.org to establish site preferences for applying NCI CIRB approvals across their Signatory Network. Site preferences can be set at the network or protocol level. Questions about establishing site preferences can be addressed to the CTSU Regulatory Office by email or calling 1-888-651-CTSU (2878).

In addition, the Site-Protocol PI (*i.e.*, the investigator on the IRB/REB approval) must meet the following criteria to complete processing of the IRB/REB approval record to be completed:

- Holds an active CTEP status,
- Active status at the site(s) on the IRB/REB approval (*applies to US and Canadian sites only*) on at least one participating organization's roster,
- If using NCI CIRB, active on the NCI CIRB roster under the applicable CIRB Signatory Institution(s) record,
- Includes the IRB number of the IRB providing approval in the Form FDA 1572 in the RCR profile,
- Lists all sites on the IRB/REB approval as Practice Sites in the Form FDA 1572 in the RCR profile; and
- Holds the appropriate CTEP registration type for the protocol.

Additional Requirements

Additional site requirements to obtain an approved site registration status include:

- An active Federalwide Assurance (FWA) number,
- An active roster affiliation with the Lead Protocol Organization (LPO) or a Participating Organization (PO),
- An active roster affiliation with the NCI CIRB roster under at least one CIRB Signatory Institution (US sites only); and
- Compliance with all protocol-specific requirements (PSRs).

4.2.1 Downloading Site Registration Documents

Download the site registration forms from the protocol-specific page located on the CTSU members' website. Permission to view and download this protocol and its supporting documents is restricted to insitutions and its associated investigators and staff on a participating roster. To view/download site registration forms: .

- Log in to the CTSU members' website (https://www.ctsu.org) using your CTEP-IAM username and password,
- Click on *Protocols* in the upper left of the screen
    - Enter the protocol number in the search field at the top of the protocol tree, or
    - Click on the By Lead Organization folder to expand, then select LAO-TX035, and protocol number 10480,
- Click on *Documents*, *Protocol Related Documents*, and use the *Document Type* filter and select *Site Registration*, to download and complete the forms provided. (Note: For sites under the CIRB, IRB data will load automatically to the CTSU.)

4.2.2 Protocol Specific Requirements For #10480 Site Registration

- Specimen Tracking System Training Requirement:
  - All data entry users (Clinical Research Associate role) at each participating site will need to complete the Theradex-led training.
  - Theradex will provide a certificate of completion, which will need to be submitted to the CTSU through the Regulatory Submission Portal.
  - The training is a one-time only requirement per individual. If an individual has previously completed the training for another ETCTN study, the training does not need to be completed again nor does the certificate of completion need to be resubmitted to the CTSU. However, new versions of the Specimen Tracking System may require new training.
  - This training will need to be completed before the first patient enrollment at a given site.
  - Please contact STS Support at Theradex for the training (STS.Support@theradex.com, Theradex phone: 609-799-7580).

4.2.3 Submitting Regulatory Documents

Submit required forms and documents to the CTSU Regulatory Office using the Regulatory Submission Portal on the CTSU members' website.

To access the Regulatory Submission Portal, log on to the CTSU members' website, go to the Regulatory section, and select Regulatory Submission.

Institutions with patients waiting that are unable to use the Regulatory Submission Portal should alert the CTSU Regulatory Office immediately by phone or email: 1-866-651-CTSU (2878), or CTSURegHelp@coccg.orgin order to receive further instruction and support.

Delegation of Tasks Log (DTL)

Each site must complete a protocol-specific DTL using the DTL application in the Delegation Log section on the CTSU members' website. The Clinical Investigator (CI) is required to review and electronically sign the DTL prior to the site receiving an approved site registration status and enrolling patients to the study. To maintain an approved site registration status the CI must re-sign the DTL at least annually and when a new version of the DTL is released; and activate new task assignments requiring CI sign-off. Any individual at the enrolling site on a participating roster may initiate the site DTL. Once the DTL is submitted for CI approval, only the designated DTL Administrators or the CI may update the DTL. Instructions on completing the DTL are available in the Help Topics button in the DTL application and include a Master Task List, which describes DTL task assignments, CI signature, and CTEP registration requirements.

The individual initiating the DTL for the site should upload the above listed training documentation when making the task assignment. The designated reviewer will accept or reject the documentation. A note regarding rejection of any training documents will display on the Site DTL Browser next to the task assignment. The DTL cannot be submitted for CI sign-off until

the minimum number of persons are assigned to the task and have met the training requirements.

4.2.4 Checking Site Registration Status

Site's registration status may be verified on the CTSU website.

- Click on *Regulatory* at the top of the screen,
- Click on *Site Registration*, and
- Enter the site's 5-character CTEP Institution Code and click on Go.
    - Additional filters are available to sort by Protocol, Registration Status, Protocol Status, and/or IRB Type.

Note: The status shown only reflects institutional compliance with site registration requirements as outlined within the protocol. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with NCI or their affiliated networks.

4.3 Patient Registration

4.3.1 OPEN / IWRS

The Oncology Patient Enrollment Network (OPEN) is a web-based registration system available on a 24/7 basis. OPEN is integrated with CTSU regulatory and roster data and with the LPOs registration/randomization systems or the Theradex Interactive Web Response System (IWRS) for retrieval of patient registration/randomization assignment. OPEN or IWRS will populate the patient enrollment data in NCI's clinical data management system, Medidata Rave.

Requirements for OPEN access:

- A valid CTEP-IAM account.
- To perform enrollments or request slot reservations: Must be on an LPO roster, ETCTN corresponding roster, or PO roster with the role of Registrar. Registrars must hold a minimum of an Associate Plus (AP) registration type.
- If a DTL is required for the study, the registrar must hold the OPEN Registrar task on the DTL for the site.
- Have an approved site registration for the protocol prior to patient enrollment.

To assign an Investigator (IVR) or Non-Physician Investigator (NPIVR) as the treating, crediting, consenting, drug shipment (IVR only), or receiving investigator for a patient transfer in OPEN, the IVR or NPIVR must list the IRB number used on the site's IRB approval on their Form FDA 1572 in RCR. If a DTL is required for the study, the IVR or NPIVR must be assigned the appropriate OPEN-related tasks on the DTL.

Prior to accessing OPEN, site staff should verify the following:

- Patient has met all eligibility criteria within the protocol stated timeframes, and
- All patients have signed an appropriate consent form and Health Insurance Portability and Accountability Act (HIPAA) authorization form (if applicable).

Note: The OPEN system will provide the site with a printable confirmation of registration and treatment information. IWRS system also sends an email confirmation of the registration. You may print this confirmation for your records.

Access OPEN at https://open.ctsu.org or from the OPEN link on the CTSU members' website. Further instructional information is in the OPEN section of the CTSU website at https://www.ctsu.org or https://open.ctsu.org. For any additional questions, contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

Patient enrollment for this study will be facilitated using the Slot Reservation System in conjunction with the registration system in OPEN. Prior to discussing protocol entry with the patient, all site staff must use the CTSU OPEN Slot Reservation System or the IWRS Slot Reservation System to ensure that a slot on the protocol is available to the patient. Once a slot reservation confirmation is obtained, site staff may then proceed to enroll the patient to this study.

4.3.2 Special Instructions for Patient Enrollment

This Study will use the ETCTN Specimen Tracking System (STS).

- All biospecimens collected for this trial must be submitted using the ETCTN Specimen Tracking System (STS) unless otherwise noted.
- The system is accessed through Rave user roles: "Rave CRA" and "Rave CRA (Labadmin)" for data entry at the treating institutions and "Biorepository" for users receiving the specimens for processing and storage at reference labs and the NCI Early-Phase and Experimental Clinical Trials Biospecimen Bank (EET Biobank, formerly known as the ETCTN Biorepository).
- Please refer to the Medidata Account Activation and Study Invitation Acceptance link on the CTSU website in the Data Management section under the Rave Home tab and then under Rave Resource Materials.
- **Important: Failure to complete required fields in STS may result in a delay in sample processing.** Any case reimbursements associated with sample submissions will not be credited if samples requiring STS submission are not logged into STS.

Detailed instructions on use of the STS can be found in Section 5.4.

4.3.3 OPEN/IWRS Questions?

Further instructional information on OPEN is provided on the OPEN link of the CTSU website at https://www.ctsu.org or at https://open.ctsu.org. For any additional questions contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

Theradex has developed a Slot Reservations and Cohort Management User Guide, which is available on the Theradex website: http://www.theradex.com/clinicalTechnologies/?National-Cancer-Institute-NCI-11. This link to the Theradex website is also on the CTSU website OPEN tab. For questions about the use of IWRS for slot reservations, contact the Theradex Helpdesk at 855-828-6113 or Theradex main number 609-799-7580; CTMSSupport@theradex.com.

4.4 General Guidelines

Following registration, patients should begin protocol treatment within 30 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 Study Coordinator should be notified of cancellations as soon as possible.

5. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

5.1 Summary Table for Specimen Collection

Time Point	Specimen	Send Specimens To:
Baseline		
Phase 1b and Phase 2	• 1 tissue cores snap frozen (mandatory)[1] • 1 tissue core in formalin (mandatory)[1] • 20 mL blood in a red top tube, processed for serum and frozen (mandatory)	EET Biobank
	• 1 fresh tissue core in X-VIVO™ 15 media (mandatory) • 1 tissue core snap frozen (mandatory)[1]	Chapkin laboratory, Texas A&M University
After completion of Cycle 2 (± 2 days)		
Phase 1b and Phase 2	• 1 tissue cores snap frozen (mandatory)[1] • 1 tissue core in formalin (mandatory)[1] • 20 mL blood in a red top tube, processed for serum and frozen (mandatory)	EET Biobank
	• 1 fresh tissue core in X-VIVO™ 15 media (mandatory) • 1 tissue core snap frozen (mandatory)[1]	Chapkin laboratory, Texas A&M University
[1] For new biopsies, **the Tissue Biopsy Verification Form (Appendix F), a copy of the radiology and/or operative reports from the tissue removal procedure *and* the diagnostic anatomic pathology report** must be sent with the tissue to the EET Biobank.		

5.2 Summary Tables for Interventional Radiologist for Research Biopsies

Biopsy #: 1
Trial Time Point: Baseline
IR Biopsy Definition: Research – No Clinical Impact (All cores from a single biopsy procedure impact research goals, but do not directly impact patient care or benefit the patient).

Core Priority	Use in the Trial	Biomarker Name(s)	Tumor Content Required	Post-Biopsy Processing
1	Integrated	Apoptosis Multiplex Immunoassay, Luminex	25-50%	Fresh frozen
2	Exploratory	Cholesterol homeostasis in cell membrane	25-50%	Fresh frozen
3	Exploratory	DNA fragmentation, ABCA1, EphA2, Ki67	25-50%	Formalin
4	Exploratory	Membrane rigidity	25-50%	Fresh in X-VIVO™ 15 media

Biopsy #: 2				
Trial Time Point: After completion of Cycle 2 (± 2 days)				
IR Biopsy Definition: Research – No Clinical Impact (All cores from a single biopsy procedure impact research goals, but do not directly impact patient care or benefit the patient).				
Core Priority	**Use in the Trial**	**Biomarker Name(s)**	**Tumor Content Required**	**Post-Biopsy Processing**
1	Integrated	Apoptosis Multiplex Immunoassay, Luminex	25-50%	Fresh frozen
2	Exploratory	Cholesterol homeostasis in cell membrane	25-50%	Fresh frozen
3	Exploratory	DNA fragmentation, ABCA1, EphA2, Ki67	25-50%	Formalin
4	Exploratory	Membrane rigidity	25-50%	Fresh in X-VIVO™ 15 media

Note: Pre-biopsy assessments will be reported and tracked through a trial-specific Case Report Form (CRF) within the CTEP Medidata Rave system (see Appendix E).

5.3 Specimen Procurement Kits and Scheduling

5.3.1 Specimen Procurement Kits

Kits for the collection and shipment of tissue cores to the EET Biobank can be ordered online via the Kit Management system: (https://kits.bpc-apps.nchri.org).

Users at the clinical sites will need to set up an account in the Kit Management system and select a specific clinical trial protocol to request a kit. Please note that protocol may include more than one type of kit. Each user may order two kits per kit type per day (daily max = 6 kits). Kits are shipped ground, so please allow 5-7 days for receipt. A complete list of kit contents for each kit type is located on the Kit Management system website.

Note: Kits or supplies are only provided for specimens shipped to the EET Biobank. Institutional supplies must be used for all other specimen collection and processing.

5.3.2 Scheduling of Specimen Collections

Please adhere to the following guidelines when scheduling procedures to collect tissue:

- Tumor tissue specimens collected during biopsy procedures and fixed in formalin must be shipped on the same day of collection.

- Tissue in formalin can be collected Monday through Wednesday and shipped overnight for arrival on Tuesday through Thursday at the EET Biobank at Nationwide Children's Hospital.

- Frozen tissue and serum can be collected on any day but must be stored frozen and shipped to the EET Biobank on Monday through Thursday. In the event that frozen specimens cannot be shipped immediately, they must be maintained in a -70°C to -80°C freezer.

5.4 Specimen Tracking System Instructions

5.4.1 Specimen Tracking System Overview and Enrollment Instructions

For the ETCTN STS, the following information will be requested:

- Protocol Number
- Investigator Identification
  - Institution and affiliate name
  - Investigator's name
- Eligibility Verification: Patients must meet all the eligibility requirements listed in Section 3.
- Additional Requirements:
  - Patients must provide a signed and dated, written informed consent form.

Upon enrolling a patient, IWRS will communicate with OPEN, assigning two separate and unique identification numbers to the patient, a Universal patient ID (UPID) and a Treatment

patient ID. The UPID is associated with the patient and used each and every time the patient engages with the portion of this or any other protocol that uses the ETCTN Specimen Tracking System. The UPID contains no information or link to the treatment protocol. IWRS will maintain an association between the UPID for ETCTN biobanking and molecular characterization and any treatment protocols the patient participates in, thereby allowing analysis of the molecular characterization results with the clinical data.

Immediately following enrollment, the institutional anatomical pathology report for the diagnosis under which the patient is being enrolled must be uploaded into Rave. The report must include the surgical pathology ID (SPID), collection date, block number, and the IWRS-assigned UPID and patient study ID for this trial. For newly acquired biopsies, the radiology and operative report(s) must also be uploaded into Rave. **Important: Remove any personally identifying information, including, but not limited to, the patient's name, date of birth, initials, medical record number, and patient contact information from the institutional pathology report prior to submission.**

Additionally, please note that the STS software creates pop-up windows when reports are generated, so you will need to enable pop-ups within your web browser while using the software.

For questions regarding the Specimen Tracking System, please contact STS Support at STS.Support@theradex.com.

The Shipping List report **must** be included with all sample submissions.

5.4.2 Specimen Labeling

5.4.2.1 Blood Specimen Labels

Include the following on blood specimens (including whole blood and frozen, processed blood products – like serum and plasma:

- Patient Study ID
- Universal Patient ID (UPID)
- Specimen ID (automatically generated by Rave)
- Time point
- Specimen type (*e.g.*, blood, serum)
- Collection date (to be added by hand)

5.4.2.2 Tissue Specimen Labels

Include the following on all tissue specimens (*e.g.*, frozen tissue) or containers (*e.g.*, formalin jar):

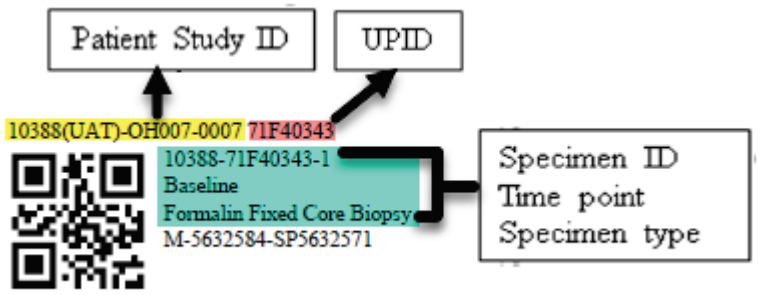
- Patient Study ID
- Universal Patient ID (UPID)
- Specimen ID (automatically generated by Rave)s
- Time point

- Specimen type (*e.g.*, Formalin Fixed Tissue, Fresh Frozen Tissue, *etc.*)
- Tissue type (P for primary, M for metastatic or N for normal)
- Surgical pathology ID (SPID) number (when applicable)
- Collection date (to be added by hand)

5.4.2.3 Example of Specimen Label Generated by STS

STS includes a label printing facility, accessed via the Print Label CRF in the All Specimens folder. A generated PDF is emailed to the user as a result of saving that form.

The following image is an example of a tissue specimen label printed on a label that is 0.5" high and 1.28" wide.



The QR code in the above example is for the Specimen ID shown on the second line.

Labels may be printed on a special purpose label printer, one label at a time, or on a standard laser printer, multiple labels per page. Theradex recommends the use of these low temperature waterproof labels for standard laser printers: https://www.labtag.com/shop/product/cryo-laser-labels-1-28-x-0-5-cl-23-colors-available/

The last line item on the label includes the following data points joined together:

1. Tissue only: Primary (P), Metastatic (M), Normal (N) tissue indicated at the beginning of the specimen ID; this field is blank if not relevant (*e.g.*, for blood)
2. Block ID or blank if not relevant
3. SPID (Surgical Pathology ID) or blank if none
4. An optional alpha-numeric code that is protocol specific and is only included if the protocol requires an additional special code classification

Space is provided at the bottom of the label for the handwritten date and optional time. The last line on the example label is for the handwritten date and optional time.

5.4.3 Overview of Process at Treating Site

5.4.3.1 OPEN Registration

All registrations will be performed using the Oncology Patient Enrollment Network (OPEN) system. OPEN communicates automatically with the Interactive Web Response System (IWRS) which handles identifier assignments, any study randomization, and any prescribed slot assignments. If specimen analysis is required to determine eligibility, the protocol will be setup with multi-step registration.

Registration without eligibility specimen analysis:

1. Site enters registration data into OPEN during one or more steps.
2. IWRS receives data from OPEN, generates the Patient Study ID and the Universal Patient ID, both of which are sent back to OPEN.
3. IWRS sends all applicable registration data directly to Rave at the end of the final registration step.

5.4.3.2 Rave Specimen Tracking Process Steps

Step 0: Log into Rave via your CTEP-IAM account, then navigate to the appropriate participant.

Step 1: Complete the **Histology and Disease** form (but do not upload reports until a specimen label can be applied to them) and the Baseline forms regarding **Prior Therapies**. Enter the initial clinical specimen data:

- **Specimen Tracking Enrollment** CRF: Enter Time Point, Specimen Category, Specimen Type, Block number, Tissue type, Surgical Path ID, and number of labels needed (include extra labels to apply to reports to be uploaded). CRF generates unique Specimen ID.

Step 2: Print labels using the **Print Labels** CRF located in the All Specimens folder, then collect specimen.

- Label specimen containers and write collection date on each label. After collection, store labeled specimens as described in Section 5.5.
- Apply an extra specimen label to *each* report before scanning. Return to the **Histology and Disease** form to upload any initial Pathology, Radiology, Molecular Reports (up to 4), and Surgical (or Operative) reports. Return to **Specimen Tracking Enrollment** CRF to upload any molecular report (one per specimen) and/or specimen specific pathology or related report (one per specimen) and/or the Tissue Biopsy Verification form (when applicable). Uploaded reports should have protected health information (PHI) data, like name, date of birth, mailing address, medical record number or social security number (SSN), redacted**. Do not redact SPID, block number, diagnosis or relevant dates (such as collection date), and include the UPID and patient study ID on each document** (either by adding a label or hand writing).

Step 3: Complete specimen data entry.

- **Specimen Transmittal** Form: Enter collection date and time and other required specimen details.

Step 4: When ready to ship, enter shipment information.

- **Shipping Status** CRF: Enter tracking number, your contact information, recipient, number of sample containers and ship date once for the first specimen in a shipment.
- **Copy Shipping** CRF: In the specimen folders for additional specimens (if any) that will be shipped with the initial specimen, please use the **Copy Shipping** form to derive common data into additional **Shipping Status** forms. A few unique fields will still need to be entered in **Shipping Status.**

Step 5: Print shipping list report and prepare to ship.

- Shipping List report is available at the site level.
- Print two copies of the shipping list, one to provide in the box, the other for your own records.
- Print pathology or other required reports to include in the box. Be sure the printed copy includes the specimen label.

Step 6: Send email notification.

- For only one of the specimens in the shipment, click "Send Email Alert" checkbox on the **Shipping Status** CRF to email recipient.

Step 7: Ship the specimen(s).

Step 8: Monitor the Receiving Status form located in each specimen folder for acknowledgment of receipt and adequacy.

5.5 Specimen Collection

Proper tissue embedding and orientation are necessary in order to support the sample and prevent tissue damage or loss during sectioning (as well as preserve diagnostic histological features). Improperly embedded tissue (e.g., needle cores) can provide incomplete information if diagnostic material is not properly sectioned in a timely manner. Improper orientation of certain samples (e.g., skin tumors) can prevent the evaluation of histological features that may affect survival or recurrence, like depth of tumor invasion and involvement of surgical margins of resection. In order to prevent tissue embedding and orientation errors, refer to the guidelines in Appendix I.

5.5.1 Fresh Tumor Biopsies

1. Label 2OZ sterile specimen container (Cat. 14-375-152C, Fisher Scientific) according to instructions in Section 5.4.2.
2. Fill with 50 ml of X-VIVO™ 15 Chemically Defined, Serum-free Hematopoietic Cell Medium (Cat. 04-744Q, Lonza).
3. Obtain 2 16-gauge or 18-gauge core needle biopsy specimens, and transfer to containers.
4. Secure the container lids and package containers into the shipping kit according to

instructions in Section 5.7. Keep containers at cold temperature by ice-pack until shipment to the Dr. Chapkin's lab via FEDEX overnight shipping.

5.5.2 Formalin-Fixed Tumor Biopsies

1. Label formalin-filled containers according to instructions in Section 5.4.2.
2. Obtain one 16-gauge or 18-gauge core needle biopsy specimens, and place one core in each cassette.
3. Snap the cassette lids closed and place cassettes into a formalin-filled pre-labeled container as soon as possible after collection to prevent air drying. Up to two cassettes may be placed in one formalin jar.
4. Secure the container lids and package containers into the shipping kit according to instructions in Section 5.6. Keep tissue in formalin jars at room temperature until shipment to the EET Biobank.

5.5.3 Collection of Snap-Frozen Biopsies

1. Follow step 5.3.1 to request specimen procurement kits for frozen sample collection before scheduled biopsy collection.
2. Biopsy specimens should be collected into pre-chilled 1.5mL Sarstedt, O-ring screw cap tubes (VWR, Cat#: 83009-010).
    a. Label Sarstedt tube(s) according to instructions in Section 5.4.2, prior to pre-chilling.
3. It is imperative that biopsies are flash frozen within **2 minutes** of collection in order to preserve key pharmacodynamic biomarkers.
4. As described in Appendix H, place the tissue in a pre-chilled cryovial and freeze the tube in liquid nitrogen or dry ice/ethanol bath. Keep frozen at -80 ºC or lower until shipment to the EET Biobank.

Sites are **strongly encouraged** to contact the NCLN PD Laboratory at NCI_PD_Support@mail.nih.gov to initiate training and clarify biopsy collection procedure.

5.5.4 Blood Collection

5.5.4.1 Collection of Blood in Red Top Tube for Serum Processing

1. Label two 10 mL red-top tubes according to the instructions in Section 5.4.2.
2. Collect 20 mL of blood in red-top tubes.
3. Gently invert the tube 8-10 times and allow blood to clot upright at room temperature for at least 30 minutes (maximum 60 minutes) prior to processing. If the blood is not immediately processed after the clotting period, then tubes should be stored (after the 30-60 minutes of clotting time) at 4°C for no longer than 4 hours. Process serum from red top tubes by centrifuging for 10 minutes at 1,200 × g at room temperature.
4. **Using a clean transfer pipette,** aliquot serum into the labeled (using the label printed from the ETCTN Specimen Tracking System or following the instructions in Section 5.4.2) cryovials at an aliquot volume of 1 mL per tube. Collect from the middle of the

plasma layer to avoid picking up red blood cells when aliquoting, keeping the pipet above the red blood cell layer and leaving a small amount of serum in the tube. Tightly secure the cap of the vials before storage. Aliquoting and freezing of serum specimens should be completed within 1 hour of centrifugation.

5. Store serum cryovials upright in a specimen box or rack in an -70°C to -90°C or colder freezer prior to delivering to laboratory. Do not allow specimens to thaw after freezing.

5.6 Shipping Specimens from Clinical Site to the EET Biobank

5.6.1 General Shipping Information

When kits are provided, the shipping container sent with kit contents should be used to ship specimens to the EET Biobank. In winter months, please include extra insulation, such as bubble wrap, inside the shipping container with the ambient specimens.

5.6.1.1 Required Forms for Specimen Submissions:

Each document submitted with the specimen must be labeled with a label printed from the STS, or the Universal ID and Patient Study ID.

Tissue	**Required Forms**
New Biopsy	1. Shipping List 2. Tissue Biopsy Verification Form 3. Diagnostic Pathology Report 4. Surgical and/or Radiology Report
Serum	1. Shipping List

5.6.2 Specimen Shipping Instructions

Tissue in formalin must be shipped on the day of collection. Collect and ship on Monday through Wednesday.

Frozen specimens may be shipped on Monday through Thursday.

5.6.2.1 Shipping Frozen and Ambient Specimens in a Dual-Chamber Kit

The Dual Chambered Specimen Procurement Kit is constructed to allow the shipment of frozen (on dry ice) and ambient (room temperature) specimens in the same container. **Dry ice may be placed in either compartment of the kit but should not be put in both.** The dual chambered kit is only used for shipments that contain both frozen and ambient specimens. If formalin-fixed tissue is shipped separately (not in the same shipment as frozen specimens), then it must be shipped using institutional shipping supplies.

Supplies for the collection of frozen serum are not provided, but serum may be shipped to the EET Biobank in the kit with the tissue cores.

1. Before packaging specimens, verify that each specimen is labeled according to the instructions in 5.4.2 and that lids of all primary receptacles containing liquid are tightly sealed.
2. Pre-fill one of the kit chambers about 1/3 with dry ice.
3. Prepare the frozen specimens for shipment:
    a. Place the specimens into zip-lock bags.
    b. Place the zip-lock bags into a biohazard envelope containing absorbent material. Expel as much air as possible before sealing the biohazard envelope.
    c. Put each biohazard envelope into a Tyvek envelope. Expel as much air as possible and then seal the Tyvek envelope.
4. Quickly place the Tyvek envelope containing frozen specimens in the kit compartment that is pre-filled with dry ice. Place the Tyvek envelope on top of the dry ice. Cover the specimens with additional dry ice until the compartment is almost completely full.
5. Place the Styrofoam lid on top to secure specimens during shipment. Do not tape the inner chamber shut.
6. Prepare the ambient specimens for shipment:
    a. Seal the lids of the formalin jars with parafilm. Place absorbent material around the primary container of each liquid specimen. Place the specimens into zip-lock bags.
    b. Place specimens inside the secondary pressure vessel with bubble wrap.
    c. Secure the lid on the secondary pressure vessel and set it inside the kit chamber.
7. Insert a copy of the required forms in the kit chamber with the ambient specimens.
8. Place the Styrofoam lid on top of the kit compartment to secure specimens during shipment. Do not tape the inner chamber shut.
9. Close the outer lid of the Specimen Procurement Kit and tape it shut with durable sealing tape. Do not completely seal the container.
10. Complete a FedEx air bill and attach to top of shipping container.
11. Complete a dry ice label.
12. Attach the dry ice label and an Exempt Human Specimen sticker to the side of the shipping container.
13. Ship specimens via overnight courier to the address below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

5.6.3 Shipping Address

Ship to the address below. Ship formalin-fixed specimens the same day of specimen collection. Do not ship specimens the day before a holiday.

EET Biobank
The Research Institute at Nationwide Children's Hospital
700 Children's Drive, WA1340
Columbus, Ohio 43205
PH: (614) 722-2865

FAX: (614) 722-2897
E-mail: BPCBank@nationwidechildrens.org

FedEx Priority Overnight service is very strongly preferred.

NOTE: The EET Biobank FedEx Account will not be provided to submitting institutions. There is no central Courier account for this study. Sites are responsible for the cost of shipments to the EET Biobank.

5.6.4 Contact Information for Assistance

For all queries, please use the contact information below:

EET Biobank
Toll-free Phone: (800) 347-2486
E-mail: BPCBank@nationwidechildrens.org

5.7 Shipping of Specimens from Clinical Site to Other Laboratories

5.7.1 Shipping of Specimens to Chapkin laboratory, Texas A&M University

5.7.1.1 Specimen Shipping Instructions

Containers in the shipping kits should be kept at cold temperature by ice-pack until shipment to the Chapkin laboratory via FEDEX overnight shipping.

5.7.1.2 Shipping Address

Department of Biochemistry and Biophysics, Texas A&M University
Attn: Dr. Robert Chapkin
Dept of Nutrition
373 Olsen Blvd., 112 Cater-Mattil Hall; 2253 TAMU
College Station, TX 77843
Texas A&M University
Tel: 979.845.0419 |
Fax: 979.458.3129
E-mail: r-chapkin@tamu.edu

5.7.1.3 Contact Information for Assistance

Dr. Robert Chapkin
Tel: 979.845.0419 |
Fax: 979.458.3129
E-mail: r-chapkin@tamu.edu

5.8 Biomarker Plan

List of Biomarker Assays in Order of Priority

Note for participating sites: Please see Section 5.1 for details on specimens to collect. The specimens tested are not always the same specimens that are submitted by the site, as processing of blood and tissue will occur at the Biobank prior to testing.

Priority	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory and Lab PI
Tissue-based Biomarkers							
1	Apoptosis Multiplex Immunoassay, Luminex	Luminex, Priority #1: Panel 3: BCL-XL:BAK heterodimer, cleaved caspase 3, MCl1:BAK heterodimer, survivin If remaining tissue: Panel 1: BAX, BAK, Lamin B (Intact and 45 KDa cleaved fragment), SMAC Panel 2: BAD, BAX: BCL2 heterodimer, BCL-XL, BIM, MCL1 CLIA: N	Integrated Response assessment To identify biomarkers of response	Frozen tumor tissue from primary or metastatic site	Phase 1b and Phase 2: Baseline and after completion of Cycle 2 (±2 days)	M	NCLN PD Assay Laboratory at Molecular Pathology Laboratory Network, Inc. Kate Ferry-Galow ferrygalowkv@mail.nih.gov
2	DNA fragmentation	TUNEL assay	Exploratory	Unstained slides from FFPE tumor	Phase 1b and Phase 2:	M	Department of Breast Medical Oncology, MD

Priority	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory and Lab PI
		CLIA: N	Hypothesis generation To evaluate biomarkers of response	tissue from primary or metastatic site	Baseline and after completion of Cycle 2 (±2 days)		Anderson Cancer Center (MDACC) Jangsoon Lee jslee@mdanderson.org
3	ABCA1	IHC CLIA: N	Exploratory Hypothesis generation To evaluate biomarkers of response	Unstained slides FFPE from tumor tissue from primary or metastatic site	Phase 1b and Phase 2: Baseline and after completion of Cycle 2 (±2 days)	M	Department of Pathology, MD Anderson Cancer Center (MDACC) Savitri Krishnamurthy skrishna@mdanderson.org
4	EphA2	IHC CLIA: N	Exploratory Hypothesis generation To evaluate biomarkers of response	Unstained slides from FFPE tumor tissue from primary or metastatic site	Phase 1b and Phase 2: Baseline and after completion of Cycle 2 (±2 days)	M	Department of Pathology, MD Anderson Cancer Center (MDACC) Savitri Krishnamurthy skrishna@mdanderson.org
5	Ki67	IHC CLIA: N	Exploratory To evaluate biomarkers of response	Unstained slides from FFPE tumor tissue from primary or metastatic site	Phase 1b and Phase 2: Baseline and after completion of Cycle 2 (±2 days)	M	Department of Breast Medical Oncology, MD Anderson Cancer Center (MDACC) Jangsoon Lee jslee@mdanderson.org
6	Membrane rigidity	Cholesterol assay CLIA: N	Exploratory Hypothesis generation To identify biomarkers of response	Fresh tumor tissue in VIVO™ 15 media from primary or metastatic site	Phase 1b and Phase 2: Baseline and after completion of Cycle 2 (±2 days)	M	Department of Biochemistry and Biophysics, Texas A&M University Robert S Chapkin r-chapkin@tamu.edu

Priority	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory and Lab PI
7	Cholesterol homeostasis in cell membrane	Cholesterol assay CLIA: N	Exploratory Hypothesis generation To identify biomarkers of response	Frozen tumor tissue from primary or metastatic site	Phase 1b and Phase 2: Baseline and after completion of Cycle 2 (±2 days)	M	Department of Biochemistry and Biophysics, Texas A&M University Robert S Chapkin r-chapkin@tamu.edu
Blood-based Biomarkers							
1	Multiplex cytokine panel	Invitrogen Immune Monitoring 65-Plex Human ProcartaPlex™ Panel M1 macrophage-derived pro-inflammatory cytokines (IL-1β, IL-6, IL-8, IL-12, IL-23, IP-10, TNF-α) M2 macrophage-derived anti-inflammatory cytokines (IL-10, CCL22) Pro-inflammatory markers (TNF-α, IL-1β)	Exploratory Response assessment	Serum	Phase 1b and Phase 2: Baseline and after completion of Cycle 2 (±2 days)	M	Department of Hematopathology, MD Anderson Cancer Center (MDACC) James M Reuben jreuben@mdanderson.org

Priority	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory and Lab PI
		CLIA: N					

5.9 Integrated Correlative Studies

5.9.1 Apoptosis Multiplex Immunoassay, Luminex

5.9.1.1 Specimen(s) Receipt and Processing at the EET Biobank

Frozen tissue will be barcoded and stored in a liquid nitrogen vapor phase freezer.

5.9.1.2 Site(s) Performing Correlative Study

This study will be conducted at the NCLN PD Assay Laboratory at Molecular Pathology Laboratory Network, Inc. under the supervision of Kate Ferry-Galow, Ph.D. (ferrygalowkv@mail.nih.gov).

5.9.1.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank to:

NCLN PD Assay Laboratory at Molecular Pathology Laboratory Network, Inc.
Attention: Donald Henley
Molecular Pathology Laboratory Network, Inc.
250 E Broadway Ave.
Maryville, TN 37804
Tel: 865-380-9746

5.9.1.4 Contact Information for Notification of Specimen Shipment

lab@geneuity.com

5.10 Exploratory/Ancillary Correlative Studies

5.10.1 DNA fragmentation

5.10.1.1 Specimen(s) Receipt and Processing at the EET Biobank

Tissue received in formalin will be processed and embedded in paraffin and stored as an FFPE tissue block. FFPE tissue blocks are stored at room temperature until sectioning unstained slides for analysis. Ten (10) slides per patient will be needed including an extra 5 µM air-dried positively charged slide.

5.10.1.2 Site(s) Performing Correlative Study

This study will be conducted at the Department of Breast Medical Oncology, MD Anderson Cancer Center (MDACC) under the supervision of Jangsoon Lee, Ph.D. (jslee@mdanderson.org).

5.10.1.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank to:

Department of Breast Medical Oncology, MD Anderson Cancer Center (MDACC)
Attention: Dr. Jangsoon Lee
Z12.5036, Breast Medical Oncology, Unit 1354
6565 MD Anderson Blvd., Houston, TX 77030
Tel: 713-563-9221
Email: jslee@mdanderson.org

5.10.1.4 Contact Information for Notification of Specimen Shipment:

Dr. Jangsoon Lee
Tel: 713-563-9221
Email: jslee@mdanderson.org

5.10.2 ABCA1

5.10.2.1 Specimen(s) Receipt and Processing at the EET Biobank

Tissue received in formalin will be processed and embedded in paraffin and stored as an FFPE tissue block. FFPE tissue blocks are stored at room temperature until sectioning unstained slides for analysis. Ten (10) slides per patient will be needed including an extra 5 µM air-dried positively charged slide.

5.10.2.2 Site(s) Performing Correlative Study

This study will be conducted at the Department of Pathology, MD Anderson Cancer Center (MDACC) under the supervision of Savitri Krishnamurthy, M.D. (skrishna@mdanderson.org).

5.10.2.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank to:

Department of Pathology, MD Anderson Cancer Center (MDACC)
Attention: Dr. Savitri Krishnamurthy
G3.3749, Hematopathology - Rsch, Unit 0053
1515 Holcombe Blvd., Houston, TX 77030
Tel: 713-794-5625

5.10.2.4 Contact Information for Notification of Specimen Shipment:

Dr. Savitri Krishnamurthy
Tel: 713-794-5625

Email: skrishna@mdanderson.org

5.10.3 EphA2

5.10.3.1 Specimen(s) Receipt and Processing at the EET Biobank

Tissue received in formalin will be processed and embedded in paraffin and stored as an FFPE tissue block. FFPE tissue blocks are stored at room temperature until sectioning unstained slides for analysis. Ten (10) slides per patient will be needed including an extra 5 µM air-dried positively charged slide.

5.10.3.2 Site(s) Performing Correlative Study

This study will be conducted at the Department of Pathology, MD Anderson Cancer Center (MDACC) under the supervision of Savitri Krishnamurthy, M.D. (skrishna@mdanderson.org).

5.10.3.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank to:

Department of Pathology, MD Anderson Cancer Center (MDACC)
Attention: Dr. Savitri Krishnamurthy
G3.3749, Hematopathology - Rsch, Unit 0053
1515 Holcombe Blvd., Houston, TX 77030
Tel: 713-794-5625

5.10.3.4 Contact Information for Notification of Specimen Shipment:

Dr. Savitri Krishnamurthy
Tel: 713-794-5625
Email: skrishna@mdanderson.org

5.10.4 Ki67

5.10.4.1 Specimen(s) Receipt and Processing at the EET Biobank

Tissue received in formalin will be processed and embedded in paraffin and stored as an FFPE tissue block. FFPE tissue blocks are stored at room temperature until sectioning unstained slides for analysis. Ten (10) slides per patient will be needed including an extra 5 µM air-dried positively charged slide.

5.10.4.2 Site(s) Performing Correlative Study

This study will be conducted at the Department of Breast Medical Oncology, MD Anderson Cancer Center (MDACC) under the supervision of Jangsoon Lee, Ph.D. (jslee@mdanderson.org).

5.10.4.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank to:

Department of Breast Medical Oncology, MD Anderson Cancer Center (MDACC)
Attn.: Dr. Jangsoon Lee
Z12.5036, Breast Medical Oncology, Unit 1354
6565 MD Anderson Blvd., Houston, TX 77030
Tel: 713-563-9221
Email: jslee@mdanderson.org

5.10.4.4 Contact Information for Notification of Specimen Shipment:

Dr. Jangsoon Lee
Tel: 713-563-9221
Email: jslee@mdanderson.org

5.10.5 Membrane rigidity

5.10.5.1 Specimen(s) Receipt and Processing at Chapkin Laboratory Texas A&M University.

Fresh tumor tissue will be received at Chapkin lab for the membrane rigidity assay.

5.10.5.2 Site(s) Performing Correlative Study

This study will be conducted at the Department of Biochemistry and Biophysics, Texas A&M University under the supervision of Robert S. Chapkin, Ph.D. (r-chapkin@tamu.edu).

5.10.5.3 Shipment of Specimens to Site Performing Correlative Study

See Section 5.7.1.

5.10.5.4 Contact Information for Notification of Specimen Shipment:

See Section 5.7.1.

5.10.6 Cholesterol homeostasis in cell membrane

5.10.6.1 Specimen(s) Receipt and Processing Chapkin Laboratory Texas A&M University.

Fresh tumor tissue will be received at Chapkin lab for the cholesterol homeostasis in cell membrane assay.

5.10.6.2 Site(s) Performing Correlative Study

This study will be conducted at the Department of Biochemistry and Biophysics, Texas A&M University under the supervision of Robert S. Chapkin, Ph.D. (r-chapkin@tamu.edu).

5.10.6.3 Shipment of Specimens to Site Performing Correlative Study

See Section 5.7.1.

5.10.6.4 Contact Information for Notification of Specimen Shipment:

See Section 5.7.1.

5.10.7 Multiplex cytokine panel

5.10.7.1 Specimen(s) Receipt and Processing at the EET Biobank

Frozen aliquots of serum will be barcoded and stored in a -80°C freezer until distribution for analysis.

5.10.7.2 Site(s) Performing Correlative Study

This study will be conducted at the Department of Hematopathology, MD Anderson Cancer Center (MDACC) under the supervision of James M. Reuben, Ph.D. (jreuben@mdanderson.org).

5.10.7.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank to:

Department of Hematopathology, MD Anderson Cancer Center (MDACC)
Attn.: Dr. Evan Cohen
Y4.6041, Hematopathology
1515 Holcombe Blvd., Houston, TX 77030
Tel: 713-745-2132
Email: encohen@mdanderson.org

5.10.7.4 Contact Information for Notification of Specimen Shipment:

Dr. Evan Cohen
Tel: 713-745-2132
Email: encohen@mdanderson.org

6. TREATMENT PLAN

6.1 Agent Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential

risks are described in Section 10. Appropriate dose modifications are described in Section 7. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Phase 1b Dose Escalation Schedule			
Dose Level	**Dose*#**		
	Dasatinib (mg)	**Icosapent ethyl (EPA) (mg)**	**Cycle Length**
Level 1*	100 mg, PO, QD	2000 mg, PO, BID	28 days
Level 2	100 mg, PO, QD	3000 mg, PO, BID	
Level 3	100 mg, PO, QD	4500 mg, PO, BID	
*Starting Dose Level. PO = Orally, QD = Once daily, BID = Twice a day #Following registration, patients should begin protocol treatment within 30 days.			

Phase 2				
Agent	**Dose**	**Route**	**Schedule**	**Cycle Length**
Dasatanib	100 mg	PO	QD	28 days
Icosapent ethyly (EPA)	MTD/RP2D	PO	BID	28 days
MTD = Maximum tolerated dose, RP2D = Recommended phase 2 dose, PO = Orally, QD = Once daily, BID = Twice a day				

6.1.1 Dasatinib

Dasatinib tablets may be taken with or without food as desired, but should be swallowed with at least 8 ounces (240 mL) of water. Taking dasatinib with grapefruit juice is prohibited. Each participant will receive the first doses of dasatinib at the clinical site and be monitored for 2 hours to accommodate safety observations. Each participant will self-administer all subsequent doses. A light meal is not required, but may improve gastric tolerance for dasatinib. Tablets must be swallowed whole and may not be broken. If vomiting occurs within 30 minutes of swallowing the tablet(s), the dose may be replaced if the tablets can be seen and counted. Four weeks (28 days) constitutes one cycle of treatment.

Patients will be provided with a Medication Diary for dasatinib (Appendix D), instructed in its use, and asked to bring the diary with them to each appointment. A new copy of the Medication Diary will be given to patients whose dose is reduced due to adverse events.

If a dose is missed, then the patient should take the next dose on the next day and in the pre-specified amount. The patient needs to record the missed dose and date in the medication diary.

The patient must not take any more than the pre-specified amount.

If tablets are accidentally crushed or broken, caregivers should wear disposable chemotherapy gloves. Pregnant women should avoid exposure to crushed and/or broken tablets.

6.1.2 Icosapent ethyl (EPA)

Icosapent ethyl capsules are administered orally (PO) twice daily (BID) with or following a meal. The capsules are to be swallowed whole and cannot be broken, crushed, dissolved, or chewed. If one dose of the daily two dose schedule is missed, the capsule should be taken as soon as possible within the same day. In the case where both doses of the daily two dose schedule are omitted, the capsules should not be doubled the next day. If vomiting occurs within 30 minutes of swallowing the capsules, then the dose may be replaced if the capsules can be seen and counted.

Patients will be provided with a Medication Diary for EPA (Appendix D), instructed in its use, and asked to bring the diary with them to each appointment. A new copy of the Medication Diary will be given to patients whose dose is reduced due to adverse events.

6.2 Definition of Dose-Limiting Toxicity

For dose-finding, DLT is defined as an inability to maintain the prescribed doses during the first 28 days as a result of treatment-related toxicity, as follows:

1. Grade 3 or 4 non-hematological toxicities >2 days while on optimal therapy
2. Grade 4 neutropenia >3 days, or grade 3 or 4 neutropenia with sepsis/fever
3. Grade 4 thrombocytopenia or bleeding requiring platelet transfusion.

Management and dose modifications associated with the above adverse events are outlined in Section 7.

Dose escalation will proceed within each cohort according to the scheme presented in Section 9.1. Dose-limiting toxicity (DLT) is defined above.

The MTD is defined as the highest dose level at which <33% of the dose cohort (0 of 3 or 1 of 6) experience a DLT in the first cycle. Up to 3 additional patients (maximum enrollment 6) will be added at the MTD level to more fully characterize the safety of the drug combination. If <33% (2) patients in this expanded cohort experience a DLT, this will be declared the MTD, and thus the RP2D. If 2 or more patients experience a DLT, we will adopt this dose level as the maximum administered dose (MAD) and drop to the dose level immediately below, for the MTD and the RP2D.

6.3 General Concomitant Medication and Supportive Care Guidelines

Because there is a potential for interaction of dasatinib with other concomitantly administered drugs, 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 for drug interactions. The study team should check a frequently-updated medical reference for a list of drugs to avoid or minimize use of. A patient wallet card listing the study agents will be provided (Appendix C).

6.3.1 Dasatinib

Dasatinib is indicated for the treatment of the following:

- Newly diagnosed adults with Philadelphia chromosome-positive (Ph+) chronic myeloid leukemia (CML) in chronic phase.
- Adults with chronic, accelerated, or myeloid or lymphoid blast phase Ph+ CML with resistance or intolerance to prior therapy including imatinib.
- Adults with Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) with resistance or intolerance to prior therapy.
- Pediatric patients with Ph+ CML in chronic phase.

The proposed research involves a change to the subject population (mTN-IBC). Since the proposed research will combine dasatinib with EPA, it may increase the risks associated with using the product. To ensure safety, the maximum tolerated dose for EPA and dasatinib in combination will be determined in phase 1b. Dasatinib is not being promoted for off-label use.

6.3.1.1 Agents or substances that strongly induce or inhibit CYP3A4 are prohibited during dasatinib treatment because the patient's exposure to dasatinib is significantly affected by such materials. For CYP3A4 inhibitors, a washout period of >7 days is required prior to starting dasatinib. The washout period should be based on the half-life of the particular CYP 3A4 inhibitor which can be substantially longer than 7 days in some cases (e.g., amiodarone). The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential to affect selected P450 isoenzymes. See Appendix G for a list of specifically prohibited CYP3A4 inhibitors and inducers.

6.3.1.2 Patients should be advised not to consume grapefruit or grapefruit juice during dasatinib treatment.

6.3.1.3 Because systemic antacids (H_2 inhibitors, proton pump inhibitors) decrease dasatinib absorption, patients who require antacids should use short-acting, locally-active agents (*e.g.*, Maalox®, Mylanta® *etc*.). However, these agents should not be taken within either 2 hours before or 2 hours after the dasatinib dose.

6.3.1.4 Use of agents with proarrhythmic potential is not permitted during the study, and a washout period of ≥7 days is required prior to starting dasatinib. The washout period should be based on the half-life of the particular proarrhythmic agent which can be substantially longer than 7 days in some cases (*e.g.*, amiodarone). See Appendix G for a list of proarrhythmic agents that are specifically prohibited during dasatinib treatment. A comprehensive list of agents with the potential to cause QTc prolongation (≤450

milliseconds) can be found at http://torsades.org.

6.3.1.5 Thrombocytopenia and hemorrhagic events can occur with dasatinib treatment. For this reason, patients may not take anticoagulants or medications that inhibit platelet function while on study including therapeutic warfarin or heparin. All such medications must have been stopped ≥7 days prior to starting dasatinib to allow an appropriate washout period. If the patient requires any surgical (including dental) procedure while on study, dasatinib should be stopped 1 day before the procedure and not reinstituted until 1 to 2 days afterward or until adequate hemostasis is achieved.

6.3.1.6 Bisphosphonate therapy should be withheld for the first 8 weeks of treatment in patients receiving such treatment pending assessment of the need for calcium supplementation (see below). If patient's serum calcium levels remain above the lower limit of normal, patients on prior bisphosphonate therapy may be restarted with caution at the investigator's discretion.

6.3.1.7 Calcium supplements (*e.g.,* calcium carbonate, 500 mg PO three times daily) may be required to maintain serum calcium levels above the lower limit of normal during dasatinib treatment. Vitamin D supplements (*e.g.*, ergocalciferol, 400 IU PO daily) may be appropriate for persistent hypocalcemia. Bisphosphonate therapy should be deferred in the presence of hypocalcemia.

6.3.1.8 The nausea, vomiting, and diarrhea that may occur with dasatinib administration can generally be managed through the use of appropriate supportive measures (anti-emetics - *e.g.,* 5-HT_3 antagonists, benzodiazepines, prochlorperazine, and anti-diarrheal medications - *e.g.*, loperamide). Granisetron, an antiemetic that does not prolong QTc intervals, should be considered early in treatment.

6.3.1.9 Fluid retention, including pleural effusions, should be controlled by early institution of diuresis (*e.g.,* furosemide, 20-40 mg PO daily and/or spironolactone, 25-50 mg PO, titrated to symptoms). Pleural effusions that remain or become symptomatic despite diuresis should be managed with thoracentesis. Steroid treatment may also be effective for pleural effusion. Chest discomfort may be related to a pericardial effusion; and an echocardiogram should be performed to investigate this possibility in such cases.

6.3.1.10 Inflammation (*e.g.*, pneumonitis, colitis, skin rash) may be appropriately managed with dasatinib interruption and short-term steroid treatment (*e.g.,* 5-7 days methylprednisolone with rapid taper). Concurrent antibiotics are appropriate if there is clinical suspicion of infection.

6.3.1.11 Therapeutic use of hematopoietic growth factors is permitted at the investigator's discretion and should follow American Society of Clinical Oncology guidelines for their use (Ozer *et al.*, 2000).

6.3.1.12 Symptoms of pulmonary arterial hypertension (PAH) include dyspnea, fatigue, hypoxia, and edema. Since other medical conditions may also cause these symptoms, non-

invasive procedures (including echocardiogram) should be done first to rule out more the common etiologies of these symptoms, such as pleural effusion, pulmonary edema, anemia, and lung infiltration.

6.3.1.13 Right heart catheterization can confirm the diagnosis of PAH. Hypertension is "pre-capillary" and not a consequence of left heart failure or chronic lung disease if there is normal pulmonary capillary wedge pressure (<15 mm Hg) but elevated pulmonary artery pressure (mean pulmonary artery pressure >25 mm Hg). Since PAH may be reversible upon discontinuation of dasatinib, a diagnostic approach of interruption of dasatinib treatment may be considered at the discretion of the treating physician; however, if PAH is confirmed, dasatinib should be permanently discontinued.

6.3.2 Icosapent ethyl (EPA)

Icosapent ethyl was associated with an increased risk of atrial fibrillation or atrial flutter requiring hospitalization in a double-blind, placebo-controlled trial of statin-treated patients with established cardiovascular disease (CVD) or diabetes plus an additional risk factor for CVD. The incidence of atrial fibrillation was greater in patients with a previous history of atrial fibrillation or atrial flutter. Thus, patients with diabetes or hypothyroidism should be monitored.

The effect of icosapent ethyl on the risk for pancreatitis in patients with severe hypertriglyceridemia has not been determined. Furthermore, icosapent ethyl has not been studied in patients with renal or hepatic impairment.

6.3.2.1 Drug Interaction Studies

In drug-drug interaction studies, icosapent ethyl (4 g/day) at steady-state did not significantly change:

- the steady-state AUC_{τ} or C_{max} of omeprazole when co-administered at 40 mg/day to steady-state
- the single dose AUC or C_{max} of rosiglitazone at 8 mg
- the single dose AUC or C_{max} of R- and Swarfarin or the anti-coagulation pharmacodynamics of warfarin when co-administered as racemic warfarin at 25 mg
- the steady-state AUC_{τ} or C_{max} of atorvastatin, 2-hydroxyatorvastatin, or 4-hydroxyatorvastatin when co-administered with atorvastatin 80 mg/day at steady-state

6.4 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
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Clinical progression
- Patient non-compliance
- Pregnancy
    - All women of child bearing potential should be instructed to contact the investigator immediately if they suspect they might be pregnant (*e.g.*, missed or late menstrual period) at any time during study participation.
    - The investigator must immediately notify CTEP in the event of a confirmed pregnancy in a patient participating in the study.
- Termination of the study by sponsor
- The drug manufacturer can no longer provide the study agent

The reason(s) for protocol therapy discontinuation, the reason(s) for study removal, and the corresponding dates must be documented in the Case Report Form (CRF).

6.5 Duration of Follow-Up

Patients will undergo in-clinic post-treatment evaluations 1 month after their last study treatment or before starting new treatment, whichever occurs first, and 3 months after their last study treatment. The 1-month and 3-month follow-up visits can be done by phone if patients prefer. Patients will then be followed by phone every 3 months for up to 2 years 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.

7. DOSING DELAYS/DOSE MODIFICATIONS

7.1 Dasatinib

The dose levels and the general approach to dose modification of dasatinib on this trial are shown below. Adverse events (AEs) should be treated with the appropriate maximum supportive care, and dose reductions should be clearly documented in the case report form.

Patients will be withdrawn from the study if they fail to recover to CTCAE grade 0-1 or tolerable grade 2 (or within 1 grade of starting values for pre-existing laboratory abnormalities) from a treatment-related toxicity within 14 days OR they experience agent- related adverse events

requiring dose modification despite two previous dose reductions (*i.e.*, would require a third dose reduction) unless the investigator and CTEP senior investigator agree that the patient should remain on the study because of evidence that the patient is/may continue deriving benefit from continued study treatment.

Dose Level	Dasatinib Dose
-2	50 mg PO QD
-1	80 mg PO QD
0	100 mg PO QD

7.1.1 Dosage Modification for Dasatinib

7.1.1.1 Selected Hematologic and Non-Hematologic Adverse Events

Event	AE Grade or Observation	Dose modification
Neutropenia	Grade 1 or 2	Maintain dose
	Grade 3 or 4 [1]	Hold dasatinib until ≤ grade 2, then reduce 1 dose level and resume treatment
Thrombocytopenia	Grade 1 or 2	Maintain dose
	Grade 3 or 4 [1]	Hold dasatinib until ≤ grade 2, then reduce 1 dose level and resume treatment
Hemorrhage/Bleeding/ Coagulopathy (without thrombocytopenia)	Grade 1	No interruption in treatment; maintain current dose. Monitor as clinically indicated
	Grade 2	Hold dasatinib until AE resolved to ≤ grade 1; reduce dose to next lower dose level, and continue treatment. If grade 2 or greater hemorrhage/ bleeding recurs following dose reduction, stop dasatinib and remove patient from study. Follow up per protocol (Section 6.5) if patient is removed from the study.
	Grade 3 or 4	Discontinue treatment and withdraw subject from study. Follow up per protocol (see Section 6.5).
QTc Prolongation	>480 but <550 msec	Review patient's concomitant medications for QT interval-prolonging agents. Correct any electrolyte abnormalities. Continue dasatinib at current dose level and repeat ECG.
	≥550 msec	Stop dasatinib and any other QT interval-prolonging agents immediately. Correct any

		electrolyte abnormalities, then 1. If there is a plausible explanation for AE other than dasatinib treatment, resume dasatinib at current dose level. 2. If dasatinib **may have contributed** to the AE: • Reduce 2 dose levels and restart dasatinib. • If QTc remains <480 msec after 14 days at reduced dose, increase one dose level and continue dasatinib.
[1] Recurrent grade 3 events require dose reduction; recurrent grade 4 events require study removal.		

7.1.1.2 General Management Guidelines for Agent-Related Non-Hematologic Toxicity

Severity	**Management**
Grade 2	1st event: Institute supportive therapy. May hold dasatinib, or continue without dose reduction, or reduce by one dose level 2nd event: Hold dasatinib and maximize supportive therapy. Decrease dose by one dose level 3rd event: Hold dasatinib. May discontinue if AE poorly controlled.
Grade 3	1st event: Hold dasatinib. Institute supportive therapy. Restart dasatinib with reduction by one dose level allowed. 2nd event: Hold dasatinib. Maximize supportive therapy. Dasatinib may be restarted or discontinued if dose already reduced.
Grade 4	1st event: Hold dasatinib. Maximize supportive therapy. Dasatinib may be restarted with dose reduction by one dose level or discontinued.

7.2 Icosapent ethyl (EPA)

EPA is a basically nutritional agent, and we do not expect severe adverse effects from the treatment. Previous clinical trials showed arthralgia was the most frequent adverse effect, but the incidence rate was less than 5%. The dosing escalation for EPA will follow the BOIN design. In case the patient had uncommon side effects, PI and clinical stuff will discuss the event and determine which drug should be reduced dosing. The table below describes the summary of dose modification and criteria for treatment interruption and re-initiation.

EPA Dose	**First Dose Reduction (Dose Level -1)**	**Second Dose Reduction (Dose Level -2)**
2000 mg PO BID	Discontinue EPA	Discontinue EPA
3000 mg PO BID	2000 mg	Discontinue EPA

4500 mg PO BID	3000 mg	2000 mg

Dose Modifications for EPA	
Worst toxicity (CTCAE 5.0 Grade)	**EPA Dose Modifications**
Arthralgia	
Grade 1 or 2	Maintain dose level
Grade 3	Omit dose until resolved to ≤ Grade 1, then decrease 1 dose level
Grade 4	Permanently discontinue patient from EPA
Coagulation abnormalities	
Grade 1 or 2	Maintain dose level
Grade 3	Omit dose until resolved to ≤ Grade 1, then decrease 1 dose level
Grade 4	Permanently discontinue patient from EPA
Pancreatitis	
Grade 1	Maintain dose level
Grade 2	Omit dose until resolved to ≤ Grade 1, then decrease 1 dose level
Grade 3	Permanently discontinue patient from EPA
High triglycerides	
Grade 1 or 2	Maintain dose level
Grade 3	Omit dose until resolved to ≤ Grade 1, then decrease 1 dose level
Grade 4	Permanently discontinue patient from EPA

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agent administered in this study can be found in Section 10.1.

8.1 CTEP Agent

8.1.1 Dasatinib (NSC #732517)

Other names: BMS-354825, Sprycel®

Chemical Name: *N*-(2-Chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2 methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide, monohydrate

Mechanism of Action*:* Dasatinib is a potent, broad spectrum ATP-competitive inhibitor of 5 critical oncogenic tyrosine kinase families: BCR-ABL, SRC family kinases, c-KIT, ephrin (EP) receptor kinases, and PDGFβ receptor. Overexpression or activation of these kinases plays critical roles in the etiology of various cancer types.

Molecular Formula: $C_{22}H_{26}ClN_7O_2S \cdot H_20$ **MW:** Dasatinib monohydrate: 506.02 daltons

Approximate Solubility: Dasatinib's solubility ranged from 18.42 mg/mL at pH 2.6 to < 0.001 mg/mL at pH 7.

How Supplied: BMS supplies and CTEP, NCI, DCTD distributes dasatinib. Dasatinib is available in the following tablet/bottle sizes:

- 20 mg biconvex, round, white to off-white film-coated tablets.
- 50 mg biconvex, oval, white to off-white film-coated tablets. The tablet is debossed with "50" on one side and "528" on the other side.

Inactive ingredients include lactose monohydrate, microcrystalline cellulose, croscarmellose sodium, hydroxypropyl cellulose, magnesium stearate. The film-coating contains hypromellulose, titanium dioxide and polyethylene glycol.

Tablets are supplied in high-density polyethylene (HDPE) bottles with desiccant and cotton. The bottles are heat-induction sealed with child-resistant caps.

Storage: Store the intact bottles at controlled room temperature (15°C-25°C) and protect from light.

Stability: Stability studies are ongoing.

Route of Administration: Oral. Tablets may be taken with or without food. They should be swallowed whole and not crushed or broken.

Potential Drug Interactions: Dasatinib is primarily metabolized by the human CYP3A4 enzyme; therefore, potent CYP3A4 inducers and inhibitors are prohibited on dasatinib trials.

Concomitant use of dasatinib and a CYP3A4 substrate may increase exposure to the CYP3A4

substrate. Therefore, caution is warranted when dasatinib is coadministered with CYP3A4 substrates of narrow therapeutic index.

Systemic antacids (both H_2 receptor antagonists and proton pump inhibitors) are prohibited on dasatinib trials. Locally acting antacids can be given up to two hours prior or two hours following dasatinib administration.

Dasatinib may prolong the QT/QTc interval. Use caution when administering dasatinib with other potential QTc-prolonging medications.

Due to the possibility of CNS, gastrointestinal, cardiac, and cutaneous hemorrhage, use caution in patients who require medications that inhibit platelet function or anticoagulants.

Special Handling: Dasatinib tablets consist of a core tablet (containing the active drug) surrounded by a film coating to prevent exposure to the active drug substance. If tablets are accidentally crushed or broken, caregivers should wear disposable chemotherapy gloves. Pregnant women should avoid exposure to crushed and/or broken tablets.

Availability

Dasatinib is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI.

Dasatinib is provided to the NCI under a Collaborative Agreement between the Pharmaceutical Collaborator and the DCTD, NCI (see Section 13.5).

8.1.2 Agent Ordering and Agent Accountability

8.1.2.1 NCI-supplied agents may be requested by eligible participating Investigators (or their authorized designee) at each participating institution. The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The eligible participating investigators at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), NCI Biosketch, Agent Shipment Form, 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 participating investigator at that institution.

Study agents must be ordered after a patient is registered since no starter supplies are being provided. Normal order processing time is two business days. Expedited orders may be shipped overnight when a site provides expedited courier information. Submit agent requests through the PMB Online Agent Order Processing (OAOP) application. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account and the maintenance of an "active" account status, a "current" password, and active person registration status. For questions about drug orders, transfers, returns, or accountability, call or email PMB any time.

Refer to the PMB's website for specific policies and guidelines related to agent management.

8.1.2.2 Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, dispensing and final disposition of all agents received from the PMB using the appropriate NCI Investigational Agent (Drug) Accountability Record (DARF) available on the CTEP forms page. Store and maintain separate NCI Investigational Agent Accountability Records for each agent, strength, formulation and ordering investigator on this protocol.

8.1.3 Investigator Brochure Availability

The current version of the dasatinib IB will be accessible to site investigators and research staff through the PMB OAOP application. Access to OAOP requires the establishment of a CTEP IAM account and the maintenance of an "active" account status, a "current" password and active person registration status. Questions about IB access may be directed to the PMB IB Coordinator via email.

8.1.4 Useful Links and Contacts

- CTEP Forms, Templates, Documents: http://ctep.cancer.gov/forms/
- NCI CTEP Investigator Registration: RCRHelpDesk@nih.gov
- PMB policies and guidelines: http://ctep.cancer.gov/branches/pmb/agent_management.htm
- PMB Online Agent Order Processing (OAOP) application: https://ctepcore.nci.nih.gov/OAOP
- CTEP Identity and Access Management (IAM) account: https://ctepcore.nci.nih.gov/iam/
- CTEP IAM account help: ctepreghelp@ctep.nci.nih.gov
- IB Coordinator: IBCoordinator@mail.nih.gov
- PMB email: PMBAfterHours@mail.nih.gov
- PMB phone and hours of service: (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET)

8.2 Commercial Agent

8.2.1 Icosapent ethyl (EPA, VASCEPA)

Product description: Icosapent ethyl (EPA, VASCEPA), a lipid-regulating agent, is supplied as either a 0.5 gram or a 1 g amber-colored, liquid-filled soft gelatin capsule for oral use. Each icosapent ethyl (EPA, VASCEPA) capsule contains either 0.5 grams of icosapent ethyl (in a 0.5 g capsule) or 1 gram of icosapent ethyl (in a 1 g capsule). Icosapent ethyl is an ethyl ester of the omega-3 fatty acid eicosapentaenoic acid (EPA). The empirical formula of icosapent ethyl is C22H34O2 and the molecular weight is 330.51. The chemical name for icosapent ethyl is ethyl all-cis-5,8,11,14,17-icosapentaenoate with the following chemical structure: icosapent ethyl (EPA, VASCEPA) capsules also contain the following inactive ingredients: tocopherol, gelatin, glycerin, maltitol, sorbitol, and purified water.

Absorption: After oral administration, icosapent ethyl (EPA, VASCEPA) is de-esterified during the absorption process and the active metabolite EPA is absorbed in the small intestine and enters the systemic circulation mainly via the thoracic duct lymphatic system. Peak plasma concentrations of EPA were reached approximately 5 hours following oral doses of icosapent ethyl (EPA, VASCEPA). Icosapent ethyl (EPA, VASCEPA) was administered with or following a meal in all clinical studies; no food effect studies were performed. Therefore, it is recommended to take icosapent ethyl (EPA, VASCEPA) with or following a meal.

Distribution: The mean volume of distribution at steady state of EPA is approximately 88 liters. The majority of EPA circulating in plasma is incorporated in phospholipids, triglycerides and cholesteryl esters, and <1% is present as the unesterified fatty acid. Greater than 99% of unesterified EPA is bound to plasma proteins.

Elimination
Metabolism
EPA is mainly metabolized by the liver via beta-oxidation similar to dietary fatty acids. Beta oxidation splits the long carbon chain of EPA into acetyl Coenzyme A, which is converted into energy via the Krebs cycle. Cytochrome P450-mediated metabolism is a minor pathway of elimination of EPA.

Excretion
The total plasma clearance of EPA at steady state is 684 mL/hr. The plasma elimination half-life (t1/2) of EPA is approximately 89 hours. icosapent ethyl (EPA, VASCEPA) does not undergo renal excretion.

Route of administration: The dose of icosapent ethyl (EPA, VASCEPA) is as assigned per Section 6.1, taken twice daily with food. Patients are advised to swallow capsules whole. Do not break open, crush, dissolve, or chew capsules.

Prior to initiation of icosapent ethyl (EPA, VASCEPA), assess lipid levels. Identify other causes (e.g., diabetes mellitus, hypothyroidism, or medications) of high triglyceride levels and manage as appropriate. Patients should engage in appropriate nutritional intake and physical activity before receiving icosapent ethyl (EPA, VASCEPA), which should continue during treatment.

Storage: Store the product bottle at 20º to 25ºC (68º to 77ºF); excursions permitted to 15º to 30ºC (59º to 86ºF).

Agent Ordering: Icosapent ethyl (VASCEPA®) is commercially available. Given that icosapent ethyl (VASCEPA®) is a dietary supplement, it will not be covered by insurance. The participating sites will purchase EPA, one of the study drugs, if it is not already in their institution's formulary. For a participating site to be reimbursed a fee-for-service agreement through Purchase Order (PO) will be established between the participating sites and the lead site (MD Anderson Cancer Center). Participating sites will invoice MD Anderson for the EPA costs for their enrolled patients.

For additional information, please refer to the package insert.

9. STATISTICAL CONSIDERATIONS

9.1 Study Design/Endpoints

This is a multicenter, single-arm, non-randomized phase 1b/2 trial of dasatinib (BMS-354825, Sprycel) and icosapent ethyl (EPA) combination therapy for patients with metastatic triple-negative inflammatory breast cancer (TN-IBC). All eligible patients will receive dasatinib and EPA combination therapy without randomization or stratification. The primary objectives of this study are: 1) To determine the MTD/RP2D for dasatinib and EPA combination therapy in patients with mTN-IBC (phase 1b) and 2) To determine the ORR of dasatinib and EPA combination therapy for patients with mTN-IBC (phase 2).

Phase 1:
The primary objective of the phase 1b trial is to determine the MTD/RP2D for dasatinib and EPA combination therapy in patients with mTN-IBC. In the phase 1b part of the study, Bayesian Optimal Interval Combination Design (BOIN), with the 3+3 design run-in, will be used to determine the MTD for the combination of EPA and dasatinib. The target DLT rate in the phase Ib part is $\phi = 0.25$, and the maximum sample size for the dose-finding is 18. We will enroll and treat patients in cohorts of 3. The BOIN design uses the following rule, optimized to minimize the probability of incorrect dose assignment, to guide dose escalation/de-escalation:

- if the observed DLT rate at the current dose is ≤ 0.197, escalate the dose to the next higher dose level;
- if the observed DLT rate at the current dose is > 0.298, de-escalate the dose to the next lower dose level;
- otherwise, stay at the current dose.

The 3+3 design run-in will be applied to override the above decision rule when the number of patients treated at the current dose is 3. That is, we will escalate the dose if 0/3 DLT, stay at the current dose if 1/3 DLT, and de-escalate the dose if >= 2/3 DLTs. For the purpose of overdose control, doses j and higher levels will be eliminated from further examination if $\Pr(p_j > 0.25 \mid \text{data}) > 0.75$ and at least 3 evaluable patients have been treated at dose level j, where p_j is the true DLT rate of dose level $j, j = 1,\cdots, 3$. This posterior probability is evaluated based on the beta-binomial model $y_j \mid p_j \sim \text{binomial}(p_j)$ with $p_j \sim \text{uniform}(0,1)$, where y_j is the number of patients experienced DLT at dose level j. When the lowest dose is eliminated, stop the trial for safety. The above dose escalation/de-escalation and elimination rule can be equivalently presented in Table 1, which will be used to conduct the trial.

The steps to conduct the trial are described as follows:

1. The dose-escalation starts by treating patients in the first cohort at dose level 1 (dasatinib = 100 mg/day; EPA = 2,000 mg twice per day).
2. To assign a dose to the next cohort of patients, we will conduct dose escalation/de-escalation according to the rule displayed in Table 1.
    - When a dose is eliminated, the dose is automatically de-escalated to the next lower level. When the lowest dose is eliminated, stop the trial for safety.

- If none of the actions is triggered, treat the new patients at the current dose.
- If the current dose was the lowest dose and the rule indicates dose de-escalation, treat the new patients at the lowest dose unless the number of DLTs reaches the elimination boundary, at which point terminates the trial for safety.
- If the current dose was the highest dose and the rule indicates dose escalation, we will treat the new patients at the highest dose.

3. We will repeat the above steps until the maximum sample size of 18 is reached or stop the trial if the number of patients treated at the current dose reaches 9 and the decision, according to Table 1 is to stay at the current dose.

Table 1. Dose escalation/de-escalation rule for the BOIN design.

Actions	The number of patients treated at the current dose			
	3	**6**	**9**	**12**
Escalate if # of DLT <=	0	1	1	2
De-escalate if # of DLT >=	2*	2	3	4
Eliminate if # of DLT >=	2	2	3	4

Note. "# of DLT" is the number of patients with at least 1 DLT. When none of the actions (i.e., escalate, de-escalate or eliminate) is triggered, stay at the current dose for treating the next cohort of patients. "NA" means that a dose cannot be eliminated before treating 3 evaluable patients. "*" indicates the 3+3 design run-in.

After the trial is completed, the MTD is selected based on isotonic regression, as specified in the previous report (Liu and Yuan, 2015). Specifically, the dose is selected as the MTD for which the isotonic estimate of the toxicity rate is closest to the target toxicity rate. If there are ties, the higher dose level is selected when the isotonic estimate is lower than the target toxicity rate; the lower dose level is selected when the isotonic estimate is greater than or equal to the target toxicity rate. The RP2D will be selected based on the safety, efficacy, and the totality of the data. The RP2D may be the MTD or a dose lower than the MTD.

The dose-finding part of the study was designed and will be conducted using the BOIN Design Desktop Program v1.0.7. Table 2 shows the operating characteristics of the trial design based on 1000 simulations of the trial using shiny app "BOIN" (BOIN V2.6.4.0) available at http://www.trialdesign.org.

Table 2. Operating characteristics of the BOIN design.

		Dose Level			
	1	2	3	Number of Patients	% Early Stopping
Scenario 1					
True DLT Rate	**0.25**	0.43	0.62		
Selection %	**44**	8	0.1		47.9
% Pts Treated	**63.6**	30.3	6.1	10.5	

Scenario 2					
True DLT Rate	0.11	**0.25**	0.39		
Selection %	42.1	**35.9**	10		12
% Pts Treated	39.6	**38.8**	21.6	14.7	
Scenario 3					
True DLT Rate	0.05	0.11	**0.25**		
Selection %	13	43.3	**41.4**		2.3
% Pts Treated	25.4	36.6	**38**	16	

Note: "% Early Stopping"" refers to early stopping due to excessive DLT.

Phase 2:

The primary objective of the phase 2 trial is to determine the ORR of dasatinib and EPA combination therapy, administered at the RP2D determined in phase 1 part, for patients with mTN-IBC. Once the MTD/RP2D has been determined in phase 1b, the trial will move to phase 2 using Bayesian optimal phase II (BOP2) design with the null hypothesis H_0: ORR = 5% vs. the alternative hypothesis H_1: ORR = 25%. In the first stage, 9 patients will be enrolled; if 1 or more responses are observed, an additional 8 patients will be enrolled. The treatment is regarded as promising if 3 or more responses out of 17 patients are observed. This design yields 81% power while controlling the one-sided type I error rate at 0.05. Under our setting, BOP2 is the same as Simon optimal two-stage design. Table 3 shows the operating characteristics of the design based on 10,000 simulations using the BOP2 web application, which is available at http://www.trialdesign.org.

Table 3. Operating characteristics for 10,000 simulations.

True ORR	**Early stopping (%)**	**Claim promising (%)**	**Sample size**
0.05	63.02	4.66	12.0
0.10	38.74	22.34	13.9
0.20	13.42	66.31	15.9
0.25	7.51	81.22	16.4
0.30	4.04	90.45	16.7

During the phase 2 part, the dose elimination rule of the BOIN design will be used to monitor toxicity. That is, if $\Pr(p_j > 0.25 \mid \text{data}) > 0.75$, the trial may be terminated for safety based on the totality of safety data. This corresponds to the following stopping boundaries:

- halt the accrual for possible trial termination if (# of toxicity) / (# of patients) >= 2/3, 3/6, 4/11 and 6/17.

Below shows the operating characteristics of the safety monitoring rule.

Toxicity rate	0.1	0.2	0.3	0.4	0.5
Early stopping (%)	4.6	22.9	50.4	74.5	90.7

Sample size	16.5	14.6	11.7	8.9	6.5

9.2 Sample Size/Accrual Rate

A maximum total of 18 patients will be enrolled in the phase 1b part of the study to determine the MTD/RP2D for the combination of EPA and dasatinib, and a maximum of 17 patients will be enrolled to the phase 2 portion of the study. The maximum sample sizes for this phase 1b/2 study will be 35 in total. An estimated 1-2 patients will be accrued per month. Total accrual duration is estimated to take 13 months for the phase 1b trial and approximately 11 months for the phase 2 trial (the duration of total accrual within 24 months).

Year	**1**				**2**				**3**				**4**			
Quarter	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Establish Phase II Dose																
Regulatory Approval																
Enroll Phase lb																
Enroll Phase ll																
Treatment duration																
Biomarker Analysis																
Follow-up																
Statistical Analysis																
Final deliberative																

PLANNED ENROLLMENT REPORT

DOMESTIC PLANNED ENROLLMENT REPORT (TREATMENT)					
Racial Categories	**Ethnic Categories**				**Total**
	Not Hispanic or Latino		**Hispanic or Latino**		
	Female	**Male**	**Female**	**Male**	
American Indian/ Alaska Native	1	0	0	0	1
Asian	1	0	0	0	1
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	4	0	0	0	4
White	25	0	2	0	27
More Than One Race	0	0	2	0	2
Total	31	0	4	0	35

9.3 Stratification Factors

N/A

9.4 Analysis of Secondary Endpoints

The secondary objectives of the phase 2 trial are: 1) To determine the clinical benefit rate (CBR) of dasatinib and EPA combination therapy for patients with mTN-IBC and 2) To determine the survival benefit of dasatinib and EPA combination therapy for patients with mTN-IBC. The definition of CBR will be the proportion of the patients with static disease (SD) ≥24 weeks, complete response (CR), and partial response (PR). The survival benefit will be determined by the progression-free survival (PFS) rate at 1 year and the overall survival rate at 2 years. PFS at 1 year is defined as the rate of patients without the progression of disease or loss to follow-up at 1 year after initiating the EPA and dastinib combination therapy. Overall survival at 2 years is the survival rate at 2 years after initiating the EPA and dasatinib combination therapy.

CBR will be estimated along with a 95% exact confidence interval. PFS and OS will be estimated using the methods of Kaplan and Meier.

Tumor apoptosis will be measured by using the Luminex Apoptosis multiplex immunoassay panel (cleaved caspase-3) as well as TUNEL assay. For the Luminex Apoptosis multiplex immunoassay panel, Panel 3, which includes cleaved caspase, will be prioritized. The tumor cells/section will be counted for apoptotic index (3,000 cells). The apoptotic index is a percentage of the number of cells displaying apoptotic bodies, scoring 0 if <0.5%, +1 if between 0.5% and 2%, and +3 if >2% (Lipponen *et al.*, 1994). The correlation between the apoptotic index by cleaved caspase-3 IHC expression and treatment response (ORR) will be evaluated using Pearson's correlation test. For the TUNEL assay, the apoptotic cells will be counted and the correlation between those apoptotic cells and ORR will be determined. Given the sample size of 17 patients, we have 82% power to detect an effect size of 0.16 in ORR across three apoptotic index groups (with score of 0, 1 and 3) at the significance level of 0.1 using Chi-square test. The effect size is defined as the variance of ORR across three apoptotic index groups, standardized by the variance of the average ORR. In addition, given 17 patients, we have 80% power to detect 1.5 SD difference in the number of apoptotic cells between respondents and non-respondents, assuming ORR=0.25 (i.e., 4 respondents and 13 non-respondents), at the 1-sided significance level of 0.05.

9.5 Analysis of Exploratory Endpoints

Changes in biomarkers corresponding to the EPA and dasatinib combination therapy will be determined in correlative studies. Cholesterol homeostasis, and cholesterol transporter in the tumor cell membrane, the tumor microenvironment, and pro-inflammatory cytokines will be assessed and analyzed using descriptive statistics. Continuous value before/after treatment will be compared by utilizing the Wilcoxon signed-rank test and paired t-test.

ABCA1 will be measured not only in tumor cells but also in immune cells, especially in M1 and M2 macrophages since ABCA1 is known to promote cholesterol efflux in macrophages (Tall *et al*., 2002). Changes in expression levels of ABCA1 in tumors are an indicator of unbalanced cholesterol metabolism. The intensity of the staining and percentage of stained cells will be scored by H-score following the formula: H Score = summation (1+i) pi, where i is the intensity score and pi is the percentage of cells with that intensity. H-score will be analyzed using descriptive statistics, including mean, standard deviation and 95% confidence interval.

EphA2-IHC positivity will be defined from 0 to 3+ by modifying the ASCO/CAP HER2 testing clinical practice guideline because EphA2 protein expressed in tumor membrane is targeted (Wolff *et al*., 2018). The modified pathological evaluation of the EphA2-IHC positivity will be as follows:1) 0 (negative) No staining is observed or membrane staining that is incomplete and is faint/barely perceptible, 2) 1+ (negative) Incomplete membrane staining that is faint/barely perceptive, 3) 2+ (equivocal) Weak to moderate complete membrane staining observed in >10% of tumor cells, and 4) 3+ (positive) Circumferential membrane staining that is complete, intense and in >10% of tumor cells. After the completion of the phase 2 part, the association between 3 levels of EphA2-IHC expression (negative, equivocal, and positive) and ORR will be analyzed using Fisher's exact test and logistic regression.

The effects of EPA and dasatinib combination therapy on membrane rigidity of tumor cells will be determined and analyzed as described previously (Torres-Adorno *et al*., 2019). Higher general polarization values are indicative of increased polarization or rigidity. Increases in cholesterol levels in cell membranes will indicate membrane stiffness in response to the combination treatment. For quantitative analysis of cholesterol in individual cells *in situ*, images will be opened in NIH ImageJ software, converted to Tiff files, and a custom macro used to quantify the average fluorescent intensity of cholesterol (Salinas *et al*., 2020). As an internal control to assess variability, serial sections of one sample will be used for staining and imaging. A minimum of 20 random regions of each sample will be acquired for quantification.

To visualize effects of EPA and dasatinib combination therapy on cholesterol homeostasis, tissue sections stained with filipin (which labels unesterified cholesterol) and Draq5 (which labels nuclei) will be imaged using confocal microscopy, and fluorescent intensity will be quantified as previously described (Salinas *et al.*, 2020).

For the cytokine/chemokine assay, the correlation between ORR and levels of cytokine and immune cell markers will be analyzed by Fisher's exact test and logistic regression (Harano *et al*., 2017).

9.6 Reporting and Exclusions

9.6.1 Evaluation of Toxicity

All patients will be evaluable for toxicity from the time of their first treatment with dasatinib.

9.6.2 Evaluation of Response

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations. Each patient will 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) should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

All conclusions should be based on all eligible patients. Sub-analyses may 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.

10. 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 10.1) and the characteristics of an observed AE (Sections 10.2 and 10.3) will determine whether the event requires expedited reporting via the CTEP Adverse Event Reporting System (CTEP-AERS) **in addition** to routine reporting.

10.1 Comprehensive Adverse Events and Potential Risks List (CAEPR)

10.1.1 CAEPRs for CTEP IND Agent

10.1.1.1 CAEPR for Dasatinib

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 2937 patients.* Below is the CAEPR for dasatinib (BMS-354825, Sprycel).

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 2.7, September 10, 2018[1]

Adverse Events with Possible Relationship to Dasatinib (BMS-354825, Sprycel) (CTCAE 5.0 Term) [n= 2937]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			***Anemia (Gr 3)***
	Febrile neutropenia		
CARDIAC DISORDERS			
		Heart failure	
		Left ventricular systolic dysfunction	
		Myocardial infarction	
	Pericardial effusion		
GASTROINTESTINAL DISORDERS			
	Abdominal distension		
	Abdominal pain		***Abdominal pain (Gr 3)***
	Anal mucositis		
	Constipation		
Diarrhea			***Diarrhea (Gr 3)***
	Dyspepsia		
	Gastrointestinal hemorrhage[2]		
	Mucositis oral		
Nausea			***Nausea (Gr 3)***
	Rectal mucositis		
	Small intestinal mucositis		
	Vomiting		***Vomiting (Gr 3)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Edema limbs		
Fatigue			***Fatigue (Gr 3)***
	Fever		***Fever (Gr 2)***
	General disorders and administration site conditions - Other (superficial edema)		***General disorders and administration site conditions - Other (superficial edema) (Gr 2)***
	Generalized edema		
	Non-cardiac chest pain		
	Pain		
INFECTIONS AND INFESTATIONS			
	Infection[3]		***Infection[3] (Gr 3)***
INVESTIGATIONS			
	Alanine aminotransferase increased		

Adverse Events with Possible Relationship to Dasatinib (BMS-354825, Sprycel) (CTCAE 5.0 Term) [n= 2937]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Aspartate aminotransferase increased		
		Electrocardiogram QT corrected interval prolonged	
Neutrophil count decreased			***Neutrophil count decreased (Gr 4)***
Platelet count decreased			***Platelet count decreased (Gr 4)***
	Weight gain		
	Weight loss		
	White blood cell decreased		***White blood cell decreased (Gr 3)***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 3)***
	Hypocalcemia		
	Hypokalemia		
	Hypophosphatemia		***Hypophosphatemia (Gr 3)***
		Tumor lysis syndrome	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		
		Growth suppression[4]	
		Musculoskeletal and connective tissue disorder - Other (epiphyses delayed fusion)[4]	
		Musculoskeletal and connective tissue disorder - Other (osteopenia)[4]	
Myalgia			***Myalgia (Gr 2)***
NERVOUS SYSTEM DISORDERS			
	Dizziness		
Headache			***Headache (Gr 3)***
		Intracranial hemorrhage	
		Leukoencephalopathy	
		Reversible posterior leukoencephalopathy syndrome	
REPRODUCTIVE SYSTEM AND BREAST DISORDERS			
		Gynecomastia[4]	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		
Dyspnea			***Dyspnea (Gr 3)***
	Laryngeal mucositis		
	Pharyngeal mucositis		
Pleural effusion			***Pleural effusion (Gr 3)***
	Pneumonitis		
		Pulmonary hypertension	
	Tracheal mucositis		
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Alopecia		

Adverse Events with Possible Relationship to Dasatinib (BMS-354825, Sprycel) (CTCAE 5.0 Term) [n= 2937]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
		Erythema multiforme	
	Pruritus		
	Rash acneiform		
Rash maculo-papular			***Rash maculo-papular (Gr 2)***
		Stevens-Johnson syndrome	
		Toxic epidermal necrolysis	
VASCULAR DISORDERS			
	Flushing		

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

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

[3]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

[4]Effects on growth and development have been observed in pediatric patients and may include epiphyses delayed fusion, osteopenia, growth retardation, and gynecomastia.

[5]Gastrointestinal ulcer includes Anal ulcer, Colonic ulcer, Duodenal ulcer, Esophageal ulcer, Gastric ulcer, Ileal ulcer, Jejunal ulcer, Rectal ulcer, and Small intestine ulcer under the GASTROINTESTINAL DISORDERS SOC.

Adverse events reported on Dasatinib (BMS-354825, Sprycel) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that Dasatinib (BMS-354825, Sprycel) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (pancytopenia)
CARDIAC DISORDERS - Atrial fibrillation; Cardiac disorders - Other (cardiomegaly); Cardiac disorders - Other (heart rate increased); Chest pain - cardiac; Myocarditis; Palpitations; Pericarditis; Sinus tachycardia; Ventricular tachycardia
CONGENITAL, FAMILIAL AND GENETIC DISORDERS - Congenital, familial and genetic disorders - Other (Keratosis follicular)
EAR AND LABYRINTH DISORDERS - Ear pain; Middle ear inflammation; Tinnitus; Vertigo
EYE DISORDERS - Blurred vision; Dry eye; Eye disorders - Other (optic nerve neuritis); Periorbital edema
GASTROINTESTINAL DISORDERS - Anal fissure; Ascites; Colitis; Dry mouth; Dysphagia; Esophagitis; Flatulence; Gastritis; Gastrointestinal disorders - Other (enteritis); Gastrointestinal disorders - Other (oral soft tissue disorder); Gastrointestinal disorders - Other (tongue eruption); Gastrointestinal ulcer[5]; Ileus; Oral pain; Pancreatitis; Periodontal disease; Stomach pain
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Edema face; Edema trunk; Flu like symptoms; Gait disturbance; General disorders and administration site conditions - Other (temperature intolerance); Localized edema; Malaise
HEPATOBILIARY DISORDERS - Cholecystitis; Hepatobiliary disorders - Other (cholestasis)

IMMUNE SYSTEM DISORDERS - Anaphylaxis
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Bruising
INVESTIGATIONS - Alkaline phosphatase increased; Blood bilirubin increased; Cardiac troponin T increased; CD4 lymphocytes decreased; CPK increased; Creatinine increased; Electrocardiogram T wave abnormal; GGT increased; Investigations - Other (bone densitometry); Investigations - Other (thermometry abnormal); Lymphocyte count decreased; Lymphocyte count increased
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hyperkalemia; Hyperuricemia; Hypoalbuminemia; Hypomagnesemia; Hyponatremia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthritis; Back pain; Bone pain; Chest wall pain; Generalized muscle weakness; Muscle cramp; Musculoskeletal and connective tissue disorder - Other (muscle stiffness); Musculoskeletal and connective tissue disorder - Other (nuchal rigidity); Musculoskeletal and connective tissue disorder - Other (tendonitis); Myositis; Osteoporosis; Pain in extremity; Rhabdomyolysis
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (hemangiomatosis)
NERVOUS SYSTEM DISORDERS - Acoustic nerve disorder NOS; Amnesia; Cognitive disturbance; Concentration impairment; Dysarthria; Dysgeusia; Ischemia cerebrovascular; Lethargy; Peripheral motor neuropathy; Peripheral sensory neuropathy; Seizure; Somnolence; Syncope; Transient ischemic attacks; Tremor
PSYCHIATRIC DISORDERS - Anxiety; Confusion; Depression; Insomnia; Libido decreased; Suicidal ideation
RENAL AND URINARY DISORDERS - Acute kidney injury; Proteinuria; Urinary frequency
REPRODUCTIVE SYSTEM AND BREAST DISORDERS – Irregular menstruation
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Adult respiratory distress syndrome; Bronchospasm; Epistaxis; Hypoxia; Oropharyngeal pain; Pulmonary edema; Sore throat
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Bullous dermatitis; Dry skin; Hair color changes; Hyperhidrosis; Nail loss; Pain of skin; Palmar-plantar erythrodysesthesia syndrome; Photosensitivity; Purpura; Skin and subcutaneous tissue disorders - Other (acute febrile neutrophilic dermatosis); Skin and subcutaneous tissue disorders - Other (panniculitis); Skin ulceration; Urticaria
VASCULAR DISORDERS - Hematoma; Hot flashes; Hypertension; Hypotension; Phlebitis; Superficial thrombophlebitis; Thromboembolic event; Vasculitis

Note: Dasatinib (BMS-354825, Sprycel) 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.

10.1.2 Adverse Event List for Commercial Agent

10.1.2.1 Adverse Event List for Icosapent Ethyl

Common adverse reactions (incidence ≥3% on icosapent ethyl and ≥1% more frequent than placebo) in a double-blind, randomized, placebo-controlled cardiovascular outcomes trial included musculoskeletal pain, peripheral edema, constipation, gout, and atrial fibrillation.

In two randomized, double-blind, placebo-controlled hypertriglyceridemia trials, adverse reactions reported with icosapent ethyl at an incidence ≥1% more frequent than placebo (based on pooled data) included arthralgia and oropharyngeal pain.

Additional adverse reactions identified during post-approval use of icosapent ethyl include:

- Diarrhea
- Blood triglycerides increased
- Abdominal discomfort
- Pain in the extremities

Please review the icosapent ethyl package insert for the comprehensive lists of adverse events.

10.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. 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 website 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 10.1) should be reported through CTEP-AERS only if the grade is above the grade provided in the SPEER.
  - Other AEs of special interest (AESIs) for the protocol that require expedited reporting are outlined in Section 10.3.4.

- **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.

10.3 Expedited Adverse Event Reporting

10.3.1 Rave-CTEP-AERS Integration

The Rave Cancer Therapy Evaluation Program Adverse Event Reporting System (CTEP-AERS) integration enables evaluation of Adverse Events (AEs) entered in Rave to determine whether they require expedited reporting and facilitates entry in CTEP-AERS for those AEs requiring expedited reporting. Sites must initiate all AEs for this study in Medidata Rave.

Treatment-emergent AEs: All AEs that occur after start of treatment are collected in Medidata Rave using the Adverse Event form, which is available for entry at each treatment course or reporting period and is used to collect AEs that start during the period or persist from the previous reporting period. AEs that occur 30 Days after the Last Administration of the Investigational Agent/Intervention are collected using the Late Adverse Event form.

Prior to sending AEs through the rules evaluation process, site staff should verify the following on the Adverse Event form in Rave:

- The reporting period (course/cycle) is correct, and
- AEs are recorded and complete (no missing fields) and the form is query-free.

The CRA reports AEs in Rave at the time the Investigator learns of the event. If the CRA modifies an AE, it must be re-submitted for rules evaluation.

Upon completion of AE entry in Medidata Rave, the CRA submits the AE for rules evaluation by completing the Expedited Reporting Evaluation form. Both NCI and protocol-specific reporting rules evaluate the AEs submitted for expedited reporting. A report is initiated in CTEP-AERS using information entered in Medidata Rave for AEs that meet reporting requirements. The CRA completes the report by accessing CTEP-AERS via a direct link on the Medidata Rave Expedited Reporting Evaluation form. Contact the CTSU Help Desk at 1-888-823-5923 or by email at ctsucontact@westat.com if you have any issues submitting an expedited report in CTEP-AERS.

In the rare occurrence that 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 that was phoned in must be entered immediately into CTEP-AERS using the direct link from Medidata Rave.

Additional information about the CTEP-AERS integration is available on the CTSU members' website:

- Study specific documents: *Protocols > Documents > Protocol Related Documents> Adverse Event Reporting* , and
- Additional resources: *Resources > CTSU Operations Information > User Guides & Help Topics.*

NCI requirements for SAE reporting are available on the CTEP website:

- NCI Guidelines for Investigators: Adverse Event Reporting Requirements is available at https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf.

10.3.2 Distribution of Adverse Event Reports

CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Principal Investigator and Adverse Event Coordinator(s) (if applicable) of the Corresponding Organization or Lead Organization, the local treating physician, and the Reporter and Submitter. CTEP-AERS provides a copy feature for other e-mail recipients.

10.3.3 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 as long as the death occurred within 30 days after the last administration of the investigational agent. 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.

Phase 1 and Early Phase 2 Studies: 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 **<u>MUST</u>** immediately report to the sponsor (NCI) **<u>ANY</u>** 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 **<u>ANY</u>** 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).

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

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

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

<u>Expedited AE reporting timelines are defined as:</u>

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

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

- All Grade 3, 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

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

Effective Date: May 5, 2011

10.3.4 Biopsy-related Adverse Events

Adverse Events related to the biopsy component of the study should be captured in Rave as they occur and should be reported expeditiously according to the table above. Four of these AEs are not currently part of the CTCAE and must be reported in Rave/CTEP-AERS using the term: Injury, poisoning and procedural complications - Other, specify. The Biopsy-related Adverse EventsTerms and Grading Table below (Section 10.3.4.1) lists the terms and their grading. The Biopsy-related Reporting Table (Section 10.3.4.2) provides instruction on how to record these events in Rave/CTEP-AERS.

10.3.4.1 Biopsy-related Adverse Events Terms and Grading

Biopsy-related Adverse Events Terms and Grading

AE	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Definition
Biopsy-related hemorrhage	Symptomatic hematoma requiring medications	Any hematoma (symptomatic or asymptomatic) requiring unplanned observation with less than 24 hours of hospitalization	Hemorrhage requiring transfusion, radiologic, endoscopic or surgical intervention or greater than 24 hours of hospitalization	Hemorrhage with life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by bleeding related to a biopsy procedure.
Biopsy-related nerve injury	Symptomatic nerve pain or injury requiring medications	Any nerve injury requiring unplanned observation with less than 24 hours of hospitalization	Any nerve injury requiring surgical intervention or greater than 24 hours of hospitalization	Nerve injury with life-threatening consequences; urgent intervention indicated	Death	A finding of damage to a nerve related to a biopsy procedure.
Biopsy-related organ injury	Symptomatic organ pain or injury requiring medications	Any organ injury requiring unplanned observation with less than 24 hours of hospitalization	Any organ injury that requires radiologic, endoscopic or surgical intervention or greater than 24 hours of hospitalization	Organ injury with life-threatening consequences; urgent intervention indicated	Death	A finding of damage to an organ related to a biopsy procedure.
Biopsy-related anesthesia (local or moderate sedation) effects	Symptomatic anesthesia effects requiring medications	Any biopsy-related anesthesia effects requiring medical intervention for reversal of symptomatic effects and/or requiring unplanned observation with less than 24 hours of hospitalization	Any anesthesia effects requiring intervention for reversal or treatment of symptomatic effects and/or requiring greater than 24 hours of hospitalization	Anesthesia effects with life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by reactions related to the administration of local or moderate sedation given for a biopsy procedure.
Pneumothorax	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; intervention indicated	Sclerosis and/or operative intervention indicated; hospitalization indicated	Life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by abnormal presence of air in the pleural cavity resulting in the collapse of the lung.
Pain	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self care ADL	-	-	A disorder characterized by the sensation of marked discomfort, distress or agony.
Wound infection	Localized, local intervention indicated	Oral intervention indicated (e.g., antibiotic, antifungal, or antiviral)	IV antibiotic, antifungal, or antiviral intervention indicated; invasive intervention indicated	Life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by an infectious process involving the wound.
Allergic reaction	Systemic intervention not indicated	Oral intervention indicated	Bronchospasm; hospitalization indicated for clinical sequelae; intravenous intervention indicated	Life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by an adverse local or general response from exposure to an allergen.

10.3.4.2 Biopsy-related Adverse Events Reporting

Biopsy-related Adverse Events Reporting

AE Term	CTCAE term to Select	Other, specify language to record:
Pneumothorax*	Pneumothorax	N/A
Pain*	Pain	N/A
Wound infection*	Wound infection	N/A
Allergic reaction*	Allergic reaction	N/A
Biopsy-related hemorrhage**	Injury, poisoning and procedural complications - Other, specify	Biopsy-related hemorrhage
Biopsy-related nerve injury**	Injury, poisoning and procedural complications - Other, specify	Biopsy-related nerve injury
Biopsy-related organ injury**	Injury, poisoning and procedural complications - Other, specify	Biopsy-related organ injury
Biopsy-related anesthesia (local or moderate sedation) effects**	Injury, poisoning and procedural complications - Other, specify	Biopsy-related anesthesia (local or moderate sedation) effects

*Select the indicated current CTCAE Term.

**Select CTCAE term, "Injury, poisoning and procedural complications - Other, specify" and fill in the 'Specify' with the AE term when prompted in Rave.

For example, if the subject experienced a biopsy-related hemorrhage, select Injury, poisoning and procedural complications - Other, specify and write in 'Biopsy-related hemorrhage' when prompted in Rave and/or CTEP-AERS.

10.4 Routine Adverse Event Reporting

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

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

10.5 Pregnancy

Although not an adverse event in and of itself, pregnancy as well as its outcome must be documented via **CTEP-AERS**. In addition, the ***Pregnancy Information Form*** included within the NCI Guidelines for Adverse Event Reporting Requirements must be completed and submitted to CTEP. Any pregnancy occurring in a patient or patient's partner from the time of consent to 90 days after the last dose of study drug must be reported and then followed for outcome. Newborn infants should be followed until 30 days old. Please see the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" (at http://ctep.cancer.gov/protocolDevelopment/adverse_effects.htm) for more details on how to report pregnancy and its outcome to CTEP.

10.6 Secondary Malignancy

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

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

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

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

10.7 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 AE reporting unless otherwise specified.

11. STUDY CALENDAR

Baseline evaluations are to be conducted within 1 week prior to start of protocol therapy. Scans and x-rays must be done ≤4 weeks prior to the start of therapy. All assessments may have a +/- 2 day window. 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.

	Pre-Study	Cycle 1				Cycle 2				Cycle 3+				Off Study[a]
		Wk 1	Wk 2	Wk 3	Wk 4	Wk 1	Wk 2	Wk 3	Wk 4	Wk 9	Wk 10	Wk 11	Wk 12	
Dasatinib		A	A	A	A	A	A	A	A	A	A	A	A	
Icosapent ethyl (EPA)		B	B	B	B	B	B	B	B	B	B	B	B	
Informed consent	X													
Demographics	X													
Medical history	X													
Concurrent meds	X	X----------X												
Physical exam	X	X				X				X				X
Vital signs	X	X				X				X				X
Height	X													
Weight	X	X				X				X				X
Performance status	X													
CBC w/diff, plts	X	X				X				X				X
Serum chemistry[b]	X	X				X				X				X
PT w/INR & PTT	X					X				X				X
Amylase and Lipase	X					X				X				X
Lipid panel	X					X				X				X
EKG[g] (at baseline and as indicated thereafter)	X													
ECHO or MUGA (at baseline and as indicated thereafter)	X													
Adverse event evaluation		X----------X												X
Tumor measurements	X	Tumor measurements are repeated every 2 cycles. Documentation (radiologic) must be provided for patients removed from study for progressive disease.												X
Radiologic evaluation	X	Radiologic measurements should be performed every 2 cycles.												X
Pregnancy test[d]	X													
Biopsy for research	X[e]								X[f]					
Blood collection for research	X[e]								X[f]					
HbA1c	X[h]													
HIV, HBV, HCV viral load	X[h]													

A: Dasatinib: 100 mg PO QD.
B: Icosapent ethyl (EPA): Dose as assigned, PO BID.
a: Off-study evaluations within 1 month after and 3 months after final study treatment.
b: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium,

	total protein, SGOT [AST], SGPT [ALT], sodium.
d:	Pregnancy test for women of childbearing potential.
e:	Baseline biopsy and blood collection to be performed within 1 week before starting study treatment.
f:	Second biopsy and blood collection to be performed ± 2 days after completing the 2nd cycle.
g.	EKG at baseline is required and later checkup will be as clinically indicated as standard of care.
h.	HbA1C at baseline for patients taken antidiabetic medication while A1c is not available only, HIV, HBV, HCV viral load for patients who have known history of positive tests/on treatment only.

12. MEASUREMENT OF EFFECT

12.1 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 4 weeks following initial documentation of objective response.

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

12.1.1 Definitions

Evaluable for Toxicity. All patients will be evaluable for toxicity from the time of their first treatment with dasatinib.

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.

12.1.2 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 (≥2 cm) by chest x-ray or as ≥10 mm (≥1 cm) with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

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

Malignant Lymph Nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm (≥1.5 cm) in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm [0.5 cm]). 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 [<1 cm] or pathological lymph nodes with ≥10 to <15 mm [≥1 to <1.5 cm] 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.

12.1.3 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 (≥1 cm) 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 (0.5 cm) or less. If CT scans have slice thickness greater than 5 mm (0.5 cm), 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.

12.1.4 Response Criteria

12.1.4.1 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 (<1 cm).

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 (0.5 cm). (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.

12.1.4.2 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 [<1 cm] 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).

12.1.4.3 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.

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

12.1.5 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.

12.1.6 Progression-Free Survival

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

12.1.7 Response Review

The Quantitative Imaging Analyzing Core (QIAC) at MD Anderson Cancer Center will independently evaluate the treatment response based on the imaging results.

13. STUDY OVERSIGHT AND DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 10 (Adverse Events: List and Reporting Requirements).

13.1 Study Oversight

This protocol is monitored at several levels, as described in this section. The Protocol Principal Investigator is responsible for monitoring the conduct and progress of the clinical trial, including the ongoing review of accrual, patient-specific clinical and laboratory data, and routine and serious adverse events; reporting of expedited adverse events; and accumulation of reported adverse events from other trials testing the same drug(s). The Protocol Principal Investigator and statistician have access to the data at all times through the CTMS web-based reporting portal.

For the Phase 1 portion of this study, all decisions regarding dose escalation/expansion/de-escalation require sign-off by the Protocol Principal Investigator through the CTMS/IWRS. In addition, for the Phase 1 portion, the Protocol Principal Investigator will have at least monthly conference calls with the Study Investigators [and, if needed, the CTEP Medical Officer(s)] to review accrual, progress, and adverse events and unanticipated problems.

For a Phase 1/2 trial, enrollment to the Phase 2 portion of the trial will not begin until a protocol amendment has been submitted which summarizes the Phase 1 results, the recommended Phase 2 dose, and the rationale for selecting it. The amendment must be reviewed and approved by CTEP before enrollment to the Phase 2 portion can begin.

All Study Investigators at participating sites who register/enroll patients on a given protocol are responsible for timely submission of data via Medidata Rave and timely reporting of adverse events for that particular study. This includes timely review of data collected on the electronic CRFs submitted via Medidata Rave.

All studies are also reviewed in accordance with the enrolling institution’s data safety monitoring plan.

13.2 Data Reporting

Medidata Rave is a clinical data management system being used for data collection for this trial/study. Access to the trial in Rave is controlled through the CTEP-IAM system and role assignments.
Requirements to access Rave via iMedidata:

- A valid account, and
- Assigned a Rave role on the LPO or PO roster at the enrolling site of: Rave CRA, Rave Read Only, Rave CRA (LabAdmin), Rave SLA, or Rave Investigator.
  Rave role requirements:
    - Rave CRA or Rave CRA (Lab Admin) role, must have a minimum of an Associate Plus (AP) registration type,
    - Rave Investigator role, must be registered as an Non-Physician Investigator (NPIVR) or Investigator (IVR), and
    - Rave Read Only role, site staff must have at a minimum an Associates (A) registration type.
- Refer to https://ctep.cancer.gov/investigatorResources/default.htm for registration types and documentation required.

If the study has a DTL, individuals requiring write access to Rave must also be assigned the appropriate Rave tasks on the DTL.

Upon initial site registration approval for the study in Regulatory Support System (RSS), all persons with Rave roles assigned on the appropriate roster will be sent a study invitation email from iMedidata. To accept the invitation, site staff must either click on the link in the email or log in to iMedidata via the CTSU members' website under *Data Management* > *Rave Home* and click to accept the invitation in the Tasks pane located in the upper right-corner of the iMedidata screen. Site staff will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings) and can be accessed by clicking on the eLearning link in the *Tasks* pane located in the upper right corner of the iMedidata screen. If an eLearning is required for a study and has not yet been taken, the link to the eLearning will appear under the study name in the Studies pane located in the center of the iMedidata screen; once the successful completion of the eLearning has been recorded, access to the study in Rave will be granted, and a *Rave EDC* link will replace the eLearning link under the study name.

Site staff that have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in RSS will receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website in the Data Management section under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website in the Data Management > Rave section at www.ctsu.org/RAVE/ or by contacting the CTSU Help Desk at 1-888-823-5923 or by email at ctsucontact@westat.com.

13.2.1 Method

This study will be monitored by the Clinical Trials Monitoring Service (CTMS). Data will be submitted to CTMS at least once every two weeks via Medidata Rave (or other modality if approved by CTEP). Information on CTMS reporting is available at http://www.theradex.com/clinicalTechnologies/?National-Cancer-Institute-NCI-11. On-site audits will be conducted three times annually (one annual site visit and two data audits). For CTMS monitored studies, after users have activated their accounts, please contact the Theradex Help Desk at (609) 619-7862 or by email at CTMSSupport@theradex.com for additional support with Rave and completion of CRFs.

13.2.2 Responsibility for Data Submission

For ETCTN trials, it is the responsibility of the PI(s) at the site to ensure that all investigators at the ETCTN Sites understand the procedures for data submission for each ETCTN protocol and that protocol specified data are submitted accurately and in a timely manner to the CTMS via the electronic data capture system, Medidata Rave.

Data are to be submitted via Medidata Rave to CTMS on a real-time basis, but no less than once every 2 weeks. The timeliness of data submissions and timeliness in resolving data queries will be tracked by CTMS. Metrics for timeliness will be followed and assessed on a quarterly basis. For the purpose of Institutional Performance Monitoring, data will be considered delinquent if it is greater than 4 weeks past due.

Data from Medidata Rave and CTEP-AERS is reviewed by the CTMS on an ongoing basis as data is received. Queries will be issued by CTMS directly within Rave. The queries will appear on the Task Summary Tab within Rave for the CRA at the ETCTN to resolve. Monthly web-based reports are posted for review by the Drug Monitors in the IDB, CTEP. Onsite audits will be conducted by the CTMS to ensure compliance with regulatory requirements, GCP, and NCI policies and procedures with the overarching goal of ensuring the integrity of data generated from NCI-sponsored clinical trials, as described in the ETCTN Program Guidelines, which may be found on the CTEP (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm) and CTSU websites.

CTMS will utilize a core set of eCRFs that are Cancer Data Standards Registry and Repository (caDSR) compliant (http://cbiit.nci.nih.gov/ncip/biomedical-informatics-resources/interoperability-and-semantics/metadata-and-models). Customized eCRFs will be included when appropriate to meet unique study requirements. The PI is encouraged to review the eCRFs, working closely with CTMS to ensure prospectively that all required items are appropriately captured in the eCRFs prior to study activation. CTMS will prepare the eCRFs with built-in edit checks to the extent possible to promote data integrity.

CDUS data submissions for ETCTN trials activated after March 1, 2014, will be carried out by the CTMS contractor, Theradex. CDUS submissions are performed by Theradex on a monthly basis. The trial's lead institution is responsible for timely submission to CTMS via Rave, as above.

Further information on data submission procedures can be found in the ETCTN Program Guidelines (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm).

13.3 Data Quality Portal

The Data Quality Portal (DQP) provides a central location for site staff to manage unanswered queries and form delinquencies, monitor data quality and timeliness, generate reports, and review metrics.

The DQP is located on the CTSU members' website under Data Management. The Rave Home section displays a table providing summary counts of Total Delinquencies and Total Queries. DQP Queries, DQP Delinquent Forms, DQP Form Status and the DQP Reports modules are available to access details and reports of unanswered queries, delinquent forms, forms with current status and timeliness reports. Review the DQP modules on a regular basis to manage specified queries and delinquent forms.

The DQP is accessible by site staff that are rostered to a site and have access to the CTSU website. Staff that have Rave study access can access the Rave study data using a direct link on the DQP.

To learn more about DQP use and access, click on the Help icon displayed on the Rave Home, DQP Queries, DQP Delinquent Forms modules, DQP Form Status, and DQP Reports.

13.4 CTEP Multicenter Guidelines

N/A

13.5 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.

13.6 Genomic Data Sharing Plan

N/A

13.7 Incidental/Secondary Findings Disclosure Procedure

N/A

14. REFERENCES

Allen, S., Y.C. Chen, J. Madden, *et al.* (2016). Macrophages Enhance Migration in Inflammatory Breast Cancer Cells via RhoC GTPase Signaling. *Sci Rep*. 6:39190.

Bays, H.E., C.M. Ballantyne, J.J. Kastelein, *et al*. (2011). Eicosapentaenoic acid ethyl ester (AMR101) therapy in patients with very high triglyceride levels (from the Multi-center, plAcebo-controlled, Randomized, double-blINd, 12-week study with an open-label Extension [MARINE] trial). *Am J Cardiol.* 108(5):682-690.

Calder, P.C. (2010). Omega-3 fatty acids and inflammatory processes. *Nutrients.* 2(3):355-374.

Cassetta, L. and T. Kitamura. (2018). Targeting Tumor-Associated Macrophages as a Potential Strategy to Enhance the Response to Immune Checkpoint Inhibitors. *Front Cell Dev Biol.* 6:38.

Chou, T.C. (2010). Drug combination studies and their synergy quantification using the Chou-Talalay method. *Cancer Res.* 70(2):440-446.

Demetri, G.D., P. Lo Russo, I.R.J. MacPherson, *et al.* (2009). Phase I dose-escalation and pharmacokinetic study of dasatinib in patients with advanced solid tumors. *Clin Cancer Res.* 15:6232-6240.

Donato, N.J., J. Wu, L.Y. Kong, *et al*. (2004). The SRC/ABL inhibitor BMS-354825 overcomes resistance to imatinib mesylate in chronic myelogenous leukemia cells through multiple mechanisms. *Blood*. 104:A1989.

Ford, N.A., E.L. Rossi, K. Barnett, *et al*. (2015). Omega-3-Acid Ethyl Esters Block the Protumorigenic Effects of Obesity in Mouse Models of Postmenopausal Basal-like and Claudin-Low Breast Cancer. *Cancer Prev Res (Phila).* 8(9):796-806.

Goldner, B., C.E. Behrendt, H.F. Schoellhammer, *et al*. (2014). Incidence of inflammatory breast cancer in women, 1992-2009, United States. *Ann Surg Oncol*. 21(4):1267-1270.

Goossens, P., J. Rodriguez-Vita, A. Etzerodt, *et al*. (2019). Membrane Cholesterol Efflux Drives Tumor-Associated Macrophage Reprogramming and Tumor Progression. *Cell Metab.* 29(6):1376-1389.

Hance, K.W., W.F. Anderson, S.S. Devesa, *et al*. (2005). Trends in inflammatory breast carcinoma incidence and survival: the surveillance, epidemiology, and end results program at the National Cancer Institute. *J Natl Cancer Inst*. 97(13):966-975.

Harano, K., T. Kogawa, J. Wu, *et al*. (2017). Thrombocytosis as a prognostic factor in inflammatory breast cancer. *Breast Cancer Res Treat.* 166(3):819-832.

Hochhaus, A., and T. Hughes. (2004). Clinical resistance to imatinib: Mechanisms and

implications. *Hematol Oncol Clin North Amer.* 18:641-656.

Horton, J.D., J.L. Goldstein, and M.S. Brown. (2002). SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest.* 109(9):1125-1131.

Huang, J., W. Hu, J. Bottsford-Miller, *et al*. (2014). Cross-talk between EphA2 and BRaf/CRaf is a key determinant of response to Dasatinib. *Clin Cancer Res.* 20(7):1846-1855.

Hunter, T. (1997). Oncoprotein networks. *Cell*. 88:333-346.

Investigator's Brochure. (2020). Dasatinib. BMS-354825. Bristol-Myers Squibb. Version No: 20. Version Date: June 2020.

Ireton, R.C. and Chen, J. (2005). EphA2 receptor tyrosine kinase as a promising target for cancer therapeutics. *Curr Cancer Drug Targets.* 5(3):149-157.

Jaureguiberry, M.S., M.A. Tricerri, S.A. Sanchez, *et al*. (2014). Role of plasma membrane lipid composition on cellular homeostasis: learning from cell line models expressing fatty acid desaturases. *Acta Biochim Biophys Sin (Shanghai).* 46(4):273-282.

Johnson, F.M., B. Saigal, M. Talpaz, and N.J. Donato. (2005). BMS-354825 tyrosine kinase inhibitor suppresses invasion and induces cell cycle arrest and apoptosis of head and neck squamous cell carcinoma (HNSCC) and non-small cell lung cancer (NSCLC) cells. *Clin Cancer Res*. 11:6924-6932.

Landen, C.N. Jr., A. Chavez-Reyes, C. Bucana, *et al*. (2005). Therapeutic EphA2 gene targeting *in vivo* using neutral liposomal small interfering RNA delivery. *Cancer Res.* 65(15):6910-6918.

Lee, F.Y., L. Lombardo, R. Borzilleri, *et al*. (2004). BMS-354825 - A potent dual SRC/ABL kinase inhibitor possessing curative efficacy against imatinib sensitive and resistant human CML models *in vivo*. *Proc Am Assoc Cancer Res*. 45:A3987.

Lee, J., R. Galloway, G. Grandjean, *et al*. (2015). Comprehensive Two- and Three-Dimensional RNAi Screening Identifies PI3K Inhibition as a Complement to MEK Inhibitor AS703026 for Combination Treatment of Triple-Negative Breast Cancer. *J Cancer.* 6(12):1306-1319.

Lehmann, B.D., B. Jovanovic, X. Chen, *et al*. (2016). Refinement of Triple-Negative Breast Cancer Molecular Subtypes: Implications for Neoadjuvant Chemotherapy Selection. *PLoS One*. 11(6):e0157368.

Li S., Y. Hu, S.J. Swerdlow, *et al.* (2004). Simultaneous targeting of SRC and BCR-ABL kinases by BMS-354825 cures Ph+ acute lymphoblastic leukemia in mice. *Blood*. 104:Abstr. 1976.

Lim, B., W.A. Woodward, X. Wang, *et al*. (2018). Inflammatory breast cancer biology: the tumour microenvironment is key. *Nature Rev Cancer.* 18(8):485-499.

Lipponen, P., S. Aaltomaa, V.M. Kosma, *et al.* (1994). Apoptosis in breast cancer as related to histopathological characteristics and prognosis. *Eur J Cancer.* 30A(14):2068-2073.

Liu, S., and Y. Yuan. (2015). Bayesian optimal interval designs for phase I clinical trials. *Journal of the Royal Statistical Society Applied Statistics Series C.* 64(3):507-523.

Lombardo, L.J., F.Y. Lee, P. Chen, *et al*. (2004). Discovery of N-(2-chloro-6-methyl-phenyl)-2-(6-(4-(2-hydroxyethyl)-piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide (BMS-354825), a dual Src/Abl kinase inhibitor with potent antitumor activity in preclinical assays. *J Med Chem*. 47:6658-6661.

Luo F.R., Z. Yang, A. Camuso, *et al.* (2005). Pharmacodynamic biomarker. *Proc Am Assoc Cancer Res.* 46:A4183.

Masuda, H., K.A. Baggerly, Y. Wang, *et al*. (2013). Comparison of molecular subtype distribution in triple-negative inflammatory and non-inflammatory breast cancers. *Breast Cancer Res.* 15(6):R112.

McAuliffe, P.F., K.W. Evans, A. Akcakanat, *et al*. (2015). Ability to Generate Patient-Derived Breast Cancer Xenografts Is Enhanced in Chemoresistant Disease and Predicts Poor Patient Outcomes. *PLoS One.* 10(9):e0136851.

Miserez, A.R., P.Y. Muller, L. Barella, *et al*. (2002). Sterol-regulatory element-binding protein (SREBP)-2 contributes to polygenic hypercholesterolaemia. *Atherosclerosis.* 164(1):15-26.

Nandi, S., L. Ma, M. Denis, *et al*. (2009). ABCA1-mediated cholesterol efflux generates microparticles in addition to HDL through processes governed by membrane rigidity. *J Lipid Res.* 50(3):456-466.

Ozer H., J.O. Armitage, C.L. Bennett, *et al*., for the American Society of Clinical Oncology Growth Factors Expert Panel. (2000). 2000 update of recommendations for the use of hematopoietic colony-stimulating factors: Evidence-based, clinical practice guidelines. *J Clin Oncol.* 18:3558-3585.

Paixao, E., A.C.M. Oliveira, N. Pizato, *et al*. (2017). The effects of EPA and DHA enriched fish oil on nutritional and immunological markers of treatment naive breast cancer patients: a randomized double-blind controlled trial. *Nutr J.* 16(1):71.

Pusztai, L., S. Moulder, M. Altan, *et al*. (2014). Gene signature-guided dasatinib therapy in metastatic breast cancer. *Clinical Cancer Res.* 20(20):5265-5271.

Rahal, O.M., A.R. Wolfe, P.K. Mandal, *et al*. (2018). Blocking Interleukin (IL)4- and IL13-Mediated Phosphorylation of STAT6 (Tyr641) Decreases M2 Polarization of Macrophages and Protects Against Macrophage-Mediated Radioresistance of Inflammatory Breast Cancer. *Int J Radiat Oncol Biol Phys.* 100(4):1034-1043.

Reddy, S.M., A. Reuben, S. Barua, *et al*. (2019). Poor Response to Neoadjuvant Chemotherapy Correlates with Mast Cell Infiltration in Inflammatory Breast Cancer. *Cancer Immunol Res.* 7(6):1025-1035.

Salinas, M.L., N.R. Fuentes, R. Choate, *et al*. (2020). AdipoRon Attenuates Wnt Signaling by Reducing Cholesterol-Dependent Plasma Membrane Rigidity. *Biophys J.* 118(4):885-897

Sawyers, C.L., N.P. Shah, H.M. Kantarjian, *et al.* (2005). A phase I study of BMS-354825 in patients with imatinib-resistant and intolerant accelerated and blast phase chronic myeloid leukemia (CML): Results from CA180002. *Proc Am Soc Clin Oncol*. 24:A6520.

Schittenhelm, M., S. Shiraga, F.Y. Lee, and M.C. Heinrich. (2006). Dasatinib (BMS-354825), a dual SRC/ABL kinase inhibitor, inhibits the kinase activity of wild-type, juxtamembrane, and activation loop mutant KIT isoforms associated with human malignancies. *Cancer Res.* 66:473-481.

Shah, N.P. (2005). Loss of response to imatinib: Mechanisms and management. *Hematology Am Soc Hematol Educ Program*. 183-187.

Shah, N.P., F.Y. Lee, R. Luo, *et al*. (2005). Dasatinib (BMS-354825) inhibits $KITD^{816V}$, an imatinib-resistant activating mutation that triggers neoplastic growth in the majority of patients with systemic mastocytosis. *Blood.* 108:286-291.

Shah, N.P., C. Tran, F.Y. Lee, and C.L. Sawyers. (2004). BMS-354825 is a novel orally bioavailable small molecule ABL tyrosine kinase inhibitor that successfully and safely inhibits the kinase activity of multiple imatinib-resistant BCR-ABL isoforms *in vitro* and *in vivo*. *Proc Am Assoc Can Res*. 45:A5624.

Tall, A.R., P. Costet, and N. Wang. (2002). Regulation and mechanisms of macrophage cholesterol efflux. *J Clin Invest.* 110(7):899-904.

Talpaz, M., N.P. Shah, H. Kantarjian, *et al.* (2006). Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. *N Engl J Med.* 354:2531-2541.

Tokarski, J.S., J. Newitt, F.Y. Lee, *et al.* (2004). The crystal structure of Abl kinase with BMS-354825, a dual SRC/ABL kinase inhibitor. *Blood*. 104:A553.

Torres-Adorno, A.M., H. Vitrac, Y. Qi, *et al*. (2019). Eicosapentaenoic acid in combination with EPHA2 inhibition shows efficacy in preclinical models of triple-negative breast cancer by disrupting cellular cholesterol efflux. *Oncogene.* 38(12):2135-2150.

VASCEPA (package insert). Bridgewater, NJ: Amarin Pharma, Inc.; 2019.

Volpato, M., S.L. Perry, G. Marston*, et al*. (2016). Changes in plasma chemokine C-C motif ligand 2 levels during treatment with eicosapentaenoic acid predict outcome in patients

undergoing surgery for colorectal cancer liver metastasis. *Oncotarget.* 7(19):28139-28150.

Wolfe, A.R., N.J. Trenton, B.G. Debeb, *et al*. (2016). Mesenchymal stem cells and macrophages interact through IL-6 to promote inflammatory breast cancer in pre-clinical models. *Oncotarget*. 7(50):82482-82492.

Wu, J.Y., N.J. Donato, D.S. Hong, *et al.* (2004). The SRC/ABL kinase inhibitor BMS-354825 induces apoptosis and overcomes imatinib resistance in chronic myelogenous leukemia cell lines and patient specimens. *Proc Am Assoc Can Res*. 45:A3850.

Zong, Z., J. Zou, R. Mao, *et al*. (2019). M1 Macrophages Induce PD-L1 Expression in Hepatocellular Carcinoma Cells Through IL-1beta Signaling. *Front Immunol.* 10:1643.

APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale	
Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
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).
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.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

APPENDIX B FORMULA TO ESTIMATE RENAL FUNCTION USING SERUM CREATININE

Formulas to estimate renal function using serum creatinine provided by the NCI's Investigational Drug Steering Committee (IDSC) Pharmacological Task Force in table below.

1. Estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (Levey *et al.*, 2009).

Formulae:

Race and Sex	Serum Creatinine (SCr), *µmol/L (mg/dL)*	Equation
Black		
Female	≤62 (≤0.7)	$GFR = 166 \times (SCr/0.7)^{-0.329} \times (0.993)^{Age}$
	>62 (>0.7)	$GFR = 166 \times (SCr/0.7)^{-1.209} \times (0.993)^{Age}$
Male	≤80 (≤0.9)	$GFR = 163 \times (SCr/0.9)^{-0.411} \times (0.993)^{Age}$
	>80 (>0.9)	$GFR = 163 \times (SCr/0.9)^{-1.209} \times (0.993)^{Age}$
White or other		
Female	≤62 (≤0.7)	$GFR = 144 \times (SCr/0.7)^{-0.329} \times (0.993)^{Age}$
	>62 (>0.7)	$GFR = 144 \times (SCr/0.7)^{-1.209} \times (0.993)^{Age}$
Male	≤80 (≤0.9)	$GFR = 141 \times (SCr/0.9)^{-0.411} \times (0.993)^{Age}$
	>80 (>0.9)	$GFR = 141 \times (SCr/0.9)^{-1.209} \times (0.993)^{Age}$

SCr in mg/dL; Output is in mL/min/1.73 m^2 and needs no further conversions.

2. eGFR using the Modification of Diet in Renal Disease (MDRD) Study (Levey *et al*., 2006).

175 x $SCr^{-1.154} \times age^{-0.203} \times 0.742$ (if female) × 1.212 (if black)
Output is in mL/min/1.73 m^2 and needs no further conversions.

3. Estimated creatinine clearance (ClCr) by the Cockcroft-Gault (C-G) equation (Cockcroft and Gault, 1976).

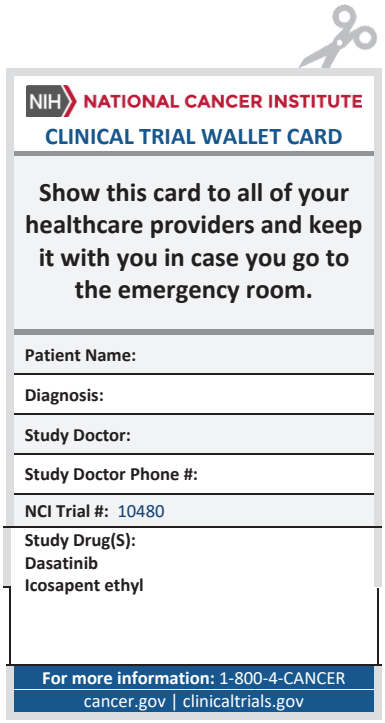
$$CLcr\ (mL/min) = \frac{[140 - age\ (years)] \times weight\ (kg)}{72 \times serum\ creatinine\ (mg\ /\ dL)} \{\times 0.85\ for\ female\ patients\}$$

Followed by conversion to a value normalized to 1.73 m^2 with the patient's body surface area (BSA).

References

1. Levey, A.S., L.A. Stevens, C.H. Schmid, *et al*. (2009). A new equation to estimate glomerular filtration rate. *Ann Inter Med*. 150:604-612.
2. Levey, A.S., J. Coresh, T. Greene, *et al*. (2006). Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med*. 145:247-254.
3. Cockcroft, D.W. and M.H. Gault. (1976). Prediction of creatinine clearance from serum creatinine. *Nephron*. 16:31-41.

APPENDIX C PATIENT CLINICAL TRIAL WALLET CARD



NIH NATIONAL CANCER INSTITUTE

CLINICAL TRIAL WALLET CARD

Show this card to all of your healthcare providers and keep it with you in case you go to the emergency room.

Patient Name:

Diagnosis:

Study Doctor:

Study Doctor Phone #:

NCI Trial #: 10480

Study Drug(S):
Dasatinib
Icosapent ethyl

For more information: 1-800-4-CANCER
cancer.gov | clinicaltrials.gov

APPENDIX D PATIENT'S MEDICATION DIARY

Appendix D1 Patient Medication Diary for Dasatinib

CTEP-assigned Protocol #_10480____

Local Protocol # _____

PATIENT'S MEDICATION DIARY

Today's date _____ **Agent __Dasatinib___ Dose** _____

Patient Name _____ (*initials acceptable)* **Patient Study ID** _____

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each cycle of treatment (4 weeks).
2. You will take ___ 20 mg tablets and ____ 50 mg tablets once at the same time each day. You should take the tablets with 8 oz. water. You may take the tablets with or without food as you wish.
3. Tablets must be swallowed whole and may not be broken.
4. If vomiting occurs within 30 minutes of swallowing the tablet(s), the dose may be replaced if the tablets can be seen and counted.
5. If a dose is missed, then take your next dose on the next day and in the pre-specified amount. You need to record the missed dose and date on the medication diary. Do not take any more than the pre-specified amount.
6. Record the date, the number of tablets you took, and when you took them.
7. If you have any comments or notice any side effects, please record them in the Comments column.
8. Please bring this form and your bottles of dasatinib tablets when you return for each appointment.

Day	Date	Time of dose	# of 20 mg tablets taken	# of 50 mg tablets taken	Comments
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					

24					
25					
26					
27					
28					

Patient's Signature_____

Physician's Office will complete this section:

1. Date patient started protocol treatment_____
2. Date patient was removed from study_____
3. Patient's planned total daily dose_____
4. Total number of tablets taken this month _____
5. Physician/Nurse/Data Manager's Signature _____

Appendix D2 Patient Medication Diary for Icosapent Ethyl

CTEP-assigned Protocol #_10480____

Local Protocol # _____

PATIENT'S MEDICATION DIARY

Today's date _____ **Agent _Icosapent ethyl_ Dose** _____

Patient Name_____ (*initials acceptable)* **Patient Study ID** _____

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each cycle of treatment.
2. You will take **icosapent ethyl (VASCEPA)** capsules twice each day with food about 12 hours apart.
   Morning dose: take ___500 mg capsules and ___ 1000 mg capsules.
   Evening dose: take ___ 500 mg capsules and ___ 1000 mg capsules.
3. The capsules are to be swallowed whole and cannot be broken, crushed, dissolved, or chewed.
4. If one dose of the daily two dose schedule is missed, the capsule should be taken as soon as possible within the same day. In the case where both doses of the daily two dose schedule are omitted, the capsules should not be doubled the next day.
5. If vomiting occurs within 30 minutes of swallowing the capsules, then the dose may be replaced if the capsules can be seen and counted.
3. Record the date, the number of capsules of each size of capsule that you took, and when you took them.
5. If you have any comments or notice any side effects, please record them in the Comments column.
6. Please bring this form and your bottles of icosapent ethyl (VASCEPA) capsules when you return for each appointment.

Day	Date	Time of morning dose	# of capsules taken		Time of evening dose	# of capsules taken		Comments
			500 mg	1000 mg		500 mg	1000 mg	
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								
24								
25								

26								
27								
28								

Patient's signature _____

Physician's Office will complete this section:

1. Date patient started protocol treatment____ _____ _____
2. Date patient was removed from study_____
3. Patient's planned total daily dose_____ _____
4. Total number of capsules taken this month _____ _____
5. Physician/Nurse/Data Manager's Signature _____

APPENDIX E PRE-BIOPSY ASSESSMENT

A pre-biopsy lesion assessment can increase trial safety and efficiency. By agreement between all investigators, an attempt at biopsy will be made if the clinical trial team determines that a biopsy poses minimal relative risk, provides potential clinical gain to the participant, and will likely yield sufficient tissue for analysis.

Pre-biopsy assessments will be reported and tracked through a trial-specific CRF within the CTEP Medidata Rave system. Additional information can be found in the Investigational Radiology SOP available at:
https://ctep.cancer.gov/initiativesPrograms/docs/ETCTN_IR_Research_Biopsy_SOP.pdf.

Individual Patient Pre-Biopsy Assessment. IR co-investigators are encouraged to apply this pre-biopsy scoring and correlation system to assist in the determination of biopsy appropriateness.

- IR co-investigators assign a subjective score of 1-3 based on likelihood of success due to lesion characteristics.

    1. Biopsy should not be done
        A. Due to safety concerns
        B. Due to lack of suitable lesion for biopsy

    2. Uncertainty about success
        A. Due to access path to lesion
        B. Due to lesion characteristics

    3. Likely successful

- Lesion characteristics to be considered
    - Size (small) (<2 cm)
    - Location/path to lesion
    - Morphologic features (necrosis, sub-solid, sclerosis, ill-defined/infiltrative)
    - PET (+/-), avidity
    - Organ/site (sclerotic bone is low yield; fine needle aspiration to be used)

APPENDIX F TISSUE BIOPSY VERIFICATION

A copy of the diagnostic pathology report must be shipped with all tissue specimens sent to the EET Biobank.

If the *corresponding* pathology report is not available for the biopsy, then a copy of the radiology report or operative report from the biopsy procedure and the diagnostic pathology report must be sent to the EET Biobank. A completed copy of this appendix (i.e., Tissue Biopsy Verification) must also be submitted to the EET Biobank.

Note: If this information is not provided with the biopsy specimen, then it will not be accepted by the EET Biobank.

Please have the Clinician* responsible for signing out this patient's case complete the following:

ETCTN Universal Patient ID: _____

ETCTN Patient Study ID: _____

Date of Procedure (mm/dd/yyyy): _____

Tissue Type (circle one): Primary Metastatic

Time point (circle one): Baseline Completion of Cycle 2 (±2 days)

Site Tissue Taken From: _____

Diagnosis: _____

I agree that this tissue may be released for research purposes only and that the release of this tissue will not have any impact on the patient's care.

_____ _____
Clinician Signature Date

_____
Clinician Printed Name

*Note: For the purposes of this form, Clinician could include the Nurse Practitioner, Registered Nurse, Pathologist, Radiologist, Interventional Radiologist, Surgeon, Oncologist, Internist, or other medical professional responsible for the patient's care.

Version: 1

APPENDIX G SUBSTANCES PROHIBITED DURING DASATINIB TREATMENT

CYP3A4 Inhibitors:

- itraconazole, ketoconazole, miconazole, voriconazole
- amprenavir, atazanavir, fosamprenavir, indinavir, nelfinavir, ritonavir
- ciprofloxacin, clarithromycin, diclofenac, doxycycline, enoxacin, imatinib, isoniazid, ketamine nefazodone, nicardipine, propofol, quinidine, telithromycin

CYP3A4 Inducers:

- aminoglutethimide, primidone, rifabutin, rifampin, St. John's wort
- carbamazepine, nevirapine, oxcarbazepine, rifapentine
- fosphenytoin, pentobarbital, phenobarbital, phenytoin

Agents with Proarrhythmic Potential

- quinidine, procainamide, disopyramide, amiodarone, sotalol, ibutilide, dofetilide
- erythromycins, clarithromycin
- chlorpromazine, haloperidol, mesoridazine, thioridazine, pimozide
- cisapride, bepridil, droperidol, methadone, arsenic, chloroquine, domperidone, halofantrine, levomethadyl, pentamidine, sparfloxacin, lidoflazine

Systemic antacids

- Both H2 receptor antagonists and proton pump inhibitors are prohibited on dasatinib trials. Locally acting antacids can be given up to two hours prior or two hours following dasatinib administration.

APPENDIX H NCLN PHARMACODYNAMICS LABORATORY FROZEN BIOPSY COLLECTION PROCEDURE

DCTD Standard Operating Procedures (SOP)

Title:	Tumor Frozen Needle Biopsy Specimen Collection, Handling and Shipment to EET Biobank				Page 1 of 13
Doc. #:	SOP340567	Revision:	-	Effective Date:	10/08/2021

Laboratory of Human Toxicology & Pharmacology

Applied/Developmental Research Directorate, Leidos Biomedical Research, Inc.

Frederick National Laboratory for Cancer Research

Technical Reviewer: Li Li _____ Date: Li Li -S (Affiliate) Digitally signed by Li Li -S (Affiliate) Date: 2021.10.08 09:55:19 -04'00'

IQC Approval: Katherine V. Ferry-Galow _____ Date: Katherine V. Ferry-galow -S (Affiliate) Digitally signed by Katherine V. Ferry-galow -S (Affiliate) Date: 2021.10.13 11:00:43 -04'00'

LHTP Approval: Ralph E. Parchment _____ Date: Ralph E. Parchment -S (Affiliate) Digitally signed by Ralph E. Parchment -S (Affiliate) Date: 2021.11.30 11:28:47 -05'00'

DCTD OD Approval: Toby Hecht _____ Date: Toby T. Hecht -S Digitally signed by Toby T. Hecht -S Date: 2021.12.06 11:58:47 -05'00'

Change History

Revision	Approval Date	Description	Originator	Approval
--	10/08/2021	New Document	LL/RA/KFG	KFG

Please check for revision status of the SOP at

http://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm

and be sure to use the current version.

TABLE OF CONTENTS

1.0 PURPOSE

Standardize the method for collecting, handling, and shipping frozen needle tumor biopsies to EET Biobank to enable measurement of pharmacodynamic (PD) markers following treatment with anti-cancer agents.

2.0 SCOPE

This procedure applies to all personnel involved in the collection and handling of frozen needle tumor biopsies for use in PD marker assays during clinical trials. The goal of this SOP and associated training is to ensure consistency in tumor needle biopsy collection and handling between clinical sites..

3.0 ABBREVIATIONS

DCTD	=	Division of Cancer Treatment and Diagnosis
EET Biobank	=	NCI Early-Phase and Experimental Clinical Trials Biospecimen Bank, also referred to as the Nationwide Biorepository or ETCTN Biorepository
FNLCR	=	Frederick National Laboratory for Cancer Research
ID	=	Identification / Identifier
IQC	=	Internal Quality Control
LHTP	=	Laboratory of Human Toxicology and Pharmacology
PADIS	=	Pharmacodynamics Assay Development & Implementation Section
PD	=	Pharmacodynamic
SOP	=	Standard Operating Procedure

4.0 INTRODUCTION

Specimen handling, shipping, and storage procedures (pre-analytical variables) can have a significant impact on the reliability of biomarker measurements in the laboratory. Following detailed steps for sample collection and handling procedures and recording any deviations from this procedure allow retrospective identification of artifactual changes in biomarker readout and increases the reliability of the data and validity of the analytical results.

5.1 ROLES AND RESPONSIBILITIES

Laboratory Director/Supervisor: The Laboratory Director/Supervisor directs laboratory operations, supervises technical personnel and reporting of findings, and is responsible for the proper performance of all laboratory procedures. Oversees the personnel who follow the SOPs in the laboratory and is responsible for ensuring the personnel are certified and have sufficient experience to handle clinical samples.

Certified Assay Operator and/or PK/PD Support Lab Personnel: An assay operator and/or PK/PD Support Lab personnel may be a Laboratory Technician/Technologist, Research Associate, or Laboratory Scientist who has been trained by DCTD personnel on this SOP. Working under the guidance of the Laboratory Director/Supervisor, this person performs laboratory procedures and examinations in accordance with thecurrent SOP(s), as well as any other procedures conducted by alaboratory, including maintaining equipment and records and performing quality assurance activities related to performance.

5.2 It is the responsibility of the Laboratory Director/Supervisor to ensure that all personnel have documented training and qualification on this SOP prior to the actual handling and processing of samples from clinical trial patients. The Laboratory Director/Supervisor is responsible for ensuring the assay operator running the SOP has sufficient experience to handle and analyze clinical samples. To become proficient with this SOP, sites are highly encouraged to reach out to NCI_PD_Support@mail.nih.gov for additional training materials.

5.3 It is the responsibility of the assay operator to confirm scheduled specimen collection time points, pre-print all labels, request access to **NCI Medidata Rave** (ETCTN Specimen Tracking System), check documentation for accuracy, request sample shipping kits from the EET Biobank and verify that the required collection tubes, supplies, and equipment are available for successful collection and handling of biopsy samples.

5.4 It is the responsibility of the assay operator to conduct the specimen collection and handling procedures following this SOP and complete the required tasks and associated documentation. The Biopsy Collection Record (Appendix 1) must be completed for each patient sample collection and filed with the study patient's other records.

5.5 The responsible personnel are to check the DCTD Biomarkers Web site (http://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm) to verify that the latest SOP version is being followed.

6.1 MATERIALS AND EQUIPMENT REQUIRED

6.2 Stopwatch, total time in minutes and seconds required

6.3 1.5-mL Sarstedt o-ring screw cap, conical bottomed tubes (Sarstedt, Cat#: 72.703.416)

6.4 Disposable, fine-tipped tweezers (e.g., VWR, Cat#: 83009-010). Tweezer tips need to easily fit to the bottom of a 1.5-mL Sarstedt tube

6.5 Printable microcentrifuge tube labels or BSI labeling system

6.6 81-place freezer boxes (e.g., Fisher Scientific, Cat#: 12-565-182)

6.7 Thermoflask cooler or polystyrene foam container

6.8 Ice bucket

6.9 Liquid nitrogen or dry ice/ethanol bath

6.10 -80°C freezer (or colder)

6.11 Specimen shipping kit from EET Biobank

7.1 OPERATING PROCEDURES

7.2 This SOP uses **NCI Medidata Rave** for sample tracking, please review the following training videos for **NCI Medidata Rave** before you start:

7.2.1 General RAVE training:
https://www.youtube.com/watch?app=desktop&v=ZRX0lSqs5zo

7.2.2 Label Printing training:
https://www.youtube.com/watch?app=desktop&v=9_Q6_k-KHHs

7.3 Sample Shipping Kits

Sample shipping kits should be requested prior to enrolling the first biopsy patient from EET Biobank by emailing BPCBank@nationwidechildrens.org. For current customers, the kits can be requested through the EET Biobank (kit management system: https://kits.bpc-apps.nchri.org/Auth/Login?ReturnUrl=%2f). Please allow 5-7 business days for kit shipment.

7.4 Labels

7.4.1 Prepare enough pre-printed specimen labels in **NCI Medidata Rave** by following steps 7.3.1.1- 7.3.1.5:

7.4.1.1 Log into **NCI Medidata Rave** and go to **Enrollment** folder and confirm the **Histology and Disease** form is complete.

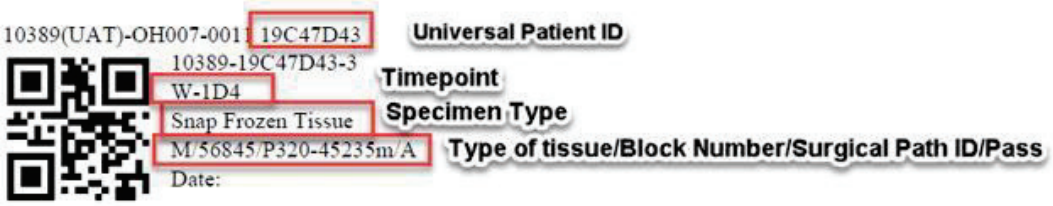
7.4.1.2 Go to **All Specimens** folder.

7.4.1.3 Complete the **Specimen Consent** form.

7.4.1.4 Complete the **Specimen Tracking Enrollment** form for each specimen.

7.4.1.5 Complete the **Print Labels** form. Labels will be sent to user's email address. For tissue specimens, apply appropriately coded label to each pass of the biopsy (see below).

Note: Five labels will be printed by default when you enter "**1**" in the "**How many labels are needed**" field. The first four will be designated with A, B, C and D to represent different passes of the biopsy procedure. Please use those accurately to label the specimens; pass A should be for the first pass, B for the second, etc. The fifth label will have no pass designation and can be used on reports to be uploaded into RAVE. See an example of pre-printed label for frozen tissue biopsy below.



7.5 Tumor Needle Biopsy Collection and Handling

7.5.1 The research nurse is to notify the laboratory of scheduled PD sample collections, preferably giving at least 24 hours of notice. Arrive at the biopsy collection site early enough to allow sufficient time to set up laboratory supplies, collect relevant clinical information, and ensure rapid transport of frozen specimens from the procedure area to the laboratory, where they will be placed into storage at -80ºC (or colder).

7.5.2 Prior to biopsy, the lesion should be assessed as to whether or not the biopsy should be performed and yield a successful outcome. Fill out the **Pre-Biopsy Lesion Score** form in **NCI Medidata Rave** using inputs from interventional radiologists and/or oncologists.

7.5.3 Bring all necessary lab supplies to the biopsy collection site, including: disposable tweezers, a minimum of four 1.5-mL Sarstedt tubes pre-cooled on liquid nitrogen or dry ice/ethanol in an insulated bucket (Sarstedt tubes will be provided in the sample shipping kit from EET Biobank; please use one tube for each whole biopsy core), the label with no pass designation to give to the research nurse for the patient record, and a printout of Appendix 1.

Note: Pre-chill additional 1.5-mL Sarstedt tubes for specimen collection in case the interventional radiologist collects additional passes, or if one of the tubes is compromised prior to collection.

7.5.4 The total time elapsed between biopsy collection and placement into the pre-chilled tube is of **key importance** to biomarker analysis; this time should be documented in **NCI Medidata Rave** for each biopsy pass. **It is important to note that all biopsies should be frozen within 2 minutes of collection.** The interventional radiologist will eject the biopsy onto a sterile slide (for optimal analyte recovery the slide should be pre-chilled). Start a stopwatch at this point (or note the time in Appendix 1) and immediately walk the slide to the sample preparation table for transfer to the pre-chilled Sarstedt tube.

7.5.5 Immediately snap freeze the biopsy by placing the tube in liquid nitrogen or a dry ice/ethanol bath (stop the stopwatch at this point). **Note:** DO NOT let the tubes tip over in the liquid nitrogen or dry ice/ethanol bath.

7.5.6 Calculate the total time elapsed from biopsy collection to biopsy freezing and record the total number of **minutes and seconds** (Appendix 1).

7.5.7 Note the specific needle type used and location of each biopsy pass collected (*e.g.*, spleen, large left upper quadrant splenic mass) (Appendix 1).

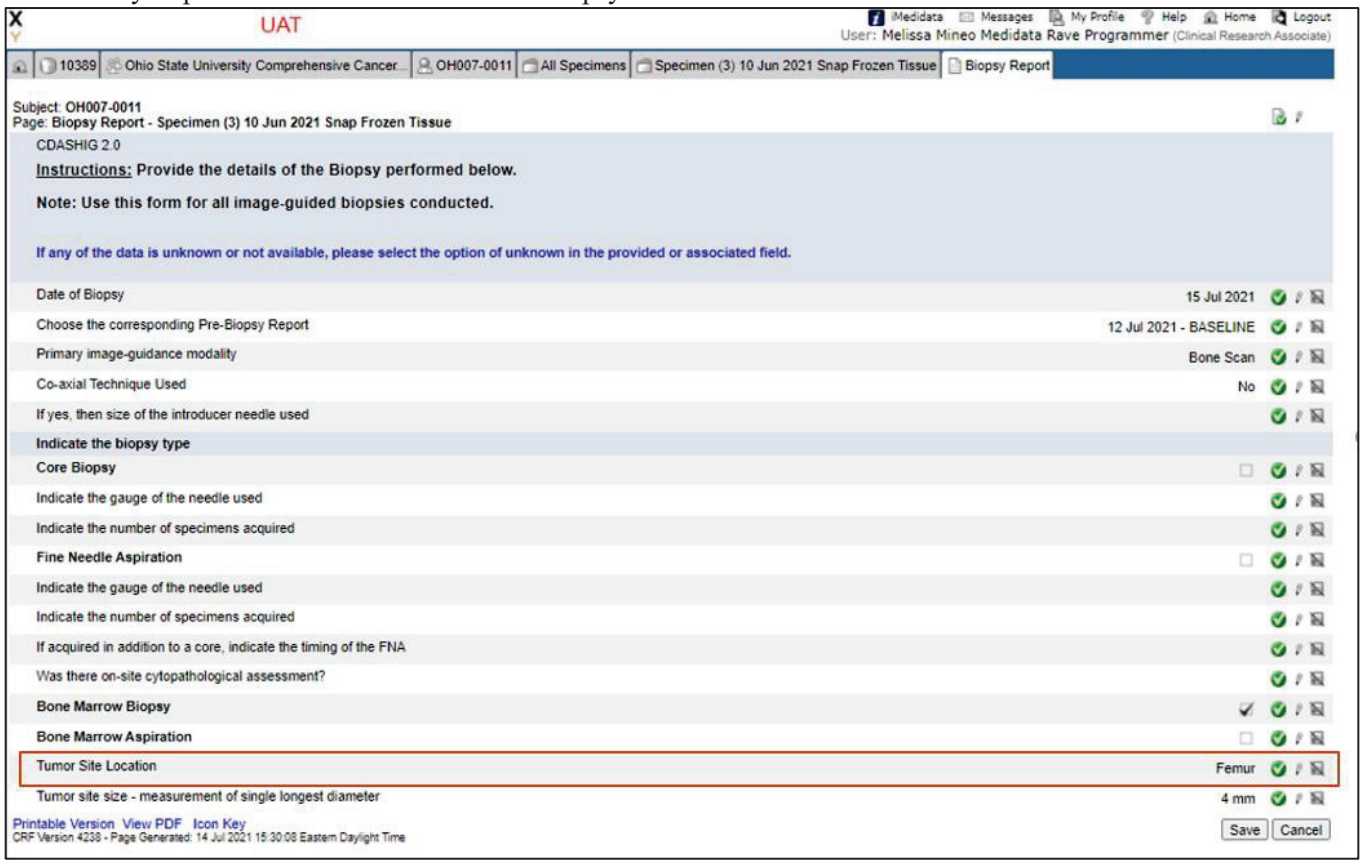
7.5.8 Note the protocol biopsy timepoint in Appendix 1.

7.5.9 Return to the sample processing laboratory and transfer the frozen biopsy specimen(s) to -80°C (or colder) for storage until shipment to the EET Biobank.

7.5.10 After biopsy collection, complete sample tracking documentation in **NCI Medidata Rave** according to notes recorded in Appendix 1.

7.5.11 Fill out **Biopsy Report** form in the **All Specimen** folder.

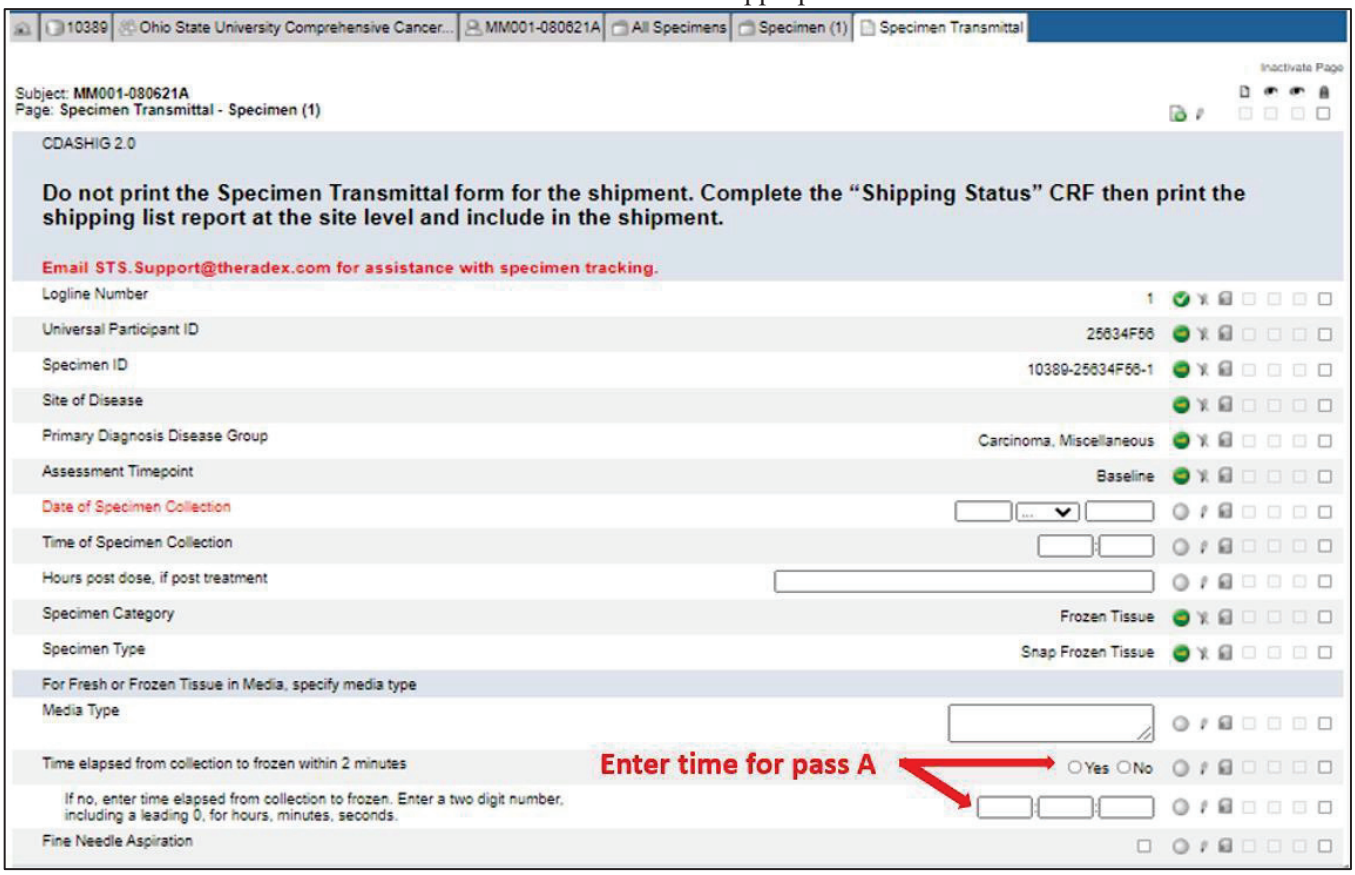
Note: It is very important to record the site of the biopsy to the **Tumor SiteLocation** field as shown below.



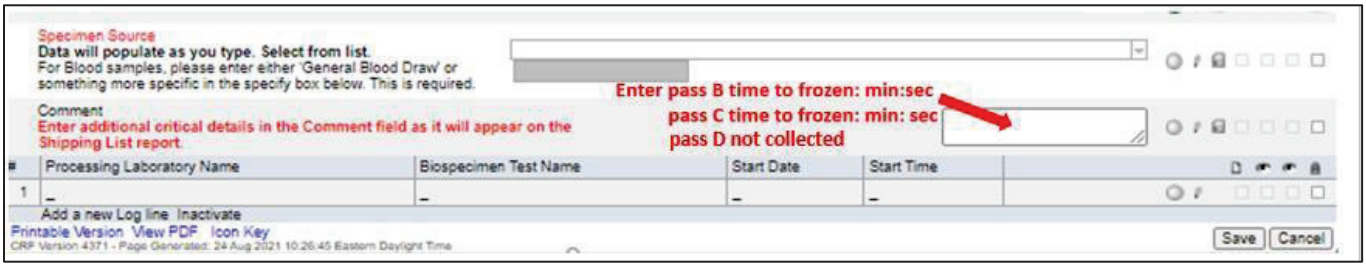
7.5.12 Complete the **Specimen Transmittal** form in the All Specimen folder.

Note: It is important to fill out the time from collection to frozen for each pass in the **Specimen Transmittal** form by following the instructions below.

Pass A time will be recorded in the appropriate fields as shown below:



Times elapsed for passes B, C and D will be recorded in the **Comment** field nearthe bottom of the Specimen Transmittal form as shown below. Biopsy passes notcollected will also be recorded in the **Comment** field as shown below.



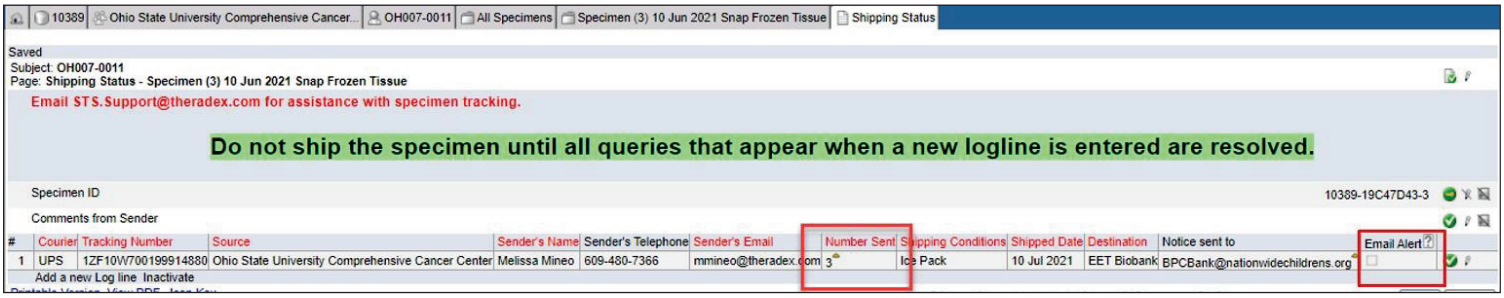
8.1 SHIP TO EET BIOBANK

8.2 When specimens are ready to be shipped, complete shipment documentation in **NCI Medidata Rave.**

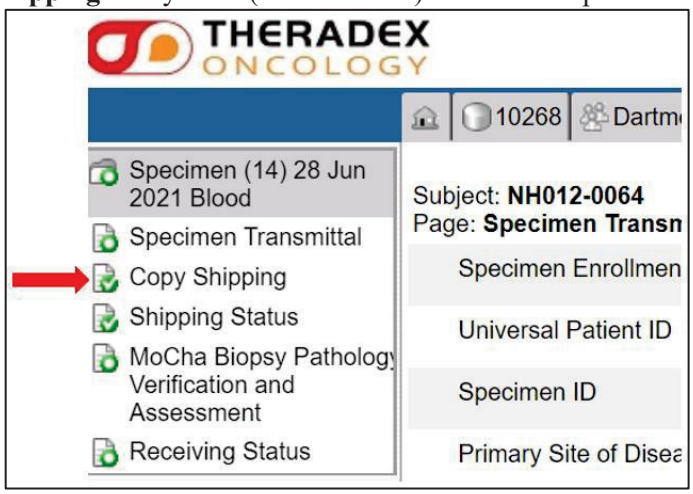
8.2.1 Complete the **Shipping Status** form.

8.2.1.1 Each field in the **Shipping Status** form should be completed as shown below and **Number Sent** (circled below) should equal the number of biopsy passes in the shipment.

8.2.1.2 **Email Alert** (circled below) is only checked for the last specimen in a shipment if multiple specimens are shipped together.

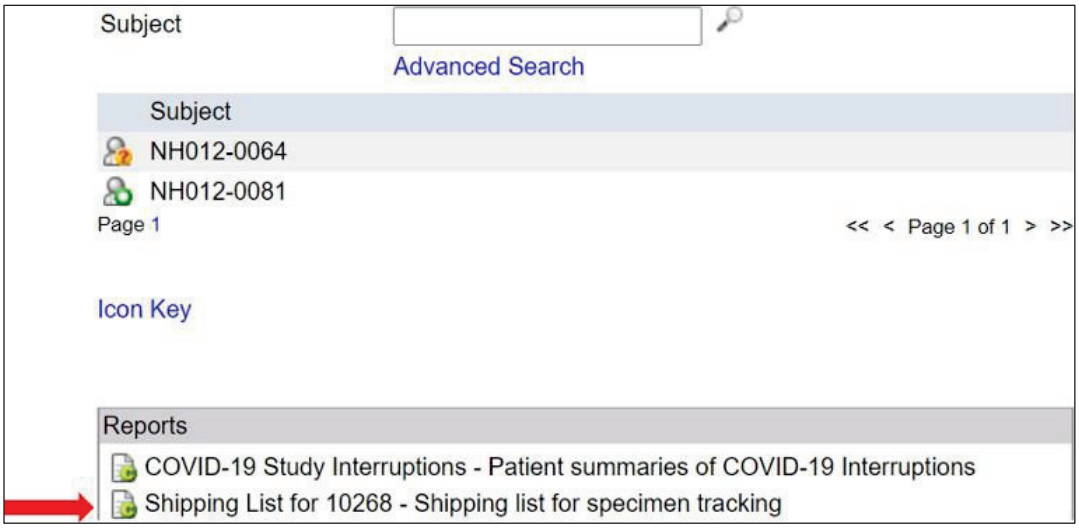


8.2.2 If there are other specimens to be shipped with the frozen biopsies, use the **Copy Shipping** utility form (shown below) in the other specimens' folder.



8.2.3 Print the **Shipping List** report and place it in the box with the specimens.

8.2.3.1 The **Shipping List** report is found in the report panel at the bottom of the window at the site level (an example shown below) since specimens from multiple patients can be included in a single shipment.



8.2.3.2 Shipment should include a hard copy (printed copy) of the **Shipping List**. An example is shown below.

Shipping List

Protocol: 10389 - UAT

Site: Ohio State University Comprehensive Cancer Center

Shipping Date: 10 Jul 2021

Tracking Number: 1ZF10W700199914880

Contact Info: Melissa Mineo
609-480-7366
mmineo@theradex.com

Please include a hardcopy of the pathology and any other relevant report in the shipment.

Protocol-Patient ID	TimePoint	Category	Samples Sent	Tissue	Sample Site	Collection Date/Time	Comments
Universal Pat. Id		Type				Processed Date/Time	
Specimen ID						Frozen in 2 min/Elapsed	
10389-OH007-0011 19C47D43 10389-19C47D43-1	Baseline	Blood Blood	1		General Blood Draw	21 Jun 202109:00 N/A	
10389-OH007-0011 19C47D43 10389-19C47D43-2	Archival	Formalin Fixed Paraffin Embedded Tissue FFPE Block	1	Metastatic	Esophagus	09 Jan 202115:00 N/A	
10389-OH007-0011 19C47D43 10389-19C47D43-3	Week -1 Day 4 (Expansion Cohort Only)	Frozen Tissue Snap Frozen Tissue	3	Metastatic	Esophagus	10 Jun 202111:10 N/A No/00:03:25	Pass B time to frozen: 02:20 ; Pass C time to frozen: 00:50

8.2.3.3 Shipment should also include a hard copy of the **TISSUE BIOPSY VERIFICATION** form found in the appendices of corresponding protocols.

8.3 Specimen shipment to EET Biobank

8.3.1 Follow the **Shipping Specimens from Clinic Site to the EET Biobank/ETCTN Biorepository** section of the clinical protocol for general instructions of sample shipment to EET Biobank.

8.3.2 Frozen biopsies should be shipped in kits provided by EET Biobank. The shipping container sent with kit contents should be used to ship specimens to EET Biobank. **Note:** It's important to include sufficient dry ice to keep the biopsy frozen for at least 96 hours.

8.3.3 Frozen specimens may be shipped on Monday through Thursday to the following address:

EET Biobank
The Research Institute at Nationwide Children's Hospital700 Children's Drive,
WA1340
Columbus, Ohio 43205
PH: (614) 722-2865
FAX: (614) 722-2897

Note: FedEx Priority Overnight service is the required shipping method. TheEET Biobank FedEx account will not be provided to submitting institutions.
Sites are responsible for all costs for shipments to the EET Biobank, so theovernight express shipment should be billed directly to the shipping institution/site.

8.4 Useful contacts for Specimen Collection, Handling and Shipment:

8.4.1 Send all questions related to this SOP or PD- assay support questions to: NCI_PD_Support@mail.nih.gov

8.4.2 Send all technical questions about the Specimen Tracking System to: STS.Support@theradex.com

8.4.3 EET Biobank queries (kit inquiries and sample shipping): BPCBank@nationwidechildrens.org

APPENDIX 1: BIOPSY COLLECTION RECORD

Note: This document lists important information to be recorded during the biopsy collection process forlater documentation in **NCI Medidata Rave.** The completed document should be filed with the study patient's other records at a predetermined location according to local policy for managing clinical trial information. Please **do not** include the document in the shipment to EET Biobank**.**

Certified Assay Operator: _____

Facility/Clinic Collecting Specimens: _____

Clinical Protocol Number: _____

Patient ID: _____

1. Biopsy Collection Information:

Note: Information collected in the table below will be entered in Medidata RAVE.

Note: Record times using military time (24-h designation); for example, specify 16:15 to indicate 4:15PM.

	Pass A	**Pass B**	**Pass C**	**Pass D**
Specimen ID				
Protocol timepoint of biopsy (Cycle, Day, and Hours post dose, if post treatment)				
Needle type				
Site of biopsy (complete for all passes or note "same" for replicate cores)				
Required: Time elapsed from collectionto placement in tube	min sec	min sec	min sec	min sec
Date biopsy collected				
Time biopsy collected	:	:	:	:
Time biopsy placed in tube	:	:	:	:

2. Notes, including any deviations from the SOP:

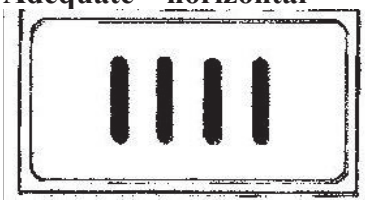
APPENDIX I EMBEDDING NEEDLE BIOPSIES

If your study has sites embedding formalin fixed tissue from needle biopsies in paraffin, include this Appendix. Customize the protocol text and reference this appendix in the body of the protocol.

Embedding needle biopsies (FFPE processing):

- These types of specimens are very thin and delicate.
- Lift gently with forceps and avoid pinching too roughly to prevent fragmentation and crush artifact.
- Because these specimens are typically very thin (diameter) it is absolutely essential that they be orientated for optimal sectioning.
- They must be embedded in the same plane, as flat as possible (including tips of long strings).
- Please select the mold size that is most suitable and will be easiest for the histotechnician to eventually position multiple FFPE tissue sections on the glass slides.
- Dipping the needle core biopsies in methylene blue or eosin renders the samples more readily visible in the tissue block (helps when tissue sections are being oriented during embedding and eventually cut following FFPE processing).
- Arrange the specimens in a horizontal plane or diagonal plane to reduce the distance that the knife blade travels across the specimen- this may reduce folds and compression.

Adequate – horizontal



Adequate – diagonal

Avoid – no orientation