Protocol Administrative Letter

October 1, 2020

"A Phase II Study of Neratinib Alone and in Combination with Fulvestrant in Metastatic HER2 Non-amplified but HER2 Mutant Breast Cancer"

The purpose of this letter is to clarify an error regarding the duration of follow-up after the last dose of protocol therapy. In Protocol Amendment 13.0, 16MAR2020, Section 6.7 Duration of Follow-up currently reads,

6.7 Duration of Follow-up
Patients will be followed every 3 months for 2 years or until death, whichever occurs first. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. Patients will be followed through either review of medical records, phone calls, or office visits.

This section is incorrect, as it should read,

6.7 Duration of Follow-up
Patients will be followed until 30 days after last dose of study therapy. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. Patients will be followed through either review of medical records, phone calls, or office visits.

If you have any questions, please contact me or the regulatory coordinator (Natalie Benford at natalienconner@wustl.edu, 314-747-8365)

Regards,

Dr. Cynthia Ma, MD, PhD.
Professor of Medicine
Washington University School of Medicine
Internal Medicine – Medical Oncology
Phone: 314-362-8903
Email: cynthiaxma@wustl.edu

A Phase II Study of Neratinib Alone and in Combination with Fulvestrant in Metastatic HER2 Non-amplified but HER2 Mutant Breast Cancer

Protocol#: 201209135
Version date: 03/16/2020

Coordinating Center: **Washington University School of Medicine**
660 S. Euclid Ave, Campus Box 8056
St. Louis, MO 63110

Principal Investigator: **Cynthia X. Ma, M.D., Ph.D.**
Washington University School of Medicine
Telephone: 314-362-9383
Email: cynthiaxma@wustl.edu

Sub-Investigators:	**Institution:**	**Division:**
Matthew J.C. Ellis, M.B., Ph.D.	Baylor College of Medicine	Medical Oncology
Ron Bose, M.D., Ph.D.	Washington University	Medical Oncology
Carey Anders, M.D.	Lineberger Cancer Center	Medical Oncology
Rachel Freedman, M.D.	Dana Farber Cancer Institute	Medical Oncology
Philippe Bedard, M.D.	Princess Margaret Cancer Centre	Medical Oncology
Kimberly Blackwell, M.D.	Duke Cancer Institute	Medical Oncology
Melody Cobleigh, M.D.	Rush University Medical Center	Medical Oncology
Andres Forero, M.D.	University of Alabama	Medical Oncology
Daniel Hayes, M.D.	University of Michigan	Medical Oncology
Janice Lu, M.D.	University of Southern California	Medical Oncology
Mark Pegram, M.D.	Stanford Medicine Cancer Institute	Medical Oncology
Timothy J. Pluard, M.D.	St. Luke's Cancer Institute	Medical Oncology
Matthew Goetz, M.D.	Mayo Clinic	Medical Oncology
Julie Nangia, M.D.	Baylor College of Medicine	Medical Oncology
Jason Jones, M.D.	Avera Cancer Institute	Medical Oncology
Marc E. Lippman, M.D.	University of Miami	Medical Oncology
Massimo Cristofanilli, M.D.	Northwestern University	Medical Oncology
Foluso Ademuyiwa, M.D.	Washington University	Medical Oncology
Michael J. Naughton, M.D.	Washington University	Medical Oncology
Rama Suresh, M.D.	Washington University	Medical Oncology
Leonel Hernandez-Aya, M.D.	Washington University	Medical Oncology
Katherine Weilbaecher, M.D.	Washington University	Medical Oncology
Ashley Frith, M.D.	Washington University	Medical Oncology
Haeseong Park, M.D.	Washington University	Medical Oncology
Matthew Cherian, M.D.	Washington University	Medical Oncology
Lindsay Peterson, M.D.	Washington University	Medical Oncology
Feng Gao, M.D., Ph.D.	Washington University	Biostatistics

Washington University Research Coordinator
Caroline Bumb
Telephone: 314-362-7249
Email: cbumb@wustl.edu

A Phase II Study of Neratinib Alone and in Combination with Fulvestrant in Metastatic HER2 Non-amplified but HER2 Mutant Breast Cancer

Study Drug(s): Neratinib (PB-272)
IND#: 116297 // **Clinical Trials.gov#:** NCT01670877

Protocol Revision History

Initial Approval Version	**09/27/12**
Amendment #1	**01/02/13**
Amendment #2	**03/22/13**
Amendment #3	**04/22/13**
Amendment #4	**09/04/13**
Amendment #5	**11/08/13**
Amendment #6	**12/26/13**
Amendment #7	**02/05/15**
Amendment #8	**12/11/15**
Amendment #9	**09/14/16**
Amendment #10	**02/07/17**
Amendment #11	**10/05/17**
Amendment #12	**06/15/18**
Amendment #13	**03/16/2020**

A Phase II Study of Neratinib Alone and in Combination with Fulvestrant in Metastatic HER2 Non-amplified but HER2 Mutant Breast Cancer

Principal Investigator Signature Page

Principal Investigator:	Cynthia X. Ma, M.D., Ph.D.	
	Signature of Investigator	Date
	Printed Name of Investigator	
	By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IRB/HRPO procedures, the Declaration of Helsinki, ICH Good Clinical Practices guidelines, and the applicable parts of the United States Code of Federal Regulations or local regulations governing the conduct of clinical studies.	

Contact Information

Ron Bose, M.D., Ph.D.
Washington University School of Medicine
Telephone: 314-747-9308
Email: rbose@wustl.edu

Matthew J. Ellis, M.B., Ph.D.
Baylor College of Medicine
Email: matthew.ellis@bcm.edu

Carey Anders, M.D.
Lineberger Comprehensive Cancer Center
University of North Carolina at Chapel Hill
Email: canders@med.unc.edu

Rachel Freedman, M.D.
Dana-Farber Cancer Institute
Harvard University
Email: rachel_freedman@dcfi.harvard.edu

Timothy Pluard, M.D.
St. Luke's Cancer Institute
Email: tpluard@saint-lukes.org

Kimberly Blackwell, M.D.
Duke Cancer Institute
Duke University Medical Center
Email: kimberly.blackwell@duke.edu

Melody Cobleigh, M.D.
Rush University Medical Center
Email: melody_cobleigh@rush.edu

Janice Lu, M.D.
Keck School of Medicine
University of Southern California
Email: Janice.Lu@med.usc.edu

Andres Forero, M.D.
University of Alabama Cancer Center
Email: aforero@uabmc.edu

Philippe Bedard, M.D.
Princess Margaret Cancer Centre
Email: philippe.bedard@uhn.ca

Daniel Hayes, M.D.
University of Michigan
Email: hayesdf@umich.edu

Mark Pegram, M.D.
Stanford Medicine Cancer Institute
Email: mpegram@stanford.edu

Marc Lippman, M.D.
University of Miami
Email: mlippman@med.miami.edu

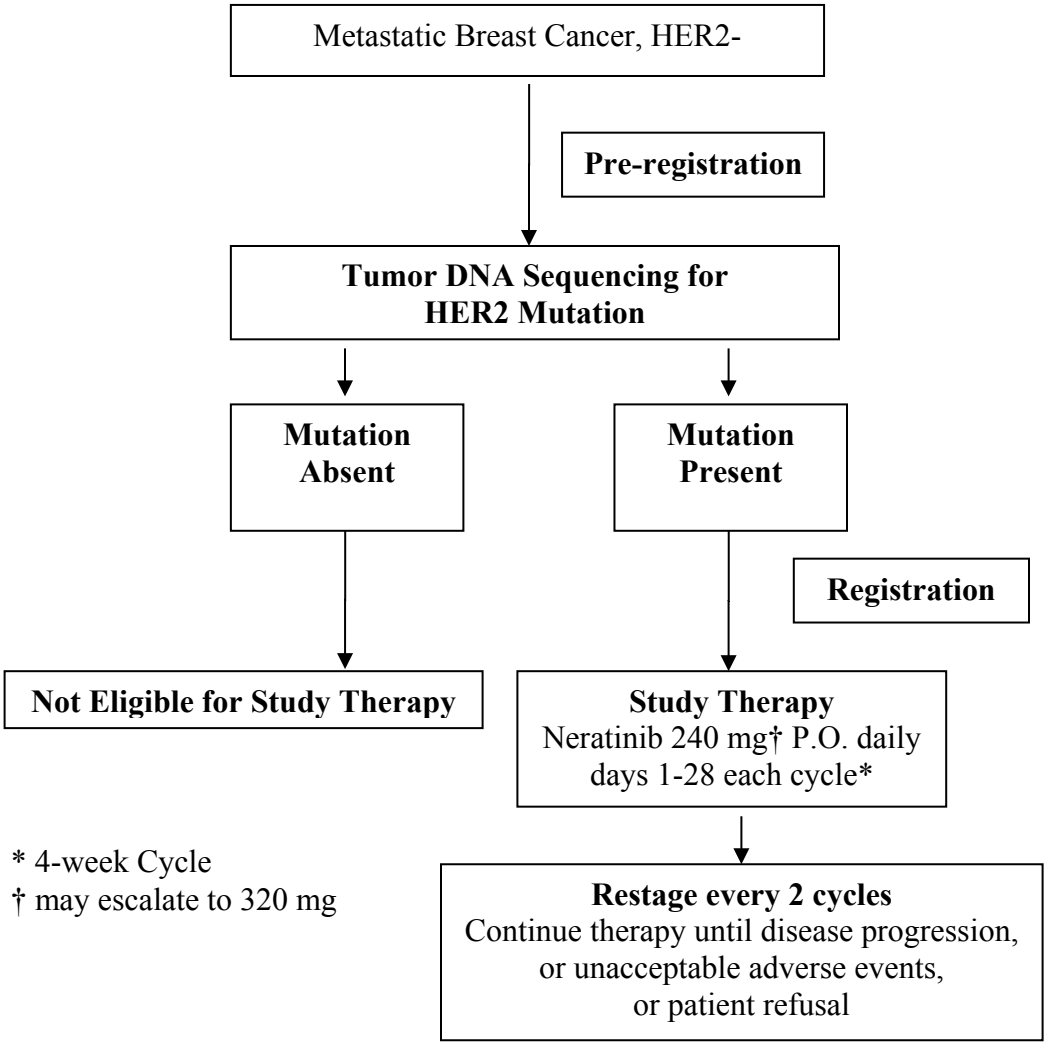
Matthew Goetz, M.D.
Mayo Clinic
Email: goetz.matthew@mayo.edu

Julie Nangia, M.D.
Baylor College of Medicine
Email: nangia@bcm.edu

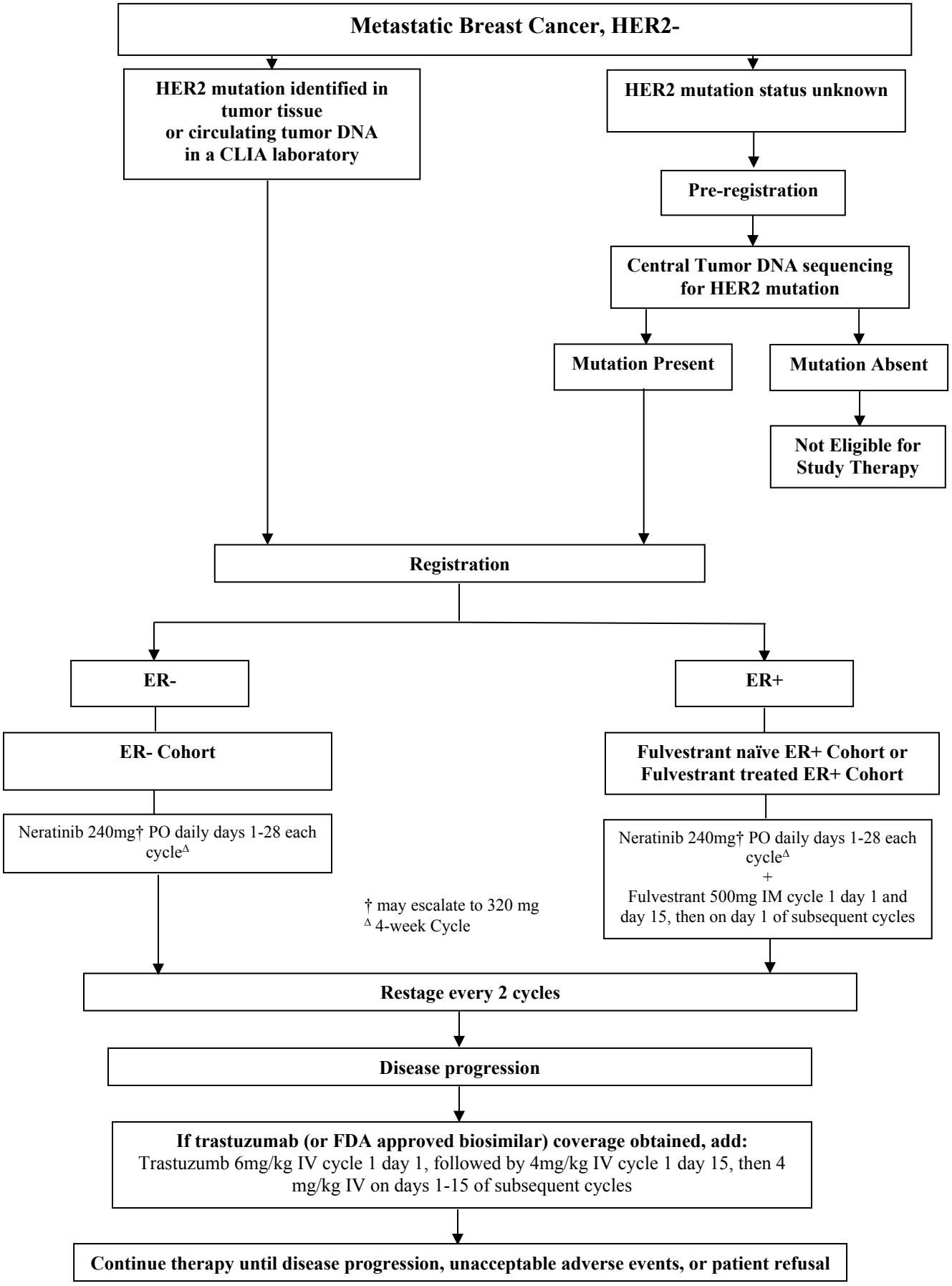
Amy Krie, M.D.
Avera Cancer Institute
Email: Amy.Krie@avera.org

Massimo Cristofanilli, M.D.
Northwestern University
Email: Massimo.Cristofanilli@nm.org

SCHEMA – Part I (closed)



SCHEMA – Part II



Glossary of Abbreviations

AE	Adverse event
ALT (SGPT)	Alanine transaminase (serum glutamate pyruvic transaminase)
ANC	Absolute neutrophil count
AST (SGOT)	Aspartate transaminase (serum glutamic oxaloacetic transaminase)
BUN	Blood urea nitrogen
CBC	Complete blood count
CLIA	Clinical Laboratory Improvement Amendments
CNS	Central nervous system
CR	Complete remission
CRF	Case report form
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
DLTs	Dose Limiting Toxicities
DNA	deoxyribonucleic acid
DSM	Data and Safety Monitoring
DSMC	Data Safety Monitoring Committee
EC	Ethics Committee
ECG (or EKG)	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EDTA	ethylenediaminetetraacetic acid
EGFR	Epidermal growth factor receptor
ER	Estrogen Receptor
FDA	Food and Drug Administration
FISH	fluorescent in situ hybridization
GCP	Good Clinical Practice
HER2	Human epidermal growth factor receptor 2
HHS	Department of Health and Human Services'
HIV	Human Immunodeficiency Virus
HRPO	Human Research Protection Office (IRB)
IHC	Immunohistochemistry
IND	Investigational New Drug
IRB	Institutional Review Board
ITT	Intent-to-treat
IULN	Institutional Upper Limit of Normal
ILLN	Institutional Lower Limit of Normal
IV	Intravenous (i.v.)
LDH	Lactate dehydrogenase
LPS	lipopolysaccharide
MedDRA	Medical Dictionary for Regulatory Activity
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose

NCCN	National Cancer Center Network
NCI	National Cancer Institute
NIH	National Institutes of Health
OHRP	Office of Human Research Protections
ORR	Overall response rate
OS	Overall survival
PB	Peripheral blood
PBMC	Peripheral blood mononuclear cell
PD	Progressive disease
PI	Principal investigator
PR	Partial response (Partial remission)
QASMC	Quality Assurance and Safety Monitoring Committee
RBC	Red blood cell (count)
RECIST	Response Evaluation Criteria in Solid Tumors (Committee)
RFS	Relapse free survival
RR	Response rate
SAE	Serious adverse event
SCC	Siteman Cancer Center
SD	Stable disease
TF	Treatment failure
TTP	Time to progression
WBC	White blood cell (count)
WHO	World Health Organization

Table of Contents

1.0 BACKGROUND AND RATIONALE

1.1 Metastatic HER2 Negative Breast Cancer

Over 80% of breast cancers are HER2 negative (HER2-), defined by the lack of *HER2* gene amplification, for which targeted therapies are lacking. The prognosis for the estrogen receptor negative (ER-)/HER2- subset is particularly poor[1]. Although patients with ER+/HER2- disease fair better, resistance to endocrine manipulation develops almost invariably[2]. The median survival is only 18-24 months when chemotherapy is necessary in the metastatic setting[3]. There is a significant unmet clinical need to uncover new drug targets to improve the outcome of these patients.

HER2/neu (ERBB2) is a well established proto-oncogene that encodes a receptor tyrosine kinase of the Epidermal Growth Factor Receptor (EGFR) family[4]. Receptor activation via heterodimerization between HER2 and EGFR and other family members leads to strong mitogenic signals and cellular proliferation[5]. HER2-targeted agents are among the most successful cancer therapeutics in the clinic, revolutionizing the care for patients with HER2+ breast cancer [6-11]. HER2+ breast cancer is defined clinically by either HER2 protein overexpression on immunohistochemistry (IHC) or HER2 gene amplification by fluorescent in situ hybridization (FISH) testing and occurs in approximately 15-20% of all breast cancer cases. HER2- breast cancers as a whole have not been shown to derive benefit from these highly effective agents[12].

Results from The Cancer Genome Atlas (TCGA) breast cancer project demonstrated that a small subpopulation of HER2- breast cancer carry mutations in HER2, with majority being in the kinase domain and compilation of data on 1,391 breast cancers documented 22 cases of somatic HER2 mutation, a mutation rate of 1.6% [13-15]. In addition, laboratory investigations indicated that many of these mutations are activating mutations, with the capacity to induce cellular transformation and importantly an enhanced susceptibility to the inhibitory effects of anti-HER2 agents (see Preliminary Investigation section). These discoveries raised the possibility that mutations in HER2 could be effectively targeted by the well established anti-HER2 agents in the clinic. The objective of this application is to establish HER2 mutation as a therapeutic target in HER2- breast cancer. We therefore propose a phase II study of neratinib, a pan HER inhibitor, in metastatic HER2-/HER2 mutated breast cancers for tumor response.

As of January 17, 2015, the study enrolled 11 patients with HER2 mutations, among which 10 patients had ER+ breast cancer. Considering the well-established signaling crosstalk between ER and HER2 in ER+ HER2+ breast cancer[16,17], we propose to add the ER down regulator fulvestrant to neratinib in the ER+ subpopulation in the second part of this trial.

Although the prevalence of HER2 mutation is low, because breast cancer is so common this would account for approximately 4,000 new cases each year in the US alone, an annual incidence that is very similar to chronic myeloid leukemia (CML), a disease transformed by the introduction of tyrosine kinase inhibitors.

1.2 Neratinib

1.2.1 Mechanism of Action

Neratinib (PB-272) is a potent irreversible pan HERB inhibitor. Neratinib is an orally available small molecule that inhibits HER-1, HER-2, and HER-4 at the intracellular tyrosine kinase domains, a mechanism of action that is different from trastuzumab[16-18]. Neratinib reduces HER-1 and HER-2 autophosphorylation, downstream signaling, and the growth of HER-1 and HER-2 dependent cell lines. Preclinical data suggest that neratinib will have antitumor activity in HER-1 - and/or HER-2-expressing carcinoma cell lines, with cellular IC50 <100 nM.

1.2.2 Preclinical Data

Neratinib is highly active against cell lines overexpressing either HER-2 or HER-1. Neratinib blocks HER receptor autophosphorylation in cells at doses consistent with inhibition of cell proliferation. Neratinib most likely inhibits kinase activity through irreversible binding at a targeted cysteine residue in the adenosine triphosphate binding pocket of the receptor. In agreement with the predicted effects of HER-2 inactivation, neratinib treatment of cells results in inhibition of downstream signal transduction events and cell cycle regulatory pathways.

In vivo, neratinib is active in HER-2- and HER-1-dependent tumor xenograft models, when administered orally on a once-a-day schedule. Overall, neratinib is less potent against HER-1 dependent tumors than HER-2-dependent tumors in vivo, even though it has equivalent activity against the 2 kinases in vitro.

1.2.3 Neratinib Phase 1 and Pharmacokinetic Data

Preliminary pharmacokinetic analyses demonstrated that neratinib absorption was relatively slow, and the maximum concentration (C_{max}) was generally attained within 3 to 6 hours. After oral administration, the neratinib C_{max} and area under the concentration versus time curve (AUC) increased in a dose-dependent manner in general. Mean steady-state C_{max} and AUC values were 70.1 ng/mL and 975 ng·h/mL for the 180-mg dose group, respectively, 73.5 ng/mL and 939 ng·h/mL for the 240-mg dose group, respectively, 90.4 ng/mL and 1333 ng·h/mL for the 320-mg dose group, respectively, and 105 ng/mL and 1704 ng·h/mL for the highest dose of 400 mg, respectively. The neratinib exposure (AUC) increased 1.2- to 2.7-fold (mean accumulation ratio) when comparing the steady-state exposure on day 21 after repeated daily administration with the exposure on day 1 after administration of 80 to 400 mg of neratinib. The mean accumulation ratio was 1.2 after a 240-mg dose, indicating no significant accumulation of neratinib after repeated daily dose administration at the dose to be used in this proposed trial. The data indicated a slow distribution of neratinib with a large apparent volume of distribution (Vz/F on day 1: about 3188 to 6181 L) after oral absorption. After oral administration on day 1, neratinib was eliminated with a mean apparent terminal half-life ($t_{½}$) of approximately 13 to 17 hours. There was moderate to large variability in neratinib $t_{½}$, C_{max}, and AUC; coefficients of variation generally ranged from 8% to 90%. In the single agent, first in human, phase 1 study 3144A1-102-US, neratinib single agent administered orally was given in increasing

doses from 40 mg to 400 mg. Diarrhea was the main dose limiting toxicity (DLT) observed. At the 400-mg dose level, 4 of 6 subjects had DLT of grade 3 diarrhea. Thus, the maximum tolerated dose (MTD) was determined to be 320 mg per day. However, due to the frequency and severity of neratinib-related diarrhea observed in subsequent subjects treated at the MTD (34% of subjects reported grade 3 diarrhea), the MTD was reevaluated to 240 mg per day. Neratinib monotherapy phase 2 data showed that oral daily doses of 240 mg of neratinib were generally well tolerated with significant antitumor activity in subjects with HER-2-positive advanced breast cancer [19]. In this trial, patients will receive 240 mg continuous daily oral doses of neratinib. Since diarrhea has been tolerable with prophylactic use of loperamide during the first cycle therapy in the first 3 patients treated in this trial, we elected to allow dose escalation to 320mg in subsequent cycles, with initial prophylactic use of loperamide, in patients who tolerated the 240mg daily dosing.

1.2.4 Single-Agent Activity of Neratinib in HER2+ Breast Cancer

Neratinib as a single agent has been studied in a phase 2 trial in subjects with metastatic HER-2 positive breast cancer [19]. Sixty-six (66) subjects with prior trastuzumab based therapy were enrolled into arm A; 70 subjects without any prior trastuzumab exposure were enrolled into arm B. Objective response rate and median progression free survival were used as estimates of antitumor activity. For patients with prior trastuzumab treatment, the objective response rate was 24% (95% CI, 14% to 36%); for patients with no prior trastuzumab treatment, the objective response rate was 56% (95% CI, 43% to 69%). Six and eight patients with and without prior trastuzumab treatment, respectively, had stable disease for at least 24 weeks that yielded clinical benefit rates of 33% and 69%. The median duration of objective tumor response was 39.3 weeks (95% CI, 32.3 weeks to 93.7 weeks) for patients with prior trastuzumab treatment and 52.4 weeks (95% CI, 33.1 weeks to not estimable) for patients with no prior trastuzumab treatment. The median onset of first complete response or partial response was rapid at 7.1 weeks for patients both with and without prior trastuzumab treatment (prior trastuzumab range, 3.0 to 24.0 weeks; no prior trastuzumab range, 3.1 to 15.6 weeks; 75th percentile, 7.1 weeks for both). Similar efficacy results were obtained by investigator assessment.

1.2.5 Adverse Event Profile of Neratinib Monotherapy

In the neratinib monotherapy phase II breast cancer study, diarrhea was the only grades 3 to 4 adverse event that occurred in more than 10% of patients (28 patients with grade 3; one patient with grade 4) and was the predominant adverse event associated with dose reductions, which were required in 29% of patients with prior trastuzumab treatment and in 4% of patients with no prior trastuzumab treatment. However, only one patient (with prior trastuzumab treatment) discontinued treatment because of grade 2 diarrhea. The onset of diarrhea occurred early in the course of therapy (median time to onset, 2 to 3 days) and lasted a median of 5 to 7 days per event. Diarrhea severity abated during multiple weeks of treatment. During study week 1, diarrhea occurred in 70% to 90% of patients and decreased to 10% to 15% of patients after month 2. Only one patient had grade 3 diarrhea after month 2. With use of antidiarrheals and dose modification, neratinib treatment was continued in 99% of patients, despite this common lower gastrointestinal adverse effect. Mild, often transient, skin rashes were observed in approximately one quarter of the patients.

Other common AEs are nausea, vomiting, fatigue, anorexia, abdominal pain, asthenia, dehydration, rash, elevated alanine aminotransferase (ALT), and elevated aspartate

aminotransferase (AST). The above AEs are considered to be adverse drug reactions for neratinib.

As opposed to treatment with other HER-2 targeted agents, such as trastuzumab, cardiotoxicity has not been observed with neratinib, even in subjects previously treated with anthracyclines and/or trastuzumab.

Interstitial lung disease, which can sometimes be fatal, has been reported with other oral tyrosine kinase inhibitors that target EGFR (HER-1) +/- HER2 (HER-2), including lapatinib, gefitinib, and erlotinib. Pneumonitis which was considered to be drug related, according to the investigator's assessment, has been reported in clinical studies of neratinib. Subjects receiving neratinib should be monitored for acute onset or worsening of pulmonary symptoms such as dyspnea, cough, and fever and treated appropriately. There has been one event of bone marrow suppression reported on December 9, 2009, in a subject who was taking neratinib monotherapy for 4 months without any other clear cause. This resulted in neutropenia, and thrombocytopenia. Refer to the most recent version of the investigator's brochure for a summary of findings from nonclinical studies that potentially have clinical significance and from clinical studies that are relevant to the study. Also, refer to the most recent version of the investigator's brochure for a summary of the known and potential risks and benefits to human subjects.

1.3 Fulvestrant

1.3.1 Fulvestrant in the Treatment of ER+ Breast Cancer

Fulvestrant (Faslodex) is an estrogen receptor antagonist that binds to ER and leads to its degradation, a distinct mechanism of action from other endocrine therapy agents that target ER. Fulvestrant blocks ER action without agonist effect. It is FDA approved for patients who have disease progression on other antiestrogen agents in the advanced breast cancer setting. In two phase III trials, fulvestrant 250 mg monthly was found to be at least as effective as anastrozole with respect to disease progression and overall survival [20, 21]. In the EFECT trial, fulvestrant 250 mg monthly was found to be at least as effective as exemestane in patients with metastatic ER+ breast cancer resistant to non-steroidal AIs [22]. In the CONFIRM trial, high-dose fulvestrant at 500 mg monthly was found to be more effective compared to the previous dosing of 250 mg monthly, which prompted FDA's subsequent approval of fulvestrant 500 mg every 28 days, following a loading dose of 500 mg on days 1 and 15 during the first month of therapy for refractory metastatic breast cancer[23].

Overall, fulvestrant demonstrated an overall response rate of approximately 10% in aromatase inhibitor resistant, fulvestrant naive ER+ HER2- breast cancers [24, 25]. There is no data at this time regarding the activity of fulvestrant in ER+ HER2- breast cancer with HER2 mutations. However its efficacy in ER+ HER2+ breast cancer has been described in a retrospective study [26]. In this study, fulvestrant resulted in an overall response rate of 9% and clinical benefit rate of 42% (43/101 patients), with anti-tumor activity observed in up to the fourth line of endocrine therapy and seventh line of overall therapy [26]. Since the response rate approximated 10% in both HER2+ and HER2- population based on the studies described above, we assume a similar response rate of 10% in fulvestrant naïve HER2 mutant patient population in this trial.

1.3.2 Fulvestrant in Combination with Receptor Tyrosine Kinase Inhibitor the Treatment of ER+ Breast Cancer

Although there is no data in the literature for the combination of fulvestrant and neratinib in ER+ breast cancer, fulvestrant was combined with the HER1/HER2 inhibitor lapatinib in a randomized phase III trial of fulvestrant with or without lapatinib in patients with AI resistant metastatic ER+ HER2 1+, 2+, 3+ [25]. The study demonstrated an overall response rate of 9% in the fulvestrant plus placebo arm and 20% in the fulvestrant plus lapatinib arm, with anticipated side effects observed with single agents alone [25].

Since fulvestrant is well tolerated, there is no obvious theoretical pharmacokinetic interaction between neratinib and fulvestrant, and the mechanism of action of neratinib is similar to lapatinib, we do not anticipate the side effects profile be different from single agent therapy with neratinib and fulvestrant.

1.3.3 Adverse Event Profile of Fulvestrant

The most common adverse reactions reported in the fulvestrant study groups, regardless of causality, were GI symptoms including nausea and vomiting (26%/13%), constipation (12.5%), diarrhea (12.3%) and abdominal pain (11.8%); as well as headache (18.9%); back pain (15.4%); hot flashes (17.7%); and pharyngitis (16.1%). Vaginal bleeding was reported in < 1% of patients and occurred most commonly during the first 6 weeks after changing from existing hormonal treatment to fulvestrant. If vaginal bleeding persists, further evaluation is required. Injection site reaction with mild transient pain (9.1-11.6%) and inflammation were seen with fulvestrant. Seven percent of patients (1% of treatments) given a single 5 ml IM injection and 27% of patients (4.6% of treatments) given 2 x 2.5 ml IM injections experienced reactions. Additional adverse reactions occurring in > 5% of patients treated with fulvestrant during clinical trials include asthenia (weakness) (68.3%), bone pain (15.8%), dyspnea (14.9%), increased cough (10.4%), pelvic pain (10%), anorexia (9%), peripheral edema (9%), rash (unspecified) (7.3%), chest pain (unspecified) (7.1%), dizziness (6.9%), insomnia (6.9%), fever (6.4%), paresthesias (6.4%), urinary tract infection (6.1%), depression (5.7%), anxiety (5%), diaphoresis (5%), and anemia (4.5%).

Other adverse events reported as fulvestrant-related and occurring infrequently (< 1%) include thromboembolism, myalgia, vertigo, and leukopenia. In addition, hypersensitivity reactions such as angioedema and urticaria have been infrequently reported.

1.4 Trastuzumab (Herceptin)

1.4.1 Mechanisms of Action

Trastuzumab is an IgG1 kappa that selectively binds to the extracellular domain of the HER2 receptor. Trastuzumab inhibits HER2+ breast cancer cell proliferation in preclinical models[27, 28]. In addition, trastuzumab is a mediator of antibody-dependent cellular cytotoxicity (ADCC)[29, 30].

1.4.2 Clinical Studies

Trastuzumab is effective either as a single agent or in combination with a variety of chemotherapy agents for HER2 positive breast cancer [31]. In a study of single agent

trastuzumab, administered 4mg/kg loading dose followed by 2mg/kg weekly, or 8mg/kg loading dose and 4mg/kg weekly, as first-line therapy for HER2-overexpressing breast cancer, the objective response rate was 35% in HER2 3+ and 34% in HER2 amplified cases[32]. In another phase II trial of single agent trastuzumab in HER2+ breast cancer, trastuzumab was administered 8 mg/kg then 6 mg/kg Q3 weeks and the clinical benefit rate was 33%[33]. In patients who had received extensive prior therapies, the objective response rate was found to be around 15% [34]. Trastuzumab also demonstrated its ability to reduce cancer recurrence and death in the adjuvant setting as demonstrated by the large multi-center randomized controlled trials [8, 35]. Trastuzumab is a recombinant DNA-derived humanized monoclonal antibody against HER2 and is approved by FDA for treatment of metastatic and early stage breast cancer that overexpresses HER2.

1.4.3 Dual Targeting in HER2+ Breast Cancer

Approximately 15% of HER2+ breast cancer patients relapse after initial therapy with trastuzumab. [36] Preclinical evidence has shown synergistic interaction between trastuzumab and other HER-2 targeted agents, and several clinical studies have shown that dual HER2 targeting may prolong time to resistance and improve survival outcomes.[37] A double-blind phase III study (CLEOPATRA) compared first-line trastuzumab plus pertuzumab plus docetaxel with trastuzumab plus placebo plus docetaxel in 808 patients with metastatic HER2+ breast cancer. The pertuzumab arm had longer median progression free survival (PFS) (19 months vs. 12 months; HR=0.62; 95% CI, 0.51-0.75; P <0.001) and overall survival (OS) (not yet reached in pertuzumab arm vs. 37.6 months; HR 0.66; 95% CI, 0.52-0.84). [38] A randomized phase III, study of single agent lapatinib vs. combination of trastuzumab plus lapatinib in patients with HER2+ metastatic breast cancer who had disease progression on prior trastuzumab-based therapy showed superior PFS (HR=0.73; 95% CI, 0.57-0.93; P =0.008) and clinical benefit rate (CBR) (24.7% in the combination arm vs. 12.4% in the monotherapy arm; P =0.01) for the combination arm. There was also a median OS advantage seen in the combination arm (14 months vs 9.5 months; HR 0.74; 95% CI 0.75-0.97; P=0.026). The side effect profile is favorable with the combination arm showing a higher incidence of diarrhea (P=0.03). [39] A meta-analysis examining the cardiac toxicity in breast cancer patients treated with dual HER2 blockade showed no significant increase in cardiotoxicity, although the trials generally only included patients with good baseline cardiac function and patients who have tolerated prior treatment with trastuzumab,[40] Long term outcomes on cardiac toxicity remain to be seen.

1.4.4 Neratinib in Combination with Trastuzumab

A phase I/II, nonrandomized, open label study of neratinib in combination with trastuzumab was performed in women with advanced HER2+ breast cancer.[41] The phase I, dose-escalation phase enrolled 8 patients. No DLT was reported and the recommended dose of neratinib was 240mg daily in combination with trastuzumab 4mg/kg loading dose followed by 2mg/kg weekly thereafter. In phase II, 37 patients received neratinib and trastuzumab at the recommended dose and 28 patients were evaluable for efficacy. In the evaluable population, the 16-week PFS rate was 44.8% (95% CI, 25.9 – 62.1%), and the median PFS was 15.9 weeks (95% CI 15.1 – 31.3), with data censored for 5 patients. Two patients had the best overall response of complete response (CR), 6 patients had partial response (PR) and 14 patients had stable disease (SD). The overall response rate was 28.6% (95% CI, 13.2 – 48.7%) and the clinical benefit rate (CBR) was 35.7% (95% CI, 18.6 – 55.9%).[41]

In this trial, trastuzumab (or FDA approved biosimilar) will be added to the study drug neratinib or neratinib and fulvestrant (ER+ cohort, Part II of the trial) at disease progression if insurance coverage is obtained to explore the activity of combined HER2 targeting in HER2 mutant breast cancer.

1.5 HER2 (ERBB2)

HER2/neu (ERBB2), located on chromosomal 17q21.1, is a well established proto-oncogene that encodes HER2, a transmembrane tyrosine kinase receptor of the EGF receptor (EGFR) family composed of four members (EGFR/HER1, HER2, HER3 and HER4) that contain an extracellular ligand-binding domain, a lipophilic transmembrane domain, and an intracellular tyrosine kinase domain[4]. Receptor activation via heterodimerization between HER2 and EGFR and other members leads to strong mitogenic signals and cellular proliferation[5].

The identification of *HER2* gene amplification and the subsequent invent of HER2-targeted agents have revolutionized the care of patients with breast cancer [11]. Approximately 20% of invasive breast cancer cases carry *HER2* gene amplification, leading to increased HER2 protein expression, an aggressive clinical behavior and a decreased disease free and overall survival [42-44]. Trastuzumab, a monoclonal antibody against HER2, was the first anti-HER2 agent which was found to markedly improve the survival of patients with HER2 positive breast cancer in both metastatic and early stage setting [6-10], establishing one of the first molecular cancer therapies. Multiple agents are currently available in clinical practice either approved by FDA or through clinical trials for patients with HER2 positive breast cancer defined by either HER2 overexpression or gene amplification [11].

1.6 Mutation in HER2 (ERBB2)

1.6.1 The Identification of HER2 Mutation in Whole Genome Sequencing Studies

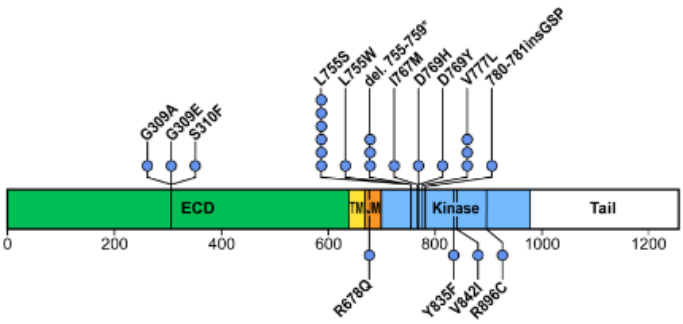


Fig. 1. HER2 somatic mutations from 22 patients

Blue circles represent individual cases.

The vast majority of HER2 mutations were identified in patients with HER2 negative disease who would not have been eligible to receive HER2 targeted drugs under the current standard of care. In addition to the TCGA-breast cancer project patients, we compiled data from 6 other breast cancer sequencing projects[15, 45-49] and identified 15 HER2 somatic mutations in 22 out of the 1391 patients, an overall incidence of 1.6% (Fig. 1). Mild enrichment for HER2 mutations may occur in lobular breast cancer as three HER2 somatic mutations were observed in 39 lobular breast cases in the TCGA study[50] and three were observed among 113 lobular cases in Shah et al., suggesting a prevalence of 4% in this histological subtype[46]. Mutations clustered in two locations, the extracellular domain (ECD) at aa 309-310 (5 of 25) and the kinase domain between aa 755-781 (17 of 25).

1.6.2 Preclinical Functional Characterization of HER2 Mutations[51]

Effects on kinase activities: We successfully expressed HER2 kinase domain constructs for WT, and V777L, D769H, V842I mutants and purified them to >80% purity. *In vitro* kinase assays demonstrated that both the D769H-HER2 and the V777L-HER2 had greater tyrosine kinase activity than WT-HER2 (Figure 2, left) and were sensitive to lapatinib in vitro (Figure 2, right). In contrast, V842I-HER2 showed modestly increased kinase activity and a small shift in lapatinib *in vitro* IC_{50} (22 vs 10 nM).

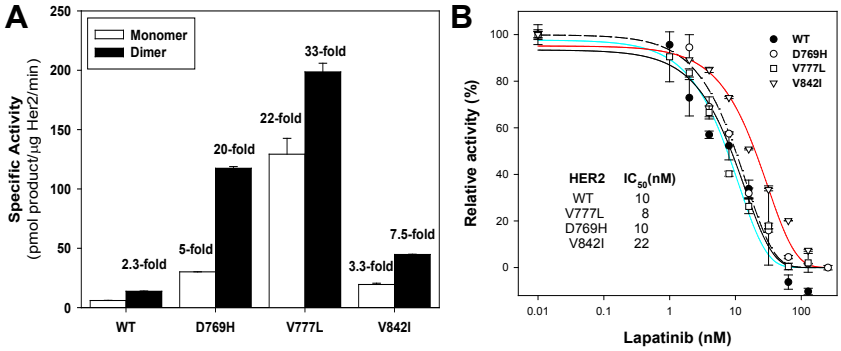


Fig. 2. *In vitro* kinase activity of HER2 mutations
(A) fold change values are relative to HER2 monomers.
(B) *In vitro* inhibition by lapatinib.

Effects on Signaling and Growth: HER2 mutations were introduced into non-transformed, MCF10A breast epithelial cells using a retroviral vector. The V777L, D769H, D769Y, P780ins, V842I, and R896C mutants are activating mutations that increased HER2 autophosphorylation and substrate protein (phospholipase γ C1, PLCγ) phosphorylation relative to WT HER2 (Fig. 3). Modest increases in HER2, PLCγ, and EGFR phosphorylation were seen with G309A, L755S, and R678Q. HER2 del.755-759 showed a marked decrease in HER2 and PLCγ phosphorylation, but increased EGFR phosphorylation, which suggests that del.755-759 has an increased ability to activate other HER/ErbB family members. The ability of various TKI drugs to block signaling from HER2 mutations was tested (not shown) and we observed that L755S mutation is a lapatinib-resistant mutation that is sensitive to neratinib and canertinib. While all three drugs are HER2/EGFR selective, lapatinib is a reversible inhibitor[52]. In contrast, neratinib and canertinib are irreversible inhibitors that form a covalent bond with a Cys residue in HER2's active site[53].

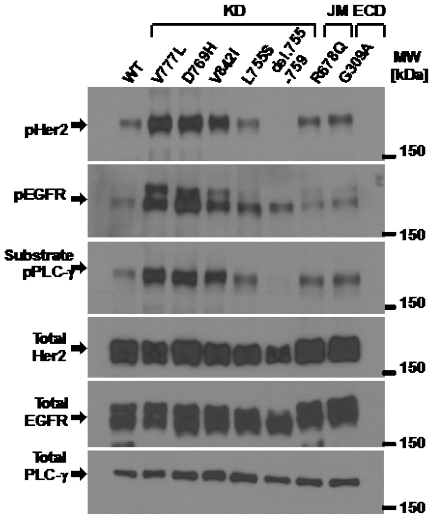


Fig. 3. Activation of downstream signaling by HER2 mutations.
KD indicates kinase domain mutations; JM: juxtamembrane; ECD: extra-cellular domain.

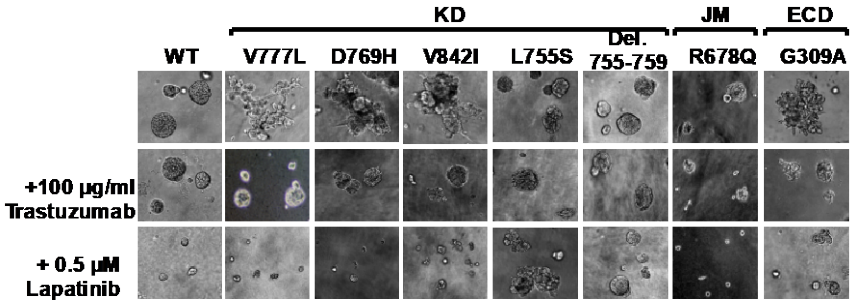


Fig. 4. HER2 mutations induce an invasive phenotype in matrigel culture.

The effect on cell growth and morphology in three dimensional cultures was measured. As previously reported[54, 55], MCF10A-HER2 WT formed spherical structures (Fig. 4, upper left). MCF10A cells bearing HER2 V777L, D769H, D769Y, P780ins, V842I, R896C, or G309A mutations formed irregular structures with spiculated, invading protrusions (Fig. 4, top row). Addition of the HER2 antibody, trastuzumab, restored the spherical morphology to these mutations (Fig. 4, middle row). HER2 L755S, del.755-759, and R678Q formed spherical structures, similar to WT HER2. MCF10A cells are an EGF-dependent cell line and require supplementary EGF in their media. Addition of lapatinib, a dual HER2/EGFR inhibitor, blocked the growth of MCF10A-HER2 WT cells and most of the HER2 mutant cells (Fig. 4, lower row). Interestingly, L755S and del.755-759 were resistant to this dose of lapatinib and continued to form large spherical structures, but they were sensitive to the irreversible inhibitor, neratinib (Fig. 5). The del.755-759 mutation was sensitive to gefitinib (Fig. 5), consistent with the increased activation of EGFR observed in HER2 del.755-759 mutant cells (Fig. 3).

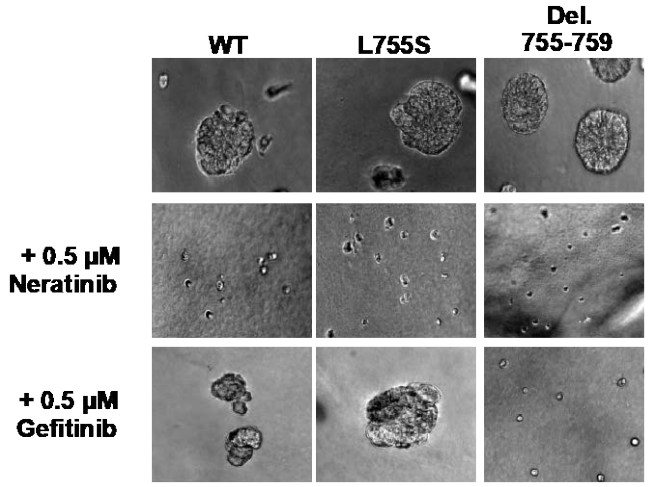


Fig. 5. Inhibitor sensitivity of lapatinib resistant mutations.

The ability of the HER2 mutations to increase tumor formation in xenografts was also tested (Fig. 6). NIH3T3-HER2 WT cells served as the control cell line. NIH3T3-HER2 V777L, D769H, and G309A mutant cell lines had more rapid tumor growth than the HER2 WT control (p-values all <0.01, as indicated in the figure by **). The L755S, V842I, R678Q and mutations showed tumor growth indistinguishable from the HER2 WT control (Fig. 6). These mutations did have more rapid tumor growth than empty vector cells, suggesting that these mutations do not impair HER2 functioning. Xenograft experiments using MCF7 cell lines are in progress (not shown).

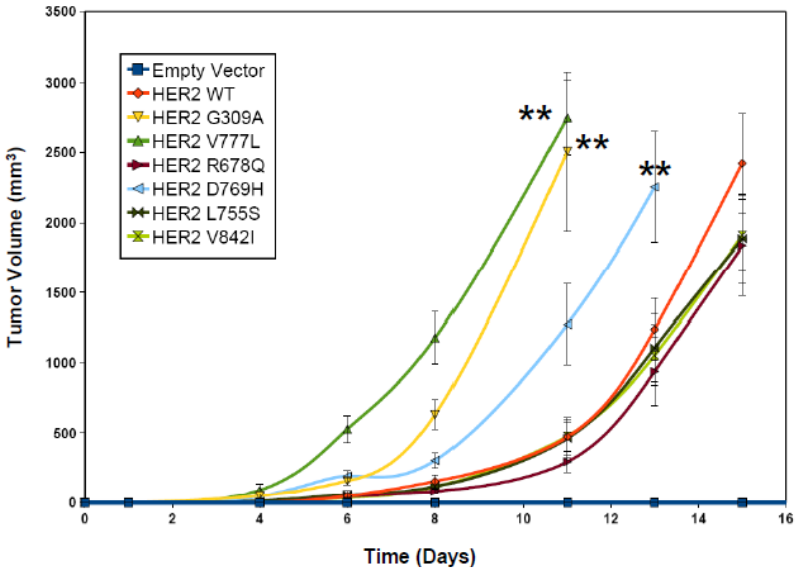


Figure 6. HER2 induces growth of tumors in nude mice.
5 x 10^5 NIH3T3 cells expressing indicated construct were injected subcutaneously.

Summary of HER2 preclinical data: To date, we have tested thirteen HER2 somatic mutations, of which seven are found to be activating mutations that are sensitive to HER2 targeted drugs: G309A, D769H, D769Y, V777L, P780ins, V842I and R896C[51]. In addition, a recent HER2 functional study by Greulich et al. also identified G309E and S310F as activating mutations[56]. Notably, the L755S mutation showed a lapatinib-resistant phenotype, but was sensitive to neratinib[51].

We hypothesize that tumors with mutations in HER2 can be effectively treated with neratinib. As a pilot study, we will conduct a 2-stage phase II single arm trial of neratinib in patients with HER2-/HER2 mutation+ metastatic breast cancer. Neratinib is chosen as it is an irreversible inhibitor of HER1, HER2 and HER4 rather than lapatinib which is a reversible inhibitor of HER1 and HER2. Importantly, neratinib has shown activity in lapatinib resistant HER2 mutations in our preclinical studies (Figure 5).

HER2 mutation is present in 1.6% of primary breast cancer overall, but certain subpopulation of breast cancer may have higher incidence. We therefore plan to analyze the clinical and pathological features of tumors with or without HER2 mutation during this trial and preferentially screen those that are most likely to carry the HER2 mutations.

1.7 Preliminary Results from Part I with Single Agent Neratinib

As of Jan 17, 2015, we have identified 16 patients with metastatic breast cancer carrying HER2 mutations (Table 1). Fifteen of these patients (94%) had ER+ disease. Among the first 10 patients with somatic Her2 mutations (9 with activating mutations and 1 with unknown significance), 1 patient with known activating mutation HER2 L755S had a partial response to neratinib. The study passed the efficacy criteria to continue enrollment to the 2nd stage (refer to statistical section). Because of the high incidence of ER positivity in HER2 mutated breast cancer and the well established crosstalk between ER and HER2 pathways, we propose to examine the combination of neratinib and fulvestrant in patients with ER+, HER2-/HER2 mutated breast cancers in Part II of the trial.

Table 1: Pathologic Characteristics of HER2 Mutant Breast Cancer Identified in Part I (N=16)

Histology	N	%
Ductal	9	56%
Lobular	6	38%
Unknown	1	6%
Biomarkers		
ER +, PR +	7	44%
ER+, PR -	8	50%
ER-, PR -	1	6%

1.8 Correlative Studies Background

Metastatic HER2- breast cancer is a heterogeneous group of diseases. Understanding the molecular and genomic structure of these tumors is the first step toward individualized therapy. In this protocol, additional tumor specimens at metastatic sites will be collected at baseline if possible when the biopsy is performed for sequencing analysis of HER2. All patients are required to consent to research on archival primary tumor specimens. Patients will be consented for research related molecular studies including genomic studies, such as whole or targeted genomic sequencing analysis, gene expression studies, for novel therapeutic target discovery. In addition, blood samples will be collected at baseline and post therapy for studies of circulating markers that correlate with breast cancer prognosis and treatment response.

2.0 OBJECTIVES

2.1 Primary Objectives

1. Part I: To determine the clinical benefit (CR+PR+SD≥6 months) of neratinib alone in patients with metastatic HER2- breast cancer that carry HER2 mutations.
2. Part II fulvestrant-naïve ER+ cohort: To examine the clinical benefit (CR+PR+SD≥6 months) of neratinib in combination with fulvestrant in patients with fulvestrant-naïve metastatic HER2-, ER+ breast cancer carrying activating HER2 mutations.
3. Part II fulvestrant-treated ER+ cohort: To examine the clinical benefit (CR+PR+SD≥6 months) of neratinib in combination with fulvestrant in patients with metastatic HER2-, ER+ breast cancer carrying activating HER2 mutations previously treated with fulvestrant.
4. Part II ER- cohort: To determine the clinical benefit rate of neratinib alone in patients with metastatic HER2-, ER- breast cancer carrying activating HER2 mutations.

2.2 Secondary Objectives

1. To determine the PFS of patients treated with neratinib alone in patients with metastatic HER2- but HER2 mutated breast cancer by ER status and by HER2 mutations (activating vs. unknown significance).

2. To assess the PFS and response rate of neratinib in combination with fulvestrant in patients with fulvestrant-naïve metastatic ER+ HER2- breast cancer carrying activating HER2 mutations.
3. To assess the PFS and response rate of neratinib in combination with fulvestrant in patients with metastatic ER+ HER2- breast cancer carrying activating HER2 mutations previously treated with fulvestrant.
4. To assess the safety profile and tolerability of neratinib in combination with fulvestrant in patients with metastatic ER+ HER2- breast cancer carrying activating HER2 mutations.
5. To correlate the presence of HER2 mutation with histology subtype (invasive lobular vs. invasive ductal cancer), tumor grade (grade 1-2 vs 3), tumor staging at initial diagnosis (I vs. II or III vs. IV), disease free survival in HER2- breast cancer.

2.3 Exploratory Objectives

1. To compare the occurrence of HER2 mutation in paired primary and metastatic sites
2. To collect peripheral blood plasma samples for circulating HER2 mutation analysis
3. To investigate other potential therapeutic targets in HER2- breast cancer
4. To explore potential mechanisms of treatment resistance
5. To explore anti-tumor response after adding trastuzumab (or FDA approved biosimilar) to neratinib or neratinib plus fulvestrant when tumor progresses on single agent neratinib or neratinib in combination with fulvestrant.

3.0 PATIENT SELECTION

3.1 Pre-registration (for patients with unknown HER2 mutation status to have tumor tissue screened centrally by Wash U GPS laboratory)

3.1.1 Inclusion Criteria for Pre-registration

1. Histologically or cytologically confirmed HER2-negative (0 or 1+ by IHC or non-amplified by FISH) breast cancer that is stage IV.
2. Agree to provide archival tumor material for research
3. There is no limitation on the number of prior lines of systemic therapy.
4. Presence of measurable or non-measurable disease by RECIST 1.1 is acceptable, except to be eligible for the Part II fulvestrant-naïve ER+ cohort, at least one measurable disease by RECIST 1.1 is required.
5. At least 18 years of age.
6. ECOG performance status ≤ 2 (see Appendix A).
7. Adequate organ function as defined below within 8 weeks of pre-registration:
   - Serum creatinine: $\leq$1.5 x ULN
   - Child-Pugh class A if with liver disease
8. Able to understand and willing to sign an IRB approved written informed consent document.

Note: HER2 mutation testing may be performed while the patient is receiving active systemic therapy for metastatic breast cancer so that the result can be used to determine eligibility for study drug therapy in the future.

3.1.2 Exclusion Criteria for Pre-registration

1. Testing for LVEF is not required for pre-registration, but patient must not have a recent LVEF < LLN or have symptoms of congestive heart failure.
2. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
3. Acute or currently active hepatic or biliary disease requiring antiviral therapy (with the exception of Gilbert's syndrome, asymptomatic gallstones, liver metastases, or stable chronic liver disease per investigator assessment).
4. History of significant cardiac disease, cardiac risk factors, or uncontrolled arrhythmias.
5. Symptomatic intrinsic lung disease or extensive tumor involvement of the lungs resulting in dyspnea at rest.

3.2 Registration (for patients positive for HER2 mutation to receive study treatments)

3.2.1 Inclusion Criteria for Registration (for patients initially pre-registered and with HER2 mutation identified by Wash U GPS laboratory)

1. Tumor tissue tested positive for HER2 mutation. See the list of mutations eligible for Part II enrollment in Table 2 (Section 5.1.6). Mutations outside the list will be assessed on a case-by-case basis by the study team to determine eligibility.
   Note: HER2 mutations listed in Table 2 detected by Guardant360 are also eligible
2. Agree to provide archival tumor material for research
3. ECOG performance status $\leq$ 2 (see Appendix A).
4. Adequate organ function as defined below within 2 weeks of registration:
   - Absolute neutrophil count: $\geq 1.5 \times 10^9$/L (1500/mm^3)
   - Platelet count: $\geq 100 \times 10^9$/L (100,000/mm^3)
   - Serum creatinine: $\leq$1.5 x ULN
   - Child-Pugh class A if with liver disease
5. The patient must have completed radiation therapy and be at least 1 week from the last systemic therapy administration, with adequate recovery of bone marrow and organ functions, before starting neratinib.
6. Presence of disease progression on the most recent disease evaluation.
7. Patients with known treated brain metastasis are eligible, but must have received radiation and be off steroids and stable (without evidence of disease progression by imaging or exam) for 3 months.
8. QTc interval $\leq$ 450 msec for men or $\leq$ 470 msec for women within 2 weeks of registration.
9. LVEF $\geq$ institutional LLN within 4 weeks of registration.
10. Women of childbearing 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 participating in this study, she must inform her treating physician immediately. Men must agree and commit to use a barrier method of contraception while on treatment and for 3 months after the last dose of the investigational product (see Section 6.3).
11. Able to understand and willing to sign an IRB approved written informed consent document.
12. There is no limitation on the number of prior lines of systemic therapy.

13. To be eligible for the Part II fulvestrant-naïve ER+ cohort, prior treatment with fulvestrant is not allowed. In addition, ER and/or PR positivity by institutional standard is required on pathology from the most recent tumor specimen if biopsy was done unless the tissue source (for example, pleural effusion or ascites or bone biopsy) may yield false negative ER and/or PR result, in which case the pathology from an earlier time point could be used and a discussion with the study chair is required.
14. To be eligible for the Part II fulvestrant-treated ER+ cohort, prior disease progression on fulvestrant is required. In addition, ER and/or PR positivity by institutional standard is required on pathology from the most recent tumor specimen unless the tissue source (for example, pleural effusion or ascites or bone biopsy) may yield false negative ER and/or PR result, in which case the pathology from an earlier time point could be used and a discussion with the study chair is required.

3.2.2 Inclusion Criteria for Registration (for patients with HER2 mutation identified at an outside CLIA certified location)

1. Histologically or cytologically confirmed HER2-negative (0 or 1+ by IHC or non-amplified by FISH) breast cancer that is stage IV.
2. Tumor tissue or circulating tumor DNA tested positive for HER2 mutation. See the list of mutations eligible for Part II enrollment in Table 2 (Section 5.1.6). Mutations outside the list will be assessed on a case-by-case basis by the study team to determine eligibility.
3. Presence of measurable or non-measurable disease by RECIST 1.1 is acceptable, except to be eligible for the Part II fulvestrant-naïve ER+ cohort, at least one measurable disease by RECIST 1.1 is required.
4. At least 18 years of age.
5. ECOG performance status $\leq$ 2 (see Appendix A).
6. Adequate organ function as defined below within 2 weeks of registration:
   - Absolute neutrophil count: $\geq 1.5 \times 10^9$/L (1500/mm^3)
   - Platelet count: $\geq 100 \times 10^9$/L (100,000/mm^3)
   - Serum creatinine: $\leq$1.5 x ULN
   - Child-Pugh class A if with liver disease
7. The patient must have completed radiation therapy and be at least 1 week from the last systemic therapy administration, with adequate recovery of bone marrow and organ functions, before starting neratinib.
8. Presence of disease progression on the most recent disease evaluation.
9. Patients with known treated brain metastasis are eligible, but must have received radiation and be off steroids and stable (without evidence of disease progression by imaging or exam) for 3 months.
10. QTc interval $\leq$ 450 msec for men or $\leq$ 470 msec for women within 2 weeks of registration.
11. LVEF $\geq$ institutional LLN within 4 weeks of registration.
12. Women of childbearing 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 participating in this study, she must inform her treating physician immediately. Men must agree and commit to use a barrier method of contraception while on treatment and for 3 months after the last dose of the investigational product (see Section 6.3).

13. Able to understand and willing to sign an IRB approved written informed consent document.
14. There is no limitation on the number of prior lines of systemic therapy.
15. To be eligible for the Part II fulvestrant-naïve ER+ cohort, prior treatment with fulvestrant is not allowed. In addition, ER and/or PR positivity by institutional standard is required on pathology from the most recent tumor specimen if biopsy was done unless the tissue source (for example, pleural effusion or ascites or bone biopsy) may yield false negative ER and/or PR result, in which case the pathology from an earlier time point could be used and a discussion with the study chair is required.
16. To be eligible for the Part II fulvestrant-treated ER+ cohort, prior disease progression on fulvestrant is required. In addition, ER and/or PR positivity by institutional standard is required on pathology from the most recent tumor specimen unless the tissue source (for example, pleural effusion or ascites or bone biopsy) may yield false negative ER and/or PR result, in which case the pathology from an earlier time point could be used and a discussion with the study chair is required.

3.2.3 Exclusion Criteria for Registration

1. Currently receiving any other investigational agents or systemic cancer therapy.
2. Currently taking medications and herbal or dietary supplements that are strong cytochrome P450 (CYP) 3A4 inducers or inhibitors (refer to Section 6.4.4). A washout period of at least 5 days is required and must have been completed prior to the start of neratinib if the patient was taking any of these agents. If unavoidable, patients taking CYP3A4 inhibitors should be monitored closely.
3. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
4. Acute or currently active hepatic or biliary disease requiring antiviral therapy (with the exception of patients with Gilbert's syndrome, asymptomatic gallstones, liver metastases, or stable chronic liver disease per investigator assessment).
5. Pregnant and/or breastfeeding.
6. History of significant cardiac disease, cardiac risk factors, or uncontrolled arrhythmias.
7. Symptomatic intrinsic lung disease or extensive tumor involvement of the lungs resulting in dyspnea at rest.
8. Experiencing grade 2 or greater diarrhea.
9. Prior treatment with neratinib.
10. Child-Pugh class B or C liver dysfunction.

3.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4.0 REGISTRATION PROCEDURES

4.1 Pre-Registration (for patients with unknown HER2 mutation status to have tumor tissue screened centrally by Wash U GPS laboratory)

Patients must not start any protocol intervention prior to pre-registration through the Siteman Cancer Center. All eligible and consenting patients will be pre-registered to this protocol for the purposes of HER2 sequencing; if HER2 mutation is identified, the patient will then be registered if eligible for study treatment.

The following steps must be taken:

1. Confirmation of patient pre-registration eligibility by Washington University
2. Pre-Registration of patient in the Siteman Cancer Center database
3. Assignment of unique patient number (UPN)

Once the patient has been entered in the Siteman Cancer Center database, the WUSM coordinator will forward verification of enrollment and the UPN via email.

4.1.1 Confirmation of Patient Eligibility for Pre-Registration

Confirm patient eligibility by faxing the information listed below to the research coordinator listed in the *Siteman Cancer Center Clinical Trials Core Protocol Procedures for Secondary Sites* packet and email Caroline Bumb (cbumb@wustl.edu) at least one business day prior to registering patient:

1. Your name and contact information (telephone number, fax number, and email address)
2. Your site PI's name, the registering MD's name, and your institution name
3. Patient's race, sex, and DOB
4. Three letters (or two letters and a dash) for the patient's initials
5. Currently approved protocol version date
6. Copy of signed consent form (patient name may be blacked out)
7. Planned date of sample shipment
8. Completed pre-registration eligibility checklist (Appendix B), signed and dated by a member of the study team
9. Copy of appropriate source documentation confirming patient eligibility

4.1.2 Patient Pre-Registration in the Siteman Cancer Center OnCore Database

Registrations may be submitted Monday through Friday between 8am and 5pm CT. Urgent late afternoon or early morning enrollments should be planned in advance and coordinated with the Washington University research coordinator. Registration will be confirmed by the research coordinator or his/her delegate by email or fax within one business day. Verification of eligibility and pre-registration should be kept in the patient chart.

Patients at all sites must be pre-registered through the Siteman Cancer Center OnCore database at Washington University.

4.1.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. Patients will also be identified by first, middle, and last initials. If the patient has no middle initial, a dash will be used on the case report forms (CRFs). All data will be recorded with this identification number on the appropriate CRFs.

4.2 Registration for Study Therapy

Patient who initially pre-registered and found to have HER2 mutation in the tumor will be registered to receive study therapy if the patient remains eligible. The same UPN will be used.

Patients with HER2 mutation identified by another CLIA certified laboratory and meet all eligibility will be registered prior to study drug therapy. An UPN will be assigned as described in Section 4.1.3.

4.2.1 Confirmation of Patient Eligibility for Registration

Confirm patient eligibility by faxing the information listed below to the research coordinator listed in the *Siteman Cancer Center Clinical Trials Core Protocol Procedures for Secondary Sites* packet and email Caroline Bumb (cbumb@wustl.edu) at least two business days prior to registering patient:

1. Your name and contact information (telephone number, fax number, and email address)
2. Your site PI's name, the registering MD's name, and your institution name
3. Patient's race, sex, and DOB
4. Three letters (or two letters and a dash) for the patient's initials
5. Currently approved protocol version date
6. Copy of signed consent form (patient name may be blacked out)
7. Planned date of treatment to start
8. Completed registration eligibility checklist (Appendix C), signed and dated by a member of the study team
9. Copy of appropriate source documentation confirming patient eligibility

4.2.2 Patient Registration in the Siteman Cancer Center OnCore Database

Registrations may be submitted Monday through Friday between 8am and 5pm CT. Urgent late afternoon or early morning enrollments should be planned in advance and coordinated with the Washington University research coordinator. Registration will be confirmed by the research coordinator or his/her delegate by email or fax within one business day if all required documentation to determine patient eligibility is provided. Verification of eligibility and registration should be kept in the patient chart.

Patients at all sites must be registered through the Siteman Cancer Center OnCore database at Washington University.

4.3 Recruitment through Army of Women

The following procedures describe how women recruited via Army of Women (AOW) will be screened and registered for this trial:

1. HRPO-approved Eblast will be sent by AOW coordinator to women signed up through AOW to receive recruitment messages.
2. Women responding to the Eblast will be referred by AOW coordinator to WUSM study coordinator at HER2MutationTrial@dom.wustl.edu.
3. WUSM study coordinator will send a HRPO-approved introductory email to each AOW recruit requesting a time to discuss the trial and attaching the version of the screening consent form drafted specifically for outside recruits.
4. WUSM study coordinator will consent the AOW recruit over the phone. If the recruit consents to be screened for this trial, the WUSM study coordinator will mail the patient info sheet and a SASE, requesting that the patient complete the info sheet and return it along with the screening consent form.
5. Once the screening consent form and patient info sheet have been received by the WUSM study coordinator, the medical records for the AOW recruit will be requested by the WUSM study coordinator.
6. Once the medical records have been received, the pre-registration process described in Section 4.1 will be followed and the pre-registration eligibility criteria described in Section 3.1 will be checked. The patient will be informed of eligibility for HER2 mutation testing via email.
7. If the patient is shown to be eligible for screening, the WUSM study coordinator will request a tumor specimen for HER2 mutation testing. The WUSM study coordinator will send the report to the patient after reviewing with the PI. If negative, the patient is not eligible to continue in the study. If positive, the PI will discuss with the patient and the patient's oncologist if the patient desires it and will refer the patient to a participating institution for consent to the main study.

4.4 Recruitment from Outside Practice

An informational card with the title of the study and contact information of the WUSM study coordinator will be made and distributed to medical oncologists from outside practices at scientific meetings and lectures. These physicians will be encouraged to refer potentially eligible patients to the WUSM study coordinator, who will begin the pre-registration screening process as described in #3 in Section 4.3. Screening and testing will continue as described in Section 4.3.

5.0 TUMOR HER2 SEQUENCING

5.1 Tumor HER2 sequencing at Washington University CLIA lab following pre-registration for eligibility

Patients meeting the pre-registration eligibility criteria with unknown tumor *HER2* mutation status could undergo tumor *HER2* sequencing centrally at the CAP/CLIA certified Genomic and Pathology Services at Washington University (GPS@WU) after pre-registration to the study. Sanger sequencing of Exons 18, 19, 20, 21, 22, 23 and 24 was initially used for the study. Since October 2014, this was switched to a next generation

sequencing (NGS) HER2 assay that covers all coding exons of the *HER2* gene.

Archival tumor specimens from the primary breast cancer or from a metastatic site is required for the sequencing analysis. Samples will be need to be shipped to the *"Clinical Support Services Office at the Washington University Department of Pathology & Immunology"*. Please refer to the Correlative Science Procedures Manual Section 1.0 for tumor sample requirement and shipping instructions. Note that sample shipment and sequencing analysis can be performed while the patient is receiving other systemic therapies so the results could be used to determine whether the patient is eligible to receive the study therapy when disease progresses on the current treatment.

The study coordinator from the participating institution listed on the sample shipment form will be notified of the results per email within 3-4 weeks. The study chair and the Washington University study coordinator have access (requiring password) to a secure folder on the WUSM pathology department network drive where the clinical reports are stored. For remotely screened participants, the reports will be faxed or emailed to the participant or the treating physician as applicable.

5.2 Tumor or circulating tumor DNA HER2 Mutation/Variants Identified at Laboratories other than GPS@WU

Patients with tumor or circulating tumor DNA *HER2* mutations meeting the criteria defined in Section 5.3 identified at other CAP/CLIA-certified laboratories are eligible for study entry if they meet all other eligibility criteria. To determine the eligibility of the reported mutation from laboratories other than Wash U GPS, the full report will need to be sent to Caroline Bumb (cbumb@wustl.edu) and Cynthia Ma (cynthiaxma@wustl.edu).

Pre-registration process is not required for patients with known mutations as above but the archival tumor sample must be submitted to Wash U Alliance/ACOSOG-CSB/TPC, not to the *Clinical Support Service Office*, for future confirmation of the *HER2* mutation and correlative studies (also refer to the Section 10 for Correlative Study). Please refer to the Correlative Science Procedure Manual regarding the sample requirement and shipping instructions for Correlative Studies.

5.3 Eligible *HER2* mutations

HER2 mutation is defined as a single nucleotide variant (SNV) or an indel variation that changes the encoded amino acid in the coding region of *HER2* or an intronic mutation known to affect the splicing of *HER2*. Identified SNVs will be compared to known single nucleotide polymorphisms (SNPs) identified in the SNP data base as well as to the variants identified in cancer mutation databases including the Catalogue Of Somatic Mutations In Cancer (COSMIC) database. *HER2* SNPs would be not considered as an *HER2* mutation. Germ-line DNA will be sequenced to rule out single nucleotide polymorphisms if needed.

All somatic *HER2* mutations were eligible for Part I of the trial. However, due to the lack of response in patients with *HER2* mutations of unknown significance observed in Part I of the trial [68], only known activating mutations listed in Table 2 are considered eligible for patients enrolling to Part II of the trial unless approved by the study chair. Mutations outside the list will be assessed on a case-by-case basis by the study team to determine eligibility.

Table 2 Known Activating HER2 Mutations	
HER2 mutation	Reference
G309A/E	[56, 57]
S310F/Y	[56]
S653C	[58]
L755S	[57, 59, 60]
L755P	[60]
Del. 755-759	[57]
D769Y/H	[57]
V777L	[57, 60]
V842I	[57]
T862A	[60]
L869R	[62]
H878Y	[60, 63, 64]
G776V/C	[65]
All exon 20 insertions, including: A771_Y772insYVMA A775_G776insYVMA P780_Y781insGSP	[66, 67]

5.4 Germline DNA Sequencing for *HER2* to determine eligibility

In cases where it is unclear whether the identified HER2 variant is somatic (tumor specific) versus germline in origin, the study team at WUSM will notify the site investigator to submit a blood sample of the patient for germline DNA sequencing at Wash U GPS laboratory. Please refer to Section 2.0 of the Correlative Science Procedure Manual for the submission and shipment.

5.5 *HER2* Mutation Testing by ctDNA

Plasma in cancer patients often carries small amounts of fragmented cell-free DNA of 160-180 base pairs, which are originated from the necrosis or apoptotic process of cancer cells. Advances in the next generation sequencing (NGS) technology and digital genomic techniques support the clinical validity of cell-free circulating DNA (ctDNA) sequencing analysis to non-invasively identify actionable genomic alterations, monitor treatment response, and investigate resistance mechanisms [69]. ctDNA sequencing is particularly helpful in cases that tumor DNA sequencing is not possible due to insufficient quality or quantity of the tumor tissue.

In collaboration with Guardant Health, Inc., we analyzed the plasma ctDNA collected from patients enrolled in this trial using the CLIA certified digital sequencing panel (Guardant 360), which utilizes hybrid capture followed by NGS of all exons in 30 genes (including HER2) and critical exons (those reported as having a somatic mutation in COSMIC) of 40 additional genes to detect and report single nucleotide variants (SNVs) and small indels in 70 genes, copy number amplifications in 18 genes, and select fusions [70]. The plasma ctDNA sequencing successfully identified HER2 mutation in 11/14 (79%; 90% CI: 53-94%) patients tested positive for HER2 mutation by tumor DNA NGS of HER2. Importantly, none of the 32 patients who were negative for HER2 mutation by tumor DNA sequencing was positive by ctDNA sequencing. Therefore, the specificity of the Guardant 360 ctDNA sequencing for HER2 mutation was 100% (90% CI: 91-100%). These data are being prepared for publication. Based on these results and the fact that tumor DNA sequencing for HER2 was unsuccessful in over 25% of patients pre-registered to this

trial, we will allow patients with HER2 mutation detected in plasma by a CLIA lab to enroll on the study to receive neratinib.

6.0 TREATMENT PLAN

To be eligible to receive neratinib, mutation in HER2 either in the primary or metastatic tumor or circulating tumor DNA is required. However, data that have been collected during pre-registration from patients who have HER2 non-mutant tumors will also be used for studies to correlate HER2 mutation with histology subtype (invasive lobular vs. invasive ductal cancer), tumor grade (grade 1-2 vs. 3), tumor staging at initial diagnosis (I vs. II or III vs. IV), and disease-free survival in HER2-negative breast cancer. The left over tumor specimens from HER2 sequencing analysis from all patients will be assayed in future studies to understand the biology of HER2-negative breast cancer. The future studies could include tumor DNA, RNA or protein analysis.

6.1 Neratinib Administration

Neratinib is administered in the outpatient setting. It will be taken by mouth with food, preferably in the morning daily, starting at 240mg per day. Neratinib will be given on a 28-day cycle (+/- 3 days). The dose modification guidelines are provided in Section 7.0. In Cycle 2 or subsequent cycles, patients may begin taking 320mg per day of neratinib with prophylactic loperamide provided the patients did not experience any intolerable grade 2 or higher treatment-related AEs during previous cycles (including diarrhea while receiving prophylactic loperamide).

6.2 Concurrent Prophylactic Administration of Loperamide

Investigators must ensure that subjects have loperamide on hand when starting to take the investigational product. Loperamide is the recommended standard therapy to treat diarrhea in this study. If alternative antidiarrheal medication is used, the reason must be documented in the source documents. Acceptable reasons are non-tolerance of loperamide or lack of efficacy. Loperamide will be dispensed directly by the site on day 1 (preferred option), or a prescription for loperamide will be provided for the subject with the instruction to have loperamide on hand prior to taking the first dose of investigational product. If none of the above is feasible, the subject must obtain loperamide "over-the-counter" prior to taking the first dose of investigational product on Day 1.

6.3 Fulvestrant Administration

Fulvestrant will be given on a 28-day cycle (+/- 3 days). It is administered at a dose of 500 mg (2 x 250 mg injections) IM on Days 1 and 15 of Cycle 1, and 500 mg IM on Day 1 of each subsequent cycle. Administer intramuscularly slowly in the buttock. (NOTE: 500 mg dose will require one 250 mg injection in each buttock). Immediately activate needle protection device upon withdrawal from patient by pushing lever arm completely forward until needle tip is fully covered. Visually confirm that the lever arm has fully advanced and the needle tip is covered. If unable to activate, discard immediately into an approved sharps container.

6.4 General Concomitant Medication and Supportive Care Guidelines

Patients should receive full supportive care, including transfusions of blood and blood products, antibiotics, antinausea/diarrhea, etc., when appropriate.

Subjects on digoxin, a P-glycoprotein substrate with a narrow therapeutic window, should be monitored closely and their digoxin dose adjusted as needed, since neratinib is an inhibitor of P-glycoprotein. Co-administration of neratinib and digoxin could result in increased digoxin levels and associated digoxin toxicity.

Elective surgery should be avoided while receiving investigational product. If surgery is required the recommendation is to hold investigational product for 3 to 4 days prior to surgery, and then resume product when the subject is tolerating a full diet post-operatively (no later than 3 weeks after holding it).

The start date and stop date and indication for concomitant treatments and/or therapies and medications given because of an AE occurring from the signing of the consent form until the end of treatment will be recorded on the Concomitant Medications Form in REDCap.

6.4.1 Prohibited Concomitant Treatment

The following treatments are prohibited during neratinib therapy:

- Any chemotherapy, radiation therapy, immunotherapy, biotherapy, or surgery for breast cancer.
- Any other investigational agent.

6.4.2 Permitted Concomitant Treatment during Neratinib Therapy

The following treatments are permitted during the study:

- Standard therapies for preexisting medical conditions, and for medical and/or surgical complications. All medication should be recorded.
- Bisphosphonates, regardless of the indication.

6.4.3 Addition of Trastuzumab (or FDA Approved Biosimilar) at Disease Progression on Neratinib or Neratinib in combination with Fulvestrant

If a patient experiences disease progression following neratinib or neratinib in combination with fulvestrant, trastuzumab may be added to the treatment regimen provided it is covered by the patient's insurance company. Trastuzumab has available biosimilar products FDA approved for use. Trastuzumab or any of the FDA approved commercially available biosimilar products for trastuzumab may be administered to patients based on their insurance requirements or institutional practice. If a biosimilar product is required, dosing, schedule of administration, and premedications are identical to the guidance described throughout the protocol for trastuzumab. Preparation of the drug product will be dependent on the biosimilar selected for use.

Patients should start trastuzumab (or FDA approved biosimilar) with their next scheduled fulvestrant injection. Neratinib does not need to be held between progression and Day 1 of trastuzumab (or FDA approved biosimilar) treatment. Palliative radiation for pain is allowed prior to starting trastuzuamb with prior approval from the Washington University PI. Neratinib should be held during radiation. AE monitoring, dose modifications for neratinib, and tumor response on combination therapy will be followed per protocol. For these patients, treatment cycle will be restarted at cycle 1 at the start of trastuzumab. Research blood collection will follow the study calendar (beginning at C1D1).

Trastuzumab (or FDA approved biosimilar) is administered intravenously with a loading dose of 6 mg/kg then 4 mg/kg every 2 weeks +/- 3 days. A cycle will be defined as 28 days. For the first (loading) dose of trastuzumab (or FDA approved biosimilar), premedication with diphenhydramine 50 mg PO or other antihistamine equivalent and acetaminophen 650 mg PO will be given.

6.4.4 Effect of Other Drugs on Neratinib

Gastric Acid Reducing Agents		
Clinical Impact	• Concomitant use of neratinib with a proton pump inhibitor (PPI, lansoprazole) resulted in a decrease of neratinib C_{max} by 71% and AUC by 65% • Concomitant use with other pH lowering agents was not studied but a decrease in neratinib AUC is also considered likely • Decreased neratinib AUC may reduce neratinib activity	
Prevention or Management	• PPIs	Avoid concomitant use
	• H2-receptor antagonists	Avoid concomitant use
	• Antacids	Separate neratinib dosing by **3 hours** after antacids
Strong and Moderate CYP3A4 Inhibitors		
Clinical Impact	• Concomitant use of neratinib with a strong CYP3A4 inhibitor (ketoconazole) increased neratinib C_{max} by 321% and AUC by 481% • Concomitant use of neratinib with other strong or moderate CYP3A4 inhibitors may increase neratinib concentrations • Increased neratinib concentrations may increase the risk of toxicity	
Preventation or Management	Avoid concomitant use of neratinib with strong or moderate CYP3A4 inhibitors.	
Examples[1]	*Strong CYP3A4 inhibitors:* boceprevir, clarithromycin, cobicistat, conivaptan, danoprevir and ritonavir, diltiazem, elvitegravir and ritonavir, grapefruit juice, idelalisib, indinavir and ritonavir, itraconazole, ketoconazole, lopinavir and ritonavir, nefazodone, nelfinavir, paritaprevir and ritonavir and (ombitasvir and/or dasabuvir), posaconazole, ritonavir, saquinavir and ritonavir, tipranavir and ritonavir, troleandomycin, voriconazole	
	Moderate CYP3A4 inhibitors: aprepitant, cimetidine, ciprofloxacin, clotrimazole, crizotinib, cyclosporine, dronedarone, erythromycin, fluconazole, fluvoxamine, imatinib, tofisopam, verapamil	
Strong or Moderate CYP3A4 Inducers		
Clinical Impact	• Concomitant use of neratinib with a strong CYP3A4 inducer (rifampin) reduced neratinib C_{max} by 76% and AUC by 87%	

	• Concomitant use of neratinib with other strong or moderate CYP3A4 inducers may decrease neratinib concentrations • Decreased neratinib AUC may reduce neratinib activity
Prevention or Management	Avoid concomitant use of neratinib with strong or moderate CYP3A4 inducers
Examples[1]	*Strong CYP3A4 inducers:* carbamazepine, enzalutamide, mitotane, phenytoin, rifampin, St. John's wort
	Moderate CYP3A4 inducers: bosentan, efavirenz, etravirine, modafinil

[1]These examples are a guide and not considered a comprehensive list of all possible drugs that may fit this category. Please contact study chair.

If unavoidable, patients taking CYP3A4 inhibitors should be monitored closely. Subjects on coumarin-derivative anticoagulants (eg, warfarin) should be monitored closely and their anticoagulant dose adjusted as needed. Subjects on chronic laxatives should be followed closely and consideration should be given to decreasing or stopping laxatives prior to starting investigational agent, given the potential for neratinib-related diarrhea to be worsened by concomitant laxative use. Drugs known to cause QTc prolongation which are being given concomitantly require close monitoring of the subject with serial ECGs.

MEDICATIONS THAT MAY CAUSE QTc PROLONGATION

The following table presents a list of drugs that prolong, may prolong, or are unlikely to prolong the QTc. Please note that this list is frequently updated. For the most current list of medications, users should be directed to the following website: *http://www.azcert.org/medical-pros/drug-lists/drug-lists.cfm*.

Patients using drugs known to cause QT/QTc prolongation should be monitored closely with serial electrocardiograms (ECG) at the Investigator's discretion.

Compound	Compound Half Life	Possible Washout Period - Hours	Possible Washout Period - Days
Alfuzocin	~10 hours		7
Amantadine	17 +/- 4 hours (10-25)		4
Amiodarone (cordarone)	58 days (15-142) 36 days (active metabolite)		180
Amitriptyline*	> 24 hours, wide interpatient variability		
Arsenic trioxide	Not characterized		
Azithromycin	40 hours		
Bepridil	42 hr (26-64)		10
Chloral hydrate	Readily converted to Trichloroethanol (active metabolite $T_{1/2}$=7-10 hour)	48	
Chloroquine	Prolonged (days to weeks)		
Chlorpromazine	30 +/- 7 hours		7
Cisapride	6 – 12 hour, up to 20 hour	60	
Clarithromycin	Non linear PK3-4 hr (250mg Q12) 5-7 hr (500mg Q12)	36	
Cloroquine	6 to 60 days; mean 20 days		
Desipramine*	> 24 hours, wide interpatient variability		
Disopyramide	6.7 hr (4-10)	36	
Dofetilide	10 hr	48	
Dolesetron	8.1 hr		

Domperidone	7-8 hr	48	
Doxepin*	> 24 hours, wide interpatient variability		
Droperidol	2.2 hours	10	
Erythromycin	* Each salt form has different Half life*		
Felbamate	20-23 hr		5
Flecainide	20 hr (12-27)		5
Foscarnet	87.5+/-41.8 hours *distribution and release from bone*		20
Fosphenytoin	12-29 hr		6
Gatifloxacin	7-14 hr	48	
Gemifloxacin	7 hours	48	
Grepafloxacin	16 hr		3
Halofantrine	6-10 days (variable among individual)		45
Haloperidol	18 +/-5 hr		5
Ibutilide	6 hours (2-12) * variable among subject*	36	
Imipramine*	> 24 hours, wide interpatient variability		
Indapamide	14 hours (biphasic elimination)		3
Isradipine	8 hours (multiple metabolites)	48	
Levofloxacin	6-8 hours	48	
Levomethadyl	Multiple compartment PK with active metabolite 2.6 day for LAAM, 2 day for nor-LAAM, 4 day for dinor-LAAM		20
Lithium	24 hour (10-50)		7
Mesoridazine	24-48 hours (animal study)		10
Methadone	15-30 hours		7
Moexipril/HCTZ	2-9 hour (include active metabolite) for moexipril; 5.6-14.8 hours for HCTZ	48	
Moxifloxacin	12 +/-1.3 hours	72	
Naratriptan	6 hours	36	
Nicardipine	~ 2 hour post IV infusion	12	
Nortriptyline*	> 24 hours, wide interpatient variability		
Octreotide	1.7 hours	12	
Ofloxacin	5 to 7.5 hours		2
Ondansetron	4 hours (IV/IM); 3 hours (PO)		1 to 3
Pentamidine	6.4+/-1.3 hours	36	
Pimozide	55 hours		10
Procainamide	3-4 hour for PA and NAPA (active metabolite)	24	
Protiptyline*	> 24 hours, wide interpatient variability		
Quetiapine	6 hours	36	
Quinidine	6-8 hours in adult; 3-4 hours in children	36	
Quinine	4-5 hours		
Risperidone	3-20 hours (extensive to poor metabolizer) 9-hydroxyrisperidone (active metabolite) T ½ =21-30 hours (extensive to poor metabolizer)		4
Salmeterol	5.5 hours (only one datum)	36	
Sotalol	12 hours	72	
Sparfloxacin	20 hours (16-30)		4
Sumatriptan	2.5 hours	12	
Tacrolimus	~34 hours in healthy; ~19 hours in Kidney transplant		7
Tamoxifen	5-7 days (biphasic)		30
Telithromycin	2-3 hr	24	
Thioridazine	20-40 hours (Phenothiazines)		7
Tizanidine	2.5 hours	12	
Vardenifil	4 to 5 hours		
Venlaflaxine	5 +/-2 hours for parent comp. 11+-2 hours for OVD (active metabolite)	60	
Voriconizole	6 hours; dose dependent		
Ziprasidone	7 hr	36	

Zolmitriptan	2.8-3.7 hours (higher in female)	18	

*Weakly associated with Torsades de Pointes and/or QT prolongation but that are unlikely to be a risk for Torsades de Pointes when used in usual recommended dosages and in patients without other risk factors (*e.g.*, concomitant QT prolonging drugs, bradycardia, electrolyte disturbances, congenital long QT syndrome, concomitant drugs that inhibit metabolism).

References:

1. **Physician's Desk Reference 2002**
2. **Facts and Comparisons (update to June 2005)**
3. **The Pharmacological Basis of Therapeutics 9th Edition, 1996**

6.5 Women of Childbearing Potential

Women of childbearing potential (women with regular menses, women with amenorrhea, women with irregular cycles, women using a contraceptive method that precludes withdrawal bleeding, and women who have had a tubal ligation) are required to have a negative serum or urine pregnancy test within 7 days prior to the first dose of neratinib.

Female and male patients (along with their female partners) are required to use two forms of acceptable contraception, including one barrier method, during participation in the study and for one month following the last dose of the neratinib. Men must agree and commit to use a barrier method of contraception while on treatment and for 3 months after the last dose of the investigational product.

If a patient is suspected to be pregnant, neratinib should be immediately discontinued. In addition a positive urine test must be confirmed by a serum pregnancy test. If it is confirmed that the patient is not pregnant, the patient may resume dosing.

If a female patient or female partner of a male patient becomes pregnant during therapy or within one month after the last dose of neratinib, the investigator must be notified in order to facilitate outcome follow-up.

6.6 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented in the case report forms.

In the absence of treatment delays due to adverse events, treatment may continue until one of the following criteria applies:

- Documented and confirmed disease progression
- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious non-compliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study

Patients who prematurely discontinue treatment for any reason will be followed as indicated in the study calendar.

6.7 Duration of Follow-up

Patients will be followed every 3 months for 2 years or until death, whichever occurs first. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. Patients will be followed through either review of medical records, phone calls, or office visits.

7.0 DOSE DELAYS/DOSE MODIFICATIONS AND AE MANAGEMENT

7.1 Neratinib Dose Administration Table

The allowed dose levels for dose reduction(s) due to toxicity which is deemed possibly, probably, or definitely related to neratinib are listed in the below dose administration table.

Dose Level	Neratinib (mg)	Number of 40 mg tablets
2	320	8
Starting dose (1)	240	6
-1	200	5
-2	160	4
-3	120	3

7.2 General Dose Adjustments for Neratinib-Related Toxicities

Dose escalation of neratinib to 320 mg per day **with prophylactic loperamide** is allowed to start at Cycle 2 or subsequent cycles in patients who experienced no intolerable grade 2 or higher treatment-related AEs during the previous cycle(s) of therapy (including diarrhea while receiving prophylactic loperamide). The guideline for diarrhea management outlined in Section 7.3 is to be followed, except that patients will be contacted over the phone within the first week of escalation to 320 mg, and then on a weekly basis thereafter during the first month at the higher dose of neratinib.

Subjects should be withdrawn from the study if the 120-mg dose level of neratinib is not tolerable, or if the subject fails to recover to NCI grade 0 to 1 (or to within baseline of starting values for preexisting laboratory abnormalities) from treatment-related toxicity, leading to treatment delay of >3 weeks. Missed dose(s) of the investigational product will not be made up. Dose adjustments are described below. **Additional clinical situations may result in dose adjustments as clinically indicated** (e.g., intolerable, persistent grade 2 drug-related AE). For general guidelines for the management of diarrhea, refer to Section 7.3. The management of LFT changes is specifically addressed in Section 7.4. For the management of LVEF declines, follow the guidelines in Section 7.5.

Event (Based on NCI CTC4.0)	Action
Pneumonitis/Interstitial Lung Disease: Symptomatic Grade 2 or Grade ≥3	• Symptomatic Grade 2: Hold the investigational product until resolution to ≤ grade 1 or baseline within 3 weeks of stopping the investigational product. Then resume the investigational product at the next lower dose level. • Grade ≥3: Discontinue the investigational product permanently.
Grade 4 (Hematologic or Nonhematologic) (Including nausea and/or vomiting despite optimal medical therapy)	• Hold the investigational product until recovery to ≤grade 1 or baseline within 3 weeks of stopping the investigational product, then resume the investigational product with 2 dose levels reduction.
Other Grade 3 Nonhematologic (Including nausea and/or vomiting despite optimal medical therapy, and asthenia lasting >3 days)	• Hold the investigational product until recovery to ≤grade 1 or baseline within 3 weeks of stopping the investigational product. Then resume the investigational product at the next lower dose level.

7.3 Guidelines for the Management of Diarrhea

Diarrhea is the major DLT of neratinib with onset typically occurring early in the course of treatment (during the first few weeks of treatment). Primary prophylactic use of antidiarrheal medication is **mandatory** for all enrolled subjects receiving neratinib at either 240mg or 320mg daily. Loperamide is the recommended standard therapy to treat diarrhea in this study. If alternative antidiarrheal medication is proposed, this should be discussed with the Principal Investigator and the reason documented in the source documents. Second-line antidiarrheal treatments and adjunctive therapies (i.e., octreotide [SANDOSTATIN®]) are also recommended for use when appropriate.

The investigator must review with the subject the patient instructions for the management of diarrhea and the Medication Diary (Appendix B) for the subject's daily recording of investigational product dose, any adverse reactions, number of stools and use of loperamide and/or other antidiarrheals. Both the subject and the investigator must sign the IRB-approved patient instructions (template is Appendix C) for the management of diarrhea. Copies of both documents are handed to the subject before leaving the site with investigational product on Day 1 with clear instructions to contact the investigator in the event of de novo onset or persistent ≥ grade 2 diarrhea to discuss the appropriate course of treatment. In addition, the Investigator or designee will contact the subject within 24, 48, and 72 hours after the first dose of study drug is administered to inquire about any diarrhea.

Documentation of any occurrences of stools or diarrhea must be as precise as possible and captured in the Medication Diary (Appendix B). For adverse event recording, documentation of "Intermittent" events of diarrhea is limited to grade 1. If events of grade 1 diarrhea are separated

by 3 days without any diarrhea, then each event must be documented as separate adverse events with corresponding start and stop dates.

The entries on the Medication Diary should be reviewed together with the subject at every visit and the data provided on the diary serves as source data for adverse event documentation on the CRF. If the subject has experienced diarrhea since the last visit, details of the daily number of unformed stools provided on the diary help to grade the diarrhea as precisely as possible (per NCI CTC 4.0). Also, daily dose of loperamide (or other antidiarrheals, if applicable) noted on the diary will be reviewed and recorded on the CRF.

Loperamide will be dispensed directly by the site (either as a script or from over the counter) on day 1 with neratinib. It is very important to initiate treatment with loperamide concomitantly with the first dose of neratinib to minimize occurrence and severity of diarrhea.

Prophylactic dosing instructions (Cycles 1 and 2)

- Inform patients that they will experience diarrhea while taking neratinib
- Administer loperamide: initial dose of 4 mg (2 tablets/capsules) with the first dose of neratinib
- For days 1 through 14, administer 4 mg (2 tablets/capsules) three times daily
- For days 15 through 56, administer 4 mg (2 tablets/capsules) twice daily
- If diarrhea occurs despite prophylaxis, treat with additional antidiarrheals, fluids and electrolytes as clinically indicated. NERLYNX dose interruptions and dose reductions may also be required to manage diarrhea
- For patients with persistent grade 1 diarrhea (<4 stools per day above baseline) on loperamide, Lomotil (diphenoxylate hydrochloride and atropine sulfate) 1 tablet (2.5 mg) every 6-8 hours may be added.
- Prophylactic loperamide administration may continue for subsequent cycles at the physician's discretion.
- For grade 2 diarrhea during cycle 1 (4 to 6 stools per day above baseline, despite intensive anti-diarrheal therapy as described above), consider adding octreotide (short-acting) 150 micrograms SC TID; or after initial dose of short-acting octreotide, if well tolerated, a single dose of octreotide LAR 20 mg IM.

For new onset uncomplicated grade 1 or grade 2 diarrhea (in Cycle 2 and beyond)

Dietetic measures

- Stop all lactose-containing products
- Drink 8 to 10 large glasses of clear liquids per day
- Eat frequent small meals
- Recommend low fat regimen enriched with bananas, rice, applesauce and toast (BRAT diet) until resolution of diarrhea.

Pharmacological Treatment

- For days 57 through 365, administer 4mg (2 tablets/capsules) as needed (not to exceed 8 tablets capsules or 16 mg per day)
- For patients with persistent grade 1 diarrhea on loperamide, Lomotil (diphenoxylate hydrochloride and atropine sulfate) 1 tablet (2.5 mg) every 6 hours to 8 hours may be added.
- For grade 2 diarrhea (4 to 6 stools per day above baseline, despite intensive anti-diarrheal therapy), consider adding octreotide (short-acting) 150 micrograms SC TID; or after initial dose of short-acting octreotide, if well tolerated, a single dose of octreotide LAR 20 mg IM.

For grade 3 or grade 4 diarrhea with complicating features (dehydration, fever, and/or grade 3-4 neutropenia

Dietetic measures (same as above)

Pharmacologic treatment

- Administer loperamide: initial dose of 4 mg (2 tablets) with the first bout of diarrhea followed by 2 mg (1 tablet) every 4 hours or after every unformed stool (maximum 16 mg a day) and continue loperamide at this frequency until diarrhea free for 12 hours. Then titrate the amount of loperamide used to keep diarrhea controlled (< 4 stools/day).
- Administer octreotide (SANDOSTAINE®) [100-150 µg SC BID or IV (25-50 µg/h) if dehydration is severe, with dose escalation up to 500 µg SC TID]
- Use intravenous fluids as appropriate
- Consider prophylactic antibiotics as needed (e.g., fluoroquinolones) especially if diarrhea is persistent beyond 24 hours or there is fever or grade 3-4 neutropenia.

Stool cultures should be done to exclude infectious causes of grade 3 or 4 diarrhea or diarrhea of any grade with complicating features (dehydration, fever, and/or grade 3 or 4 neutropenia) per the investigator's discretion. Results from occult blood, fecal leukocyte stain, Clostridium difficile, Campylobacter, Salmonella, and Shigella testing, when performed, should be reported.

Subjects with significant diarrhea who are unresponsive to medical treatment may require treatment interruption or dose reduction.

Gastrointestinal Toxicities Requiring Dose Adjustment of Neratinib

NCI CTCAE V4.0	Action
Grade 1 Diarrhea [Increase of < 4 stools per day over baseline; mild increase in ostomy output compared to baseline.] OR **Grade 2 Diarrhea** [Increase of 4-6 stools per day over baseline; moderate increase in ostomy output compared to baseline;] **lasting ≤ 5 days** OR **Grade 3 Diarrhea** [Increase of ≥7 stools per day over baseline; incontinence; hospitalization indicated; severe increase in ostomy output compared to baseline limiting self-care activities of daily living {ADL};] **lasting ≤ 2 days**	• Adjust anti-diarrheal treatment, as per the guidelines for management of diarrhea at the first onset of diarrhea. • Continue **neratinib** at full dose. • Instruct patient to follow dietetic recommendations in the guidelines for management of diarrhea. • Fluid intake of ~2L should be maintained to avoid dehydration. • Once the event resolved to ≤ grade 1 or baseline, start loperamide 4 mg with each subsequent **neratinib** administration.
Persisting and intolerable **Grade 2 Diarrhea** lasting >5 days despite being treated with optimal medical therapy, or associated with fever, dehydration, **or grade 3-4 neutropenia** OR **Grade 3 Diarrhea** lasting > 2 days despite being treated with optimal medical therapy, or	• Adjust anti-diarrheal treatment, as per the guidelines for management of diarrhea at the first onset of diarrhea. • Hold **neratinib** until recovery to ≤ grade 1 or baseline. • Instruct patient to follow dietetic recommendations of the guidelines for management of diarrhea.

NCI CTCAE V4.0	Action
associated with fever, dehydration, **or grade 3-4 neutropenia** OR **Any Grade 4 diarrhea** [Life-threatening consequences; urgent intervention indicated]	• Fluid intake of ~2L should be maintained, intravenously if needed. • If recovery occurs: o ≤1 week after withholding treatment, resume same dose of **neratinib**. o Within 1-3 weeks after withholding treatment, reduce **neratinib** dose to the next lower dose level. • If event recurs and the **neratinib** dose has not already been decreased, reduce **neratinib** dose to the next lower dose level. • If subsequent events occur, reduce **neratinib** dose to the next lower dose level. • Once the event resolved to ≤ grade 1 or baseline, start loperamide 4 mg with each subsequent **neratinib** administration. • If event recurs and the **neratinib** dose has already been reduced at the lowest level, discontinue **neratinib**.

7.4 Guidelines for the Management of Changes in Liver Function Tests

Changes in LFTs have been reported in subjects taking neratinib. Subjects experiencing grade 3 or 4 diarrhea requiring IV hydration and associated with any signs or symptoms of hepatotoxicity such as worsening of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash or eosinophilia should be evaluated for LFT changes.

Abnormal values in aspartate transaminase (AST) and/or alanine transaminase (ALT) concurrent with abnormal elevations in total bilirubin that meet the criteria in the table below in the absence of other causes of liver injury are considered potential cases of drug-induced liver injury (potential Hy's Law cases) and should always be considered important medical events.

The possibility of hepatic neoplasia (primary or secondary) should also be considered.

During evaluation of potential hepatotoxicity, bilirubin should be fractionated and prothrombin time should be measured.

Also, liver imaging should be obtained for subjects with any signs or symptoms of hepatotoxicity and/or grade 3 or greater LFT elevations, or as clinically indicated.

Event (Based on NCI CTC4.0)	Action
Grade 3 ALT (>5-20x ULN), **or** Grade 4 ALT (>20x ULN), **or** Grade 3 Bili (>3-10x ULN) and direct bili ≥ 35% of total bili, **or** Grade 4 Bili (>10x ULN) and direct bili ≥ 35% of total bili.	• Permanently discontinue investigational product (IP) • Evaluate alternative causes.
ALT > 3x ULN **and** Total bilirubin > 2x ULN **and** Alkphos < 2x ULN.	• Hold IP and immediately contact the PI to discuss next steps, including evaluation of alternative causes, and management of IP. • Report as SAE.
Signs or symptoms related to liver injury (abdominal pain, fever, jaundice, rash, eosinophilia, or drop in performance status) with either: Grade 2 ALT (>3-5x ULN), **or** Grade 2 ALT (>3-5x ULN) **and** Grade 2 Bili (>1.5-3x ULN) and direct bili ≥ 35% of total bili.	• Permanently discontinue IP. • Evaluate alternative causes.

7.5 Guidelines for the Management of LVEF Declines

LVEF assessments will be performed at the completion of every four cycles during the treatment period. It is strongly recommended to use the same method of cardiac evaluation (ECHO or MUGA) at each time point for each subject.

In case of symptomatic heart failure, the investigational product must be discontinued permanently.

Asymptomatic decline in LVEF that warrants intervention is defined as:

- LVEF < LLN AND absolute decrease in LVEF of ≥ 10% compared with pre-treatment LVEF, OR
- Absolute decrease in LVEF of ≥ 15% compared with pre-treatment LVEF

If asymptomatic decline in LVEF that warrants intervention is experienced, then:

- Temporarily HOLD neratinib and
- Repeat LVEF in 3-4 weeks

If asymptomatic decline in LVEF as defined above persists, permanently discontinue neratinib, repeat LVEF in 3-4 weeks and consider cardiology consult. If LVEF recovers to ≥ LLN and absolute decrease in LVEF of resolves to < 15%, resume neratinib at the same dose, and resume LVEF monitoring per this protocol.

If a subject has a second episode of asymptomatic decline in LVEF that meets either of the above criteria, permanently discontinue neratinib, repeat LVEF in 3-4 weeks and consider cardiology consult.

Note that, for adverse events other than asymptomatic LVEF decline, if neratinib is held for > 3 weeks, subject should be withdrawn from the treatment phase of the study. In case of asymptomatic LVEF decline, subjects may resume neratinib within one week after LVEF recovery is documented as above, even if the timeframe exceeds 3 weeks. If a site does not provide normal ranges for ECHO or MUGA, the LLN should be defaulted to 50%.

7.6 Subject Compliance

Compliance is monitored by study personnel at the site by using subject-completed diaries and verbal information from the subject that is recorded on source documents, the drug inventory record, and CRFs. A subject must have taken the prescribed investigational product dosage 75% of the days in the treatment period to be compliant. Dose adjustments must follow the recommendations in this section.

Site personnel will review subject-completed diaries at every visit and will document on drug accountability forms provided to the sites. Dose administration will be recorded on sponsor drug accountability forms.

7.7 Fulvestrant Dose Modifications

There are no planned dose modifications for fulvestrant. Dosing may be modified or discontinued at the discretion of the treating physician.

7.8 Trastuzumab (or FDA Approved Biosimilar) Dose Modifications

There are no planned dose modifications for trastuzumab (or FDA approved biosimilar). Dosing may be modified or discontinued at the discretion of the treating physician.

8.0 REGULATORY AND REPORTING REQUIREMENTS

The entities providing oversight of safety and compliance with the protocol require reporting as outline below. Please refer to Appendix E for definitions and Appendix F for a grid of reporting timelines.

Adverse events will be tracked from start of treatment up to 28 days following the last day of study treatment. All adverse events must be recorded on the toxicity tracking case report form (CRF) with the exception of:

- Baseline adverse events, which shall be recorded on the medical history CRF

Refer to the data submission schedule in Section 12 for instructions on the collection of AEs in the EDC.The Washington University Human Research Protection Office (HRPO) requires that all events meeting the definition of unanticipated problem or serious noncompliance be reported as outlined in Section 8.1.5 and 8.1.6.

The FDA requires that all serious and unexpected adverse events considered related to the study drug be reported as outlined in Section 8.6. In addition, any fatal or life-threatening adverse experiences where there is a reasonable possibility of relationship to study intervention must be reported.

Puma Biotechnology requires that all adverse events be reported as outlined in Section 8.7.

8.1 Reporting to the Human Research Protection Office (HRPO) at Washington University

Reporting will be conducted in accordance with Washington University IRB Policies.

Pre-approval of all protocol exceptions must be obtained prior to implementing the change.

8.2 Reporting to the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The Sponsor-Investigator (or designee) is required to notify the QASMC of any unanticipated problem involving risks to participants or others occurring at WU or any BJH or SLCH institution that has been reported to and acknowledged by HRPO. Unanticipated problems reported to HRPO and withdrawn during the review process need not be reported to QASMC.

QASMC must be notified within **10 days** of receipt of IRB acknowledgment via email to qasmc@wustl.edu. Submission to QASMC must include the MyIRB form and any supporting documentation sent with the form.

For events that occur at secondary sites, the Washington University Sponsor Investigator (or designee) is required to notify the QASMC within 10 days of Washington University notification via email to qasmc@wustl.edu. Submission to QASMC must include either the myIRB form and supporting documentation or (if not submitted to myIRB) the date of occurrence, description of the event, whether the event is described in the currently IRB approved materials, the event outcome, determination of relatedness, whether currently enrolled participants will be notified, and whether the informed consent document and/or any study procedures will be modified as a result of this event.

8.3 Secondary Sites Reporting Requirements

The research team at each secondary site is required to promptly notify the Washington University Sponsor-Investigator and designee of all serious adverse events (refer to Appendix E) **within 1 working day** of the occurrence of the event or notification of the secondary site's PI of the event. This notification may take place via email if there is not yet enough information for a formal written report (using FDA Form 3500a (MedWatch) and Washington University's cover sheet (Appendix G). A formal written report must be sent to the Washington University Sponsor-Investigator and designee **within 4 calendar days** (for fatal or life-threatening suspected adverse reactions) or **11 calendar days** (for serious unexpected suspected adverse reactions) of the occurrence of the event or notification of the secondary site's PI of the event.

The research team at a secondary site is responsible for following its site's guidelines for reporting applicable events to its site's IRB according to its own institutional guidelines. The research team at Washington University is responsible for reporting all applicable events to the FDA, IBC, and Puma Biotechnology, Inc. as needed.

Washington University pre-approval of all protocol exceptions must be obtained prior to implementing the change. Local IRB approval must be obtained as per local guidelines. Washington University IRB approval is not required for protocol exceptions occurring at secondary sites.

8.4 Reporting to Secondary Sites

The Washington University Sponsor-Investigator (or designee) will notify the research team at each secondary site of all reportable events that have occurred at other sites within **10 working days** of the occurrence of the event or notification of the Sponsor-Investigator of the event. This includes events that take place both at Washington University and at other secondary sites, if applicable. Refer to Section 16.0 (Multicenter Management) for more information.

8.5 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. **PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC.** It is the responsibility of the Washington University principal investigator to report to the FDA as follows:

- Report any unexpected fatal or life-threatening suspected adverse experiences (Refer to Appendix E for definitions) no later than **7 calendar days** after initial receipt of the information.

- Report a suspected adverse reaction that is both serious and unexpected (SUSAR, refer to Appendix E) no later than **15 calendar days** after it is determined that the information qualifies for reporting. Report an adverse event (refer to Appendix E) as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event, such as:
  - A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure
  - One or more occurrences of an event that is not commonly associated with drug exposure but is otherwise uncommon in the population exposed to the drug
  - An aggregate analysis of specific events observed in a clinical trial that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group
- Report any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies that suggest a significant risk in humans exposed to the drug no later than **15 calendar days** after it is determined that the information qualifies for reporting.
- Report any findings from animal or in vitro testing that suggest significant risk in humans exposed to the drug no later than **15 calendar days** after it is determined that the information qualifies for reporting.
- Report any clinically important increase in the rate of a serious suspected adverse reaction of that listed in the protocol or IB within **15 calendar days** after it is determined that the information qualifies for reporting.

Submit each report as an IND safety report in a narrative format or on FDA Form 3500A or in an electronic format that FDA can process, review, and archive. Study teams must notify the Siteman Cancer Center Protocol Development team of each potentially reportable event within 1 business day after initial receipt of the information, and must bring the signed 1571 and FDA Form 3500A to the Siteman Cancer Center Protocol Development team no later than 1 business day prior to the due date for reporting to the FDA.

Each notification to FDA must bear prominent identification of its contents ("IND Safety Report") and must be transmitted to the review division in the Center for Drug Evaluation

and Research (CDER) or in the Center for Biologics Evaluation and Research (CBER) that has responsibility for review of the IND. Relevant follow-up information to an IND safety report must be submitted as soon as the information is available and must be identified as such ("Follow-up IND Safety Report").

8.6 Reporting to Puma Biotechnology, Inc.

Any serious adverse events which occur during the clinical study or within 28 days of receiving the last dose of study medication, whether or not related to the study drug, must be reported by the investigator. In addition, any SAEs which occur as a result of protocol specific diagnostic procedures or interventions must also be reported.

All serious adverse events, in addition to being reported to the FDA by the investigator, must be reported by email to **Puma Biotechnology, Inc.** as follows:

For expedited reports, (Site) will send the MEDWATCH report to the Company no later than seven (7) days for initial life-threatening and death reports, and fifteen (15) days for all other initial or follow-up serious and unexpected suspected adverse reaction (SUSAR) as assessed by the investigator for causality and by the Investigator-Sponsor for causality and expectedness based on the adverse reaction table in the most current Investigator Brochure, from the time of receipt of the SAE by (Site). For non-expedited reports (i.e., unrelated to study drugs or listed/expected event), (Site) will send the MEDWATCH report to the Company no later than thirty (30) days from the time of receipt of the SAE by (Site).

By e-mail to PumaSAE@parexel.com

SAEs brought to the attention of the investigator at any time after cessation of neratinib and considered by the investigator to be related or possibly related to neratinib must be reported to **Puma Biotechnology, Inc.** if and when they occur. Additionally, in order to fulfill international reporting obligations, SAEs that are related to study participation (e.g., procedures, invasive tests, changes from existing therapy) or are related to a concurrent medication will be collected and recorded from the time the subject consents to participate in the study until he/she is discharged from the study.

8.7 Timeframe for Reporting Required Events

Adverse events will be tracked for 28 days following the last day of study treatment.

9.0 PHARMACEUTICAL INFORMATION

9.1 Neratinib

9.1.1 Description

Neratinib (PB-272) is a potent irreversible pan erbB inhibitor. Neratinib is an orally available small molecule that inhibits erbB-1, erbB-2, and erbB-4 at the intracellular tyrosine kinase domains, a mechanism of action that is different from trastuzumab.

9.1.2 Clinical Pharmacology

Please refer to section 1.2.3.

9.1.3 Supplier(s)

Neratinib will be provided free of charge by **Puma Biotechnology, Inc**.

9.1.4 Dosage Form and Preparation

Neratinib 40-mg tablets will be supplied by the sponsor. There are 210 pills per bottle. Repackaging of product is not allowed.

9.1.5 Storage and Stability

Investigational products will be stored by sites in a secure location at the storage conditions listed on the label; store at 25°C (77°F) or below with desiccant (bottle contains the dessicant); do not freeze. Correspondingly, subjects must be instructed to store investigational products in a safe place at these same storage conditions. Storage in a refrigerator or cold room is acceptable as long as the temperature remains above 0 °C.

The investigational product must be stored as indicated. Deviations from the storage requirements, including any actions taken, must be documented and reported to the sponsor. Once a deviation is identified, the investigational product must be quarantined and not used until the sponsor provides documentation of permission to use the investigational product.

The investigational product is only to be administered to subjects who have provided informed consent. Once the investigational product has been assigned to a subject it must not be reassigned to another subject.

9.1.6 Administration

The number of tablets of neratinib taken will depend on the prescribed dose per the dose administration table. The investigational product will be taken by mouth with food, preferably in the morning.

9.1.7 Special Handling Instructions

None.

9.2 Fulvestrant

9.2.1 Description

Fulvestrant (Faslodex) is an estrogen receptor antagonist which down-regulates the ER protein in breast cancer cells.

9.2.2 Availability

Fulvestrant injection is commercially available as a sterile single-patient pre-filled syringe containing 250 mg at a concentration of 50 mg/ml. The solution is a clear, colorless to yellow, viscous liquid. In addition to the fulvestrant, each injection contains as inactive ingredients alcohol, USP, benzyl alcohol, NF, and benzyl benzoate, USP as co-solvents and castor oil, USP as a co-solvent and release rate modifier.

For patients who cannot obtain fulvestrant commercially, such as those who are without medical insurance or with a limited income, AstraZeneca Foundation Patient Assistance Program can be reached at 1-800-424-3727 or by downloading a PDF application from the AstraZeneca US web site at www.astrazeneca-us.com. Completed applications should be mailed to:

AstraZeneca Foundation Patient Assistance Program
PO Box 66551
St. Louis, MO 63166-66551

9.2.3 Storage and Stability

The syringes of fulvestrant for all cycles of treatment should be stored in the original container and refrigerated at 2°- 8°C (36°- 46°F).

9.2.4 Preparation

Remove glass syringe barrel from tray and check that it is not damaged. Peel open the safety needle (SafetyGlide™) outer packaging. Break the seal of the white plastic cover on the syringe luer connector to remove the cover with the attached rubber tip cap. Twist to lock the needle to the luer connector. Remove needle sheath. Remove excess air from the syringe (a small gas bubble may remain).

9.2.5 Administration

Fulvestrant will be administered at a dose of 500 mg (2 x 250 mg injections) IM on Cycle 1 Day 1 and Day 15, then on Day 1 of each subsequent cycle. Administer intramuscularly slowly in the buttock. (NOTE: 500 mg dose will require one 250 mg injection in each buttock.) Immediately activate needle protection device upon withdrawal from patient by pushing lever arm completely forward until needle tip is fully covered. Visually confirm that the lever arm has fully advanced and the needle tip is covered. If unable to activate, discard immediately into an approved sharps container.

9.2.6 Drug Interactions

In vitro studies using human hepatocytes, fulvestrant was metabolized predominantly by conjugation. The metabolites thus formed are thought to possess no estrogenic activity and minimal anti-estrogenic activity. In studies using human liver microsomes, fulvestrant inhibited the activity of CYP1A2, 2C9 and 3A4 minimally. CYP3A4 did metabolize fulvestrant in these studies, but the human hepatocyte studies noted above indicate conjugation is a more important metabolic pathway. In addition, studies in healthy volunteers indicate that fulvestrant metabolism is not significantly affected by inducers or inhibitors of CYP3A4, nor does fulvestrant affect the metabolism of 3A4 substrates. Thus, fulvestrant is not expected to be involved in significant drug interactions mediated by CYP3A4.

10.0 CORRELATIVE STUDIES

10.1 Required archival tumor sample for research:

- Archival tumor specimens that are left over from the HER2 mutation analysis at Washington University are not returned and will be used for investigations of potential therapeutic targets in HER2-negative breast cancer.
- Additional archival tumor specimens may be requested for studies that compare mutations in primary versus metastatic disease sites, and predictors of treatment response to neratinib.
- For patients who had *HER2* mutation identified in another laboratory and registered to the study, please submit archival tumor specimens to Wash U Alliance/ACOSOG-CSB/TPC.
- Please refer to the Correlative Science Procedure Manual **S**ection 3.1 for sample requirement and shipping instructions.

10.2 Required research blood collection

Peripheral blood samples will be collected in patients who have *HER2* mutation and are registered to receive study drug therapy. Blood will be collected at baseline, during therapy as well as at disease progression (Also refer to the **Correlative Science Calendar Section 10.5**), for **ctDNA sequencing** and other circulating markers that may affect disease outcome. **Please ship samples to Wash U Alliance/ACOSOG-CSB/TPC.**

Please refer to the Correlative Science Procedure Manual Section 3.2 for sample requirement and shipping instructions.

10.3 Optional research tumor biopsy (kits will be provided):

If a patient is registered to this trial to receive neratinib and has tumors that are safe for biopsy, we encourage consenting for tumor collection prior to study drug administration and/or at the time of disease progression on neratinib for investigations that assess predictors of response to therapy and mechanisms of resistance.

Note that the tumor biopsy collected at disease progression on study treatment will be subjected to the CLIA NGS panel of 122 genes (the Comprehensive Cancer Gene Set, verion 3.2 (CCGSv3.2)), at the Wash U GPS and a clinical report will be issued to the site investigator to provide to the treating physician and the patient within 3-4 weeks of receiving the specimen. The sequencing report will provide interpretations of genomic findings, but it is at the discretion of the treating physician and the patient in regards to any need for referral to clinical genetic counseling service. The report may be helpful in assessing patient eligibility for clinical trials that require mutation screening.

Please refer to the Correlative Science Procedure Manual Section 3.3 in regards to sample requirements and shipping instructions.

10.4 Reporting of a CLIA multi-gene NGS on tumor biopsy collected at disease progression on study drug:

This will follow a full clinical workflow including evaluation of the FFPE biopsy specimen by a qualified surgical pathologist (WU Anatomic and Molecular Pathology Core lab), extraction of DNA, NGS library preparation and sequencing, NGS data analysis and variant interpretation for the CCGSv3.2 Solid Tumor gene set (122 genes). Cases with negative results (no level 1 or 2 variants identified) will proceed to additional reflex testing for the hematopoietic disorders gene set (54 genes).

10.5 Correlative Science Study Calendar

Specimen Type	Collection/Submission Time Point				Shipping Condition	Ship To
	Pre-registration	Pre-treatment / C1D1 (prior to starting treatment)	C1D15, C2D1, C3D1 and day 1 of each odd cycle	EOT / Prog		
Archival Tissue for HER2 mutation testing (see CSPM 1.0)	X				Ambient	GPS
10 ml Whole Blood in EDTA for germline DNA sequencing (see CSPM 2.0)	X[1]				Ambient	GPS
Archival Tissue for correlative studies (See CSPM 3.0)		X[2]			Ambient	TPC
10 ml Whole Blood in EDTA tube		X			Ambient	TPC
3 ml plasma (from 10 ml EDTA tube)		X	X	X	Frozen[3]	TPC
3 ml serum (from 10 ml SST tube)		X	X	X	Frozen[3]	TPC
(2) 8 ml Streck tubes		X	X	X	Ambient	TPC
Optional Fresh Biopsies (see CSPM 3.3)		X		X	Frozen and Ambient	TPC

[1] Only required if notified by WUSM study team

[2] FFPE tumor rich block/slides are required – due within 60 days of registration for patients who receive treatment on trial.

[3]Store frozen samples at -70ºC to -80ºC. Ship frozen samples quarterly on dry ice.

CSPM: Correlative Science Procedure Manual

11.0 STUDY CALENDAR

Evaluations for pre-registration should be conducted within 8 weeks prior to pre-registration and specimen submission. Evaluations for registration should be conducted within 2 weeks prior to registration, with the following exceptions: echocardiogram, scans, and x-rays, which should be performed within 4 weeks of registration. Study drug therapy should start within 7 days of registration.

Screening Through Cycle 1											
	Pre-Screening[W] -56 to 0 days	**Pre-Registration**	**Screening** -14 to 0 days unless otherwise noted	**Registration**	**C1D1** (- 7)	**C1D2**	**C1D3**	**C1D4**	**C1D8** (+/- 1)	**C1D15** (+/- 1)	**C1D22** (+/- 1)
Informed Consent	X		X[V]								
Medical History	X		X								
Physical Exam w/ECOG	X[I]		X		X				X	X	X
Vital Signs[A]			X		X				X[L]	X[L]	X[L]
Safety Phone Call[K]						X	X	X			
Adverse Events					X----------						----------X
CBC w/Differential	X		X		X[M]						
Blood Chemistry	X		X		X[M]						
Pregnancy Test[H]			X								
Blood for Research[S]					X					X	
ECG			X								
Echocardiogram or MUGA[C]			X[E]								
Tumor Assessment (CT or MRI)[D]			X[E]								
Tumor Biopsy (optional)			X[O]								
Archival Tumor	X[U]		X[T]								
HER2 Mutation Report			X[B]								
Patient Instructions for Diarrhea Management (Appendix C)					X						
Neratinib[N]					X----------						----------X
Fulvestrant[N, R]					X					X	
Medication Diary (Appendix B)					X----------						----------X
Concomitant Medications			X		X----------						----------X
Serious Adverse Events	X[J]		X[J]		X----------						----------X

Cycle 2 Through End of Study						
	C2D1 (+/- 3)	**C3D1** (+/- 3)	**Day 1 of Subsequent Even Cycles** (+/- 3)	**Day 1 of Subsequent Odd Cycles** (+/- 3)	**End of Treatment** (+ 7)	**30 Day Follow Up** (+/- 3)
Physical Exam w/ECOG	X	X	X	X	X	
Vital Signs	X	X	X	X	X	
Adverse Events	X----------					----------X
Follow Up Phone Call						X
CBC w/Differential	X	X	X	X	X	
Blood Chemistry	X	X	X	X	X	
Blood for Research[S]	X	X		X		
Tumor Assessment (CT or MRI)[D]		X[F]		X[F]		
Tumor Biopsy (optional)					X[P]	
Medication Diary (Appendix B)						
Neratinib[G]	X----------					----------X
Fulvestrant[R]	X	X	X	X		
Trastuzumab (or FDA approved biosimilar)[Q]		X----------		----------X		
Concomitant Medications	X----------					----------X
Serious Adverse Events	X----------					----------X

A: Height to be recorded only at baseline

B: The sequencing report identifying the eligible HER2 mutation is required.

C: Pre-registration and HER2 mutation testing, ECHO or MUGA is not required, but a patient with a known recent LVEF < LLN or with symptoms of congestive heart failure is not eligible for Pre-registration and HER2 mutation testing. ECHO or MUGA is required for all patients continuing to the treatment portion of the trial.

D: CT of the chest, abdomen and pelvis with contrast is required for the study. If contrast could not be administered, CT of the chest without contrast, and MRI of abdomen and pelvis would be acceptable. The same method should be used at each evaluation. Patients with skin lesions should have photographs taken at baseline and each evaluation. Please have a CD copy of each radiology assessment at the completion of the exam or upload the image in the "drop box" specified in the secondary site instructions. Please use either the tumor measurement form in Appendix D for RECIST 1.1 response evaluation or an institutional RECIST 1.1 report for upload to the EDC.

E: Within 28 days prior to Registration.

F: Radiology test for tumor response assessment is to be performed at baseline and every 2 cycles (+/- 7 days).

G: Neratinib is to be self-administered daily on each day of every 28-day cycle (+/- 3 days). Please see Section 6.1 for dosing instructions.

H: Premenopausal women of childbearing potential only

I: Only performance status is required

J: Determined to be related to screening procedures.

K: Safety calls to the patient are to be conducted 24, 48, and 72 hours after first dose administration and can be performed by study coordinator.

L: Physical exam on C1D8, C1D15, and C1D22 may be performed by MD or research NP or RN.

M: Cycle 1 Day 1 labs do not need to be repeated if done within 7 days of treatment.

N: To accommodate calls to patients at 24, 48, and 72 hours post-drug initiation, the first dose of neratinib +/- fulvestrant could be started any time within one week following the C1D1 visit.

O: Optional baseline biopsy must be performed within 4 weeks before C1D1 or on C1D1 prior to starting neratinib +/- fulvestrant. Please refer to Correlative Science Procedure Manual

P: The biopsy at progression must occur prior to starting the next line of cancer therapy. Note that a clinical sequencing report will be provided to the treating physician in 3-4 weeks from Wash U. Please refer to the Correlative Science Procedure Manual.

Q: May be administered if patient progresses on neratinib +/- fulvestrant; refer to Section 6.4.3 for dosing and schedule. The +/- 3 day window applies to all treatment days. Trastuzumab or any of the FDA approved commercially available biosimilar products for trastuzumab may be administered to patients based on their insurance requirements or institutional practice.

R: In Part II ER+ cohorts, fulvestrant will be administered at a dose of 500 mg (2 x 250 mg injections) IM on Days 1 and 15 of Cycle 1, then on Day 1 of each subsequent cycle. Please see Section 6.3 for dosing instructions. Patients already on fulvestrant 500mg every 28 days prior to registration to the fulvestrant-treated cohort do not need to receive the loading dose on C1D15. In these patients, it is acceptable that C1D1 neratinib does not coincide with fulvestrant administration schedule per discretion of treating physician.

S: Research blood is collected at baseline, on Cycle 1 day 15, Cycle 2 day 1, on Cycle 3 Day 1, followed by Day 1 of every other cycles (including Cycles 5, 7, etc), and EOT. Please refer to Correlative Science Procedure Manual.

T: Due within 60 days of Registration. If sample was submitted for HER2 sequencing after pre-registration a new block may not be required. See Correlative Science Procedure Manual.

U: See Correlative Science Procedure Manual for instructions for submitting archival tissue for HER2 sequencing

V: Consent can be obtained earlier than the 14 day screening window

W: Patients with known HER2 mutations (see Section 5.2) are not required to complete pre-screening and pre-registration.

12.0 DATA SUBMISSION SCHEDULE

Case report forms with appropriate source documentation will be completed within 60 days of the schedule listed in this section.

Electronic data management systems will be used in this trial in collaboration with the Institute for Informatics (I^2) at Washington University. REDCap is a web-based clinical studies data management system that will be used for capture of clinical data from this trial. The case report forms developed for this trial will be transformed to electronic format. An electronic study calendar will drive the study's data collection workflow. Each center has access only to data from its own participants. Washington University, as the data coordinating center, has access to data from all sites. Contact the WUSTL study coordinator for instructions on how to request access.

Case Report Form	**Submission Schedule**
Only for patients being screened for HER2 mutation at Washington University Pre-registration Consent Form Pathology Source Documents	Prior to HER2 mutation testing (send to main site registrar to confirm eligibility)
Biospecimen Submission Form (CSPM Appendix 2) GPS Submission Form (CSPM Appendix 1)	Sent with tumor sample
Biospecimen Submission Form (CPSM Appendix 2)	Sent with peripheral blood
Consent Form Pathology Source Documents (only for patients who were previously screened for HER2 mutation at outside location) Eligibility Checklist	Prior to starting treatment
Within REDCap	
Pre-Study Form Prior Therapy Form Vital Signs Form CBC Form Chemistries Form Tissue Collection Form	Enter prior to treatment start
Vital Signs Form CBC Form Chemistries Form Treatment Form	Enter within 2 week of the end of each cycle
Peripheral Blood Collection Form	Due within 2 weeks of collection
Toxicity Form Concomitant Medications Form	Continuous
Treatment Summary Form	Completion of treatment
Follow Up Form	30 Day follow up
Tumor Measurement Form	Baseline End of every other cycle End of treatment
MedWatch Form	See Section 8.0 for reporting requirements

Any queries generated by Washington University must be responded to within 14 days of receipt by the participating site. The Washington University research team will conduct a regular review of data status at all secondary sites, with appropriate corrective action to be requested as needed.

13.0 MEASUREMENT OF EFFECT

13.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every 8 weeks (2 cycles).

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). 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.

13.2 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as >20 mm by chest x-ray, as >10 mm with CT scan, or >10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Tumor lesions from a previously irradiated area may only be used as index lesions if they have demonstrated clear progression since completion of the radiation.

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

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

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

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

Target lesions: All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the

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

Non-target lesions: All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

13.3 Methods for Evaluation of Measurable Disease

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

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

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

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

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

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

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

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

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

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

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

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

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

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

- 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.

13.4 Response Criteria

13.4.1 Evaluation of Target Lesions

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

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

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

13.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

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

13.4.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	>4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	>4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once >4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.
Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "symptomatic deterioration." Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

13.4.4 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.

14.0 DATA AND SAFETY MONITORING

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, an independent Data and Safety Monitoring Board (DSMB) will be specifically convened for this trial to review toxicity data. A DSMB will consist of no fewer than 3 members including 2 clinical investigators and a biostatistician. DSMB members must be employed by Washington University, Barnes-Jewish Hospital, or St. Louis Children's Hospital. Like investigators, DSMB members are subject to the Washington University School of Medicine policies regarding standards of conduct. Individuals invited to serve on the DSMB will disclose any potential conflicts of interest to the trial principal investigator and/or appropriate university officials, in accordance with institution policies. Potential conflicts that develop during a trial or a member's tenure on a DSMB must also be disclosed.

Until such a time as the first secondary site enrolls its first patient, a semi-annual DSM report to be prepared by the study team will be submitted to the Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months after study activation at Washington University (if at least one patient has been enrolled) or one year after study activation (if no patients have been enrolled at the six-month mark).

The DSM report for the DSMB will be prepared by the study team with assistance from the study statistician, will be reviewed by the DSMB, and will be submitted to the QASM Committee. The DSMB must meet at least every six months beginning six months after study activation at Washington University or six months after enrollment of the first patient at a secondary site, no more than one month prior to the due date of the DSM report to QASMC. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual including numbers from participating sites
- Protocol activation date at each participating site
- Average rate of accrual observed in year 1, year 2, and subsequent years at each participating site
- Expected accrual end date, accrual by site
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities at all participating sites
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

Further DSMB responsibilities are described in the DSMB charter.

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

Refer to the Washington University Quality Assurance and Data Safety Monitoring Committee Policies and Procedures for full details on the responsibilities of the DSMC. This is located on the QASMC website at https://siteman.wustl.edu/research/clinical-research-resources/protocol-office-prmcqasmc/

14.1 Independent Research Monitor

Dr. Brian Van Tine, Associate Professor of Medicine and Sarcoma Program Director at Washington University School of Medicine, will serve as the independent research monitor for the trial.

The independent research monitor may discuss the research protocol with the investigators, interview human subjects, and consult with others outside of the study about the research. The research monitor shall have authority to stop the research protocol in progress, remove individual human subjects from the research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report. The research monitor has the responsibility to promptly report his/her findings to the IRB or other designated official and to the US Army Medical Research and Materiel Command, Office of Research Protections, Human Research Protection Office.

In addition, the independent research monitor is required to review all unanticipated problems involving risk to subjects or others, serious adverse events, and all subject deaths associated with

the protocol and provide an unbiased written report of the event. At a minimum, the independent research monitor must comment on the outcomes of the event or problem and in case of a serious adverse event or death, comment on the relationship to participation in the study. The independent research monitor must also indicate whether he/she concurs with the details of the report provided by the principal investigator.

15.0 AUDITING

Since Washington University is the coordinating center, each site will be audited annually by Siteman Cancer Center personnel (QASMC) unless the outside institution has an auditing mechanism in place and can provide a report. The outside sites will be asked to send copies of all audit materials, including source documentation. The audit notification will be sent to the Washington University Research Patient Coordinator, who will obtain the audit materials from the participating institution.

Notification of an upcoming audit will be sent to the research team one month ahead of the audit. Once accrual numbers are confirmed, and approximately 30 days prior to the audit, a list of the cases selected for review (up to 10 for each site) will be sent to the research team. However, if during the audit the need arises to review cases not initially selected, the research team will be asked to provide the additional charts within two working days.

Additional details regarding the Auditing Policies and procedures can be found at https://siteman.wustl.edu/wp-content/uploads/2015/QASMC-Policies-and-Procedures-03.31.2015.pdf

16.0 STATISTICAL CONSIDERATIONS

16.1 Study Objectives

16.1.1 Primary Objectives

1. To determine the clinical benefit (CR+PR+SD$\geq$6 months) of neratinib alone in patients with metastatic HER2- breast cancer that carry HER2 mutations (Part I)
2. To examine the clinical benefit (CR+PR+SD$\geq$6 months) of neratinib in combination with fulvestrant in patients with fulvestrant naïve metastatic HER2-, ER+ breast cancer carrying activating HER2 mutations (Part II fulvestrant naïve ER+ cohort)
3. To examine the clinical benefit (CR+PR+SD$\geq$6 months) of neratinib in combination with fulvestrant in patients with metastatic HER2-, ER+ breast cancer carrying activating HER2 mutations previously treated with fulvestrant (Part II fulvestrant treated ER+ cohort)
4. To determine the clinical benefit rate of neratinib alone in patients with metastatic HER2-, ER- breast cancer carrying activating HER2 mutations (Part II ER- cohort)

16.1.2 Secondary Objectives

1. To determine the PFS of patients treated with neratinib alone in patients with metastatic HER2-, but HER2 mutated breast cancers by ER status and by HER2 mutations (activating vs unknown significance)

2. To assess the PFS and RR of neratinib in combination with fulvestrant in patients with fulvestrant naïve metastatic ER+ HER2- breast cancer carrying activating HER2 mutations
3. To assess the PFS and response rate for neratinib in combination with fulvestrant in patients with metastatic HER2-, ER+ breast cancer carrying activating HER2 mutations previously treated with fulvestrant
4. To correlate the presence of HER2 mutation with histology subtype (invasive lobular vs. invasive ductal cancer), tumor grade (grade 1-2 vs 3), tumor staging at initial diagnosis (I vs. II or III vs. IV), disease free survival in HER2- breast cancer
5. To assess the safety profile and tolerability of neratinib in combination with fulvestrant in patients with metastatic ER+, HER2- breast cancer carrying activating HER2 mutations

16.1.3 Exploratory Objectives

1. To compare the occurrence of HER2 mutation in paired primary and metastatic sites.
2. To collect peripheral blood plasma samples for circulating HER2 mutation analysis.
3. To investigate other potential therapeutic targets in HER2- breast cancer.
4. To explore potential mechanisms of treatment resistance.
5. To explore anti-tumor response of trastuzumab in combination with neratinib alone or neratinib and fulvestrant combination when tumor progresses on single agent neratinib or neratinib and fulvestrant combination, respectively.

16.2 Study Design and Sample Size Justification

Sample size justification for Part I of the study with neratinib alone in metastatic HER2- breast cancer carrying HER2 mutations:

Patients with any somatic HER2 mutations identified are eligible for the study. Based on Simon's Optimal 2-stage design, our ultimate goal is to enroll a total of 29 patients with HER2 mutations. This will allow us 80% power at a 1-sided 0.05 significance level to detect an anticipated 20% clinical benefit rate against the null hypothesis of 5%. Ten patients will be enrolled in the first stage. Accrual will continue until 10 patients are considered evaluable for clinical benefit, at which time a decision will be made whether to continue enrollment to the 2nd stage. If none of the 10 patients in the first stage had clinical benefit, we will continue the trial until we enroll 10 patients with known activating mutations by preclinical studies. If 1 or more of the 10 patients in the first stage have shown clinical benefit, a total of 19 patients (with or without known activating mutations, depending on the enrollment criterion in stage 1) will be enrolled in the 2nd stage. In a multicenter study such as this, however, it is difficult for a trial to reach the planned sample exactly. The investigators would not turn away patients who have already been approached for participation just because the number of patients needed has been met. Therefore, we adopted a flexible Phase II design (Chen and Ng 1998) to allow the actual number at final stage to deviate slightly from what is planned. That is, if 4 or more cases with clinical benefit are observed out of 29-34 patients, or 5 or more cases with clinical benefit are observed out of 35 or more patients, we would conclude that preliminary evidence for efficacy exists and that further investigation of the treatment is warranted.

As of Jan 17th 2015, 10 evaluable patients with HER2 mutations, including 9 with activating mutations, were enrolled in the study. Among these patients, 1 patient with activating mutation

derived clinical benefit with a partial response. However, since majority of patients (15 of 16) found to have HER2 mutations in the study had ER+ breast cancer, we propose to add fulvestrant to neratinib in the ER+ population in the second part of the study to evaluate the overall response rate for this combination. The first part of the trial will close once the amendment (amendment 7) for the second part of the trial is approved. As of August 25th, 2015, amendment 7 has been approved by all participating institutions; therefore enrollment is only open to the second part trial since that date.

Sample size justification for the second part of the trial with neratinib alone in patients with metastatic ER- HER2- breast cancer carrying HER2 mutations: We will enroll 10 patients. This will provide us a reasonable precision to estimate the clinical benefit (CR+PR+SD≥6 months) of neratinib alone in patients with metastatic ER-/HER2- breast cancer that carry HER2 mutations. If 2 clinical benefits are observed out of 10 patients, for example, we will have 80% confidence that the true rate will be above 5%. Conversely, if the "true" response rate is above 20%, there will only 10% chance to observe none clinical benefit out of 10 patients.

Sample size justification for the Part II fulvestrant naïve ER+ Cohort: Based on the phase III trials PALOMA 3 (fulvestrant alone vs fulvestrant + palbo), the CBR for fulvestrant naïve endocrine resistant population is 40% and adding palbo increase CBR to 67%. We propose a sample size of 26 evaluable patients. Based on Simon's Minimax design, this will allow us 80% power at a 1-sided 0.05 significance level to detect an anticipated 65% response rate for fulvestrant plus neratinib combination therapy against the null hypothesis of 40%. Specifically, 12 patients will be enrolled in the first stage. If 6 or more clinical benefits are observed out of these 12 patients, additional 14 patients will be enrolled to the 2nd-stage. If 15 or more cases with clinical benefits are observed out of 26 patients, we would conclude that preliminary evidence for efficacy exists and that further investigation of the treatment is warranted. If the "true" response rate is 40% or less, this design allows us 67% chance to stop the trial earlier.

Sample size justification for the Part II fulvestrant treated ER+ Cohort: Patients enrolled in this cohort has had disease progression on prior fulvestrant, therefore we anticipate fulvestrant alone would be ineffective. Since neratinib alone resulted in clinical benefit rate of 38.5% (5 of 13 evaluable patients with metastatic ER+ breast cancer that carry activating HER2 mutations) based on the preliminary efficacy data observed in the part I of this trial, we propose a sample size of 41 evaluable patients. Based on Simon's Minimax design, this will allow us 80% power at a 1-sided 0.05 significance level to detect an anticipated 55% clinical benefit rate for fulvestrant plus neratinib combination therapy against the null hypothesis of 35%. Specifically, 21 patients will be enrolled in the first stage. If 9 or more clinical benefits are observed out of 21 patients, additional 20 patients will be enrolled to the 2nd-stage. If 20 or more cases with clinical benefit are observed out of 41 patients, we would conclude that preliminary evidence for efficacy exists and that further investigation of the treatment is warranted. If the "true" benefit rate is 35% or less, this design allows us 70% chance to stop the trial earlier.

In total, 77 patients (10 ER-, 26 ER+ fulvestrant naïve, 41 ER+ fulvestrant treated) will be enrolled in Part II of this study.

16.3 Data Analysis

Data analysis will be performed for Parts I and II separately. Only patients with tumors tested positive for HER2 mutations are eligible for study therapy but the clinical and pathology data will

be collected for all screened patients. Subset analysis will also be performed on patients with known activating mutations if needed.

For patients with HER2 mutation, the number and percentage of responses to neratinib alone (Part I, and Part II ER- cohort) or neratinib in combination with fulvestrant (Part II, fulvestrant naïve and fulvestrant treated cohorts) will be tabulated. The rate of overall clinical benefit (CR, PR, or SD $\geq$ 6 months) and its 90% confidence interval (CI) will be calculated in each cohort separately. The time to progression or death will be listed, and the progression-free survival (PFS) will be estimated using Kaplan-Meier product limit method in each cohort. For all the enrolled patients, the number and percentage of patients with HER2 mutation will be presented, and its 95% CI will also be calculated. The agreement for the occurrence of HER2 mutation in paired primary and metastatic sites will be described using contingency tables and assess by McNemar test. The association of the presence of HER2 mutation with histology subtype (invasive lobular vs invasive ductal cancer), tumor grade, tumor staging at initial diagnosis (I vs II or III vs IV), and disease-free survival (DFS) will be assessed using Fisher's exact test, Mann-Whitney rank sum test, or log-rank test as appropriate.

17.0 MULTICENTER REGULATORY REQUIREMENTS

Washington University requires that each participating site sends its informed consent document to be reviewed and approved by the Washington University Principal Investigator (or designee) prior to IRB/IEC submission.

Each participating institution must have the following documents on file at Washington University prior to first subject enrollment:

- Documentation of IRB approval of the study in the form of a letter or other official document from the participating institution's IRB. This documentation must show which version of the protocol was approved by the IRB.
- Documentation of IRB approval of an informed consent form. The consent must include a statement that data will be shared with Washington University, including the Quality Assurance and Safety Monitoring Committee (QASMC), the DSMC (if applicable), and the Washington University study team.
- Documentation of FWA, signed FDA Form 1572, and signed and dated CVs of all participating investigators.
- Documentation of training in protection of human subjects by all participating investigators.
- Protocol signature page signed and dated by the investigator at each participating site.

The Principal Investigator is responsible for disseminating to the participating sites all study updates, amendments, reportable adverse events, etc. There will be one current version of the protocol document at any given time and each participating institution will utilize that document. Protocol/consent modifications and IB updates will be forwarded electronically to the secondary sites within 2 weeks of obtaining Washington University IRB approval with acknowledgement of receipt requested. Secondary sites are to submit protocol/consent/IB modifications to their local IRBs within 4 weeks of receipt, and confirmation of submission must be forwarded to the appropriate contact person on the Washington University study team at the time of submission. Upon the secondary sites obtaining local IRB approval, documentation of such shall be sent to the Washington University study team within 2 weeks of receipt of approval.

Documentation of participating sites' IRB approval of annual continuing reviews, protocol amendments or revisions, all SAE reports, and all protocol violations/deviations/exceptions must be kept on file at Washington University.

The investigator or a designee from each institution must participate in a regular conference call to update and inform regarding the progress of the trial.

18.0 REFERENCES

1. Ma CX, Luo J, Ellis MJ: **Molecular profiling of triple negative breast cancer**. *Breast disease* 2010, **32**(1):73-84.
2. Mouridsen H, Gershanovich M, Sun Y, Perez-Carrion R, Boni C, Monnier A, Apffelstaedt J, Smith R, Sleeboom HP, Jaenicke F *et al*: **Phase III study of letrozole versus tamoxifen as first-line therapy of advanced breast cancer in postmenopausal women: analysis of survival and update of efficacy from the International Letrozole Breast Cancer Group**. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2003, **21**(11):2101-2109.
3. Kiely BE, Soon YY, Tattersall MH, Stockler MR: **How long have I got? Estimating typical, best-case, and worst-case scenarios for patients starting first-line chemotherapy for metastatic breast cancer: a systematic review of recent randomized trials**. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2011, **29**(4):456-463.
4. Yarden Y: **The EGFR family and its ligands in human cancer. signalling mechanisms and therapeutic opportunities**. *Eur J Cancer* 2001, **37 Suppl 4**:S3-8.
5. Harari PM, Allen GW, Bonner JA: **Biology of interactions: antiepidermal growth factor receptor agents**. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2007, **25**(26):4057-4065.
6. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M *et al*: **Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2**. *N Engl J Med* 2001, **344**(11):783-792.
7. Perez EA, Romond EH, Suman VJ, Jeong JH, Davidson NE, Geyer CE, Jr., Martino S, Mamounas EP, Kaufman PA, Wolmark N: **Four-year follow-up of trastuzumab plus adjuvant chemotherapy for operable human epidermal growth factor receptor 2-positive breast cancer: joint analysis of data from NCCTG N9831 and NSABP B-31**. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2011, **29**(25):3366-3373.
8. Romond EH, Perez EA, Bryant J, Suman VJ, Geyer CE, Jr., Davidson NE, Tan-Chiu E, Martino S, Paik S, Kaufman PA *et al*: **Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer**. *N Engl J Med* 2005, **353**(16):1673-1684.
9. Smith I, Procter M, Gelber RD, Guillaume Sb, Feyereislova A, Dowsett M, Goldhirsch A, Untch M, Mariani G, Baselga J *et al*: **2-year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: a randomised controlled trial**. *The Lancet*, **369**(9555):29-36.
10. Robert N, Leyland-Jones B, Asmar L, Belt R, Ilegbodu D, Loesch D, Raju R, Valentine E, Sayre R, Cobleigh M *et al*: **Randomized Phase III Study of Trastuzumab, Paclitaxel, and Carboplatin Compared With Trastuzumab and Paclitaxel in Women With HER-2Â–Overexpressing Metastatic Breast Cancer**. *Journal of Clinical Oncology* 2006, **24**(18):2786-2792.
11. Abramson V, Arteaga CL: **New Strategies in HER2-Overexpressing Breast Cancer: Many Combinations of Targeted Drugs Available**. *Clinical Cancer Research*, **17**(5):952-958.
12. Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, Slamon DJ, Murphy M, Novotny WF, Burchmore M *et al*: **Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer**. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2002, **20**(3):719-726.

13. Shah SP, Morin RD, Khattra J, Prentice L, Pugh T, Burleigh A, Delaney A, Gelmon K, Guliany R, Senz J *et al*: **Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution**. *Nature* 2009, **461**(7265):809-813.
14. Lee JW, Soung YH, Seo SH, Kim SY, Park CH, Wang YP, Park K, Nam SW, Park WS, Kim SH *et al*: **Somatic mutations of ERBB2 kinase domain in gastric, colorectal, and breast carcinomas**. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2006, **12**(1):57-61.
15. Kan Z, Jaiswal BS, Stinson J, Janakiraman V, Bhatt D, Stern HM, Yue P, Haverty PM, Bourgon R, Zheng J *et al*: **Diverse somatic mutation patterns and pathway alterations in human cancers**. *Nature* 2010, **466**(7308):869-873.
16. Rabindran SK: **Antitumor activity of HER-2 inhibitors**. *Cancer Letters* 2005, **227**(1):9-23.
17. Tsou H-R, Overbeek-Klumpers EG, Hallett WA, Reich MF, Floyd MB, Johnson BD, Michalak RS, Nilakantan R, Discafani C, Golas J *et al*: **Optimization of 6,7-Disubstituted-4-(arylamino)quinoline-3-carbonitriles as Orally Active, Irreversible Inhibitors of Human Epidermal Growth Factor Receptor-2 Kinase Activity**. *Journal of Medicinal Chemistry* 2005, **48**(4):1107-1131.
18. Rabindran SK, Discafani CM, Rosfjord EC, Baxter M, Floyd MB, Golas J, Hallett WA, Johnson BD, Nilakantan R, Overbeek E *et al*: **Antitumor activity of HKI-272, an orally active, irreversible inhibitor of the HER-2 tyrosine kinase**. *Cancer research* 2004, **64**(11):3958-3965.
19. Burstein HJ, Sun Y, Dirix LY, Jiang Z, Paridaens R, Tan AR, Awada A, Ranade A, Jiao S, Schwartz G *et al*: **Neratinib, an irreversible ErbB receptor tyrosine kinase inhibitor, in patients with advanced ErbB2-positive breast cancer**. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2010, **28**(8):1301-1307.
20. Howell A, Pippen J, Elledge RM, Mauriac L, Vergote I, Jones SE, Come SE, Osborne CK, Robertson JF: **Fulvestrant versus anastrozole for the treatment of advanced breast carcinoma: a prospectively planned combined survival analysis of two multicenter trials**. *Cancer* 2005, **104**(2):236-239.
21. Robertson JF, Osborne CK, Howell A, Jones SE, Mauriac L, Ellis M, Kleeberg UR, Come SE, Vergote I, Gertler S *et al*: **Fulvestrant versus anastrozole for the treatment of advanced breast carcinoma in postmenopausal women: a prospective combined analysis of two multicenter trials**. *Cancer* 2003, **98**(2):229-238.
22. Chia S, Gradishar W, Mauriac L, Bines J, Amant F, Federico M, Fein L, Romieu G, Buzdar A, Robertson JF *et al*: **Double-blind, randomized placebo controlled trial of fulvestrant compared with exemestane after prior nonsteroidal aromatase inhibitor therapy in postmenopausal women with hormone receptor-positive, advanced breast cancer: results from EFECT**. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2008, **26**(10):1664-1670.
23. Di Leo A, Jerusalem G, Petruzelka L, Torres R, Bondarenko IN, Khasanov R, Verhoeven D, Pedrini JL, Smirnova I, Lichinitser MR *et al*: **Results of the CONFIRM phase III trial comparing fulvestrant 250 mg with fulvestrant 500 mg in postmenopausal women with estrogen receptor-positive advanced breast cancer**. *J Clin Oncol* 2010, **28**(30):4594-4600.
24. Di Leo A, Jerusalem G, Petruzelka L, Torres R, Bondarenko IN, Khasanov R, Verhoeven D, Pedrini JL, Smirnova I, Lichinitser MR *et al*: **Results of the CONFIRM phase III trial comparing fulvestrant 250 mg with fulvestrant 500 mg in postmenopausal women with estrogen receptor-positive advanced breast cancer**. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2010, **28**(30):4594-4600.
25. Burstein HJ, Cirrincione CT, Barry WT, Chew HK, Tolaney SM, Lake DE, Ma C, Blackwell KL, Winer EP, Hudis CA: **Endocrine Therapy With or Without Inhibition of Epidermal**

Growth Factor Receptor and Human Epidermal Growth Factor Receptor 2: A Randomized, Double-Blind, Placebo-Controlled Phase III Trial of Fulvestrant With or Without Lapatinib for Postmenopausal Women With Hormone Receptor-Positive Advanced Breast Cancer-CALGB 40302 (Alliance). *J Clin Oncol* 2014, **32**(35):3959-3966.
26. Robertson JF, Steger GG, Neven P, Barni S, Gieseking F, Nole F, Pritchard KI, O'Malley FP, Simon SD, Kaufman B *et al*: **Activity of fulvestrant in HER2-overexpressing advanced breast cancer**. *Ann Oncol* 2010, **21**(6):1246-1253.
27. Hudziak RM, Lewis GD, Winget M, Fendly BM, Shepard HM, Ullrich A: **p185HER2 monoclonal antibody has antiproliferative effects in vitro and sensitizes human breast tumor cells to tumor necrosis factor**. *Mol Cell Biol* 1989, **9**(3):1165-1172.
28. Baselga J, Norton L, Albanell J, Kim YM, Mendelsohn J: **Recombinant humanized anti-HER2 antibody (Herceptin) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/neu overexpressing human breast cancer xenografts**. *Cancer research* 1998, **58**(13):2825-2831.
29. Pegram MD, Baly D, Wirth C, Gilkerson E, Slamon DJ, Sliwkowski MX: **Antibody dependent cell-mediated cytotoxicity in breast cancer patients in Phase III clinical trials of a humanized anti-HER2 antibody [abstract]**. *Proc Am Assoc Cancer Res* 1997, **38**:602.
30. Hotaling TE, Reitz B, Wolfgang-Kimball D, K B, Fox JA: **The humanized anti-HER2 antibody rhuMAb HER2 mediates antibody dependent cell-mediated cytotoxicity via FcgR III [abstract].** *Proc Annu Meet Am Assoc Cancer Res* 1996, **37**:471.
31. Hudis CA: **Trastuzumab â€" Mechanism of Action and Use in Clinical Practice**. *New England Journal of Medicine* 2007, **357**(1):39-51.
32. Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, Slamon DJ, Murphy M, Novotny WF, Burchmore M *et al*: **Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer**. *Journal of Clinical Oncology* 2002, **20**(3):719-726.
33. Baselga J, Carbonell X, CastaÃ±eda-Soto N-J, Clemens M, Green M, Harvey V, Morales S, Barton C, Ghahramani P: **Phase II Study of Efficacy, Safety, and Pharmacokinetics of Trastuzumab Monotherapy Administered on a 3-Weekly Schedule**. *Journal of Clinical Oncology* 2005, **23**(10):2162-2171.
34. Cobleigh MA, Vogel CL, Tripathy D, Robert NJ, Scholl S, Fehrenbacher L, Wolter JM, Paton V, Shak S, Lieberman G *et al*: **Multinational Study of the Efficacy and Safety of Humanized Anti-HER2 Monoclonal Antibody in Women Who Have HER2-Overexpressing Metastatic Breast Cancer That Has Progressed After Chemotherapy for Metastatic Disease**. *Journal of Clinical Oncology* 1999, **17**(9):2639.
35. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, Goldhirsch A, Untch M, Smith I, Gianni L, Baselga J, Bell R, Jackisch C *et al*: **Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer**. *N Engl J Med* 2005, **353**(16):1659-1672.
36. Nahta R, Esteva FJ: **Herceptin: mechanisms of action and resistance**. *Cancer Lett* 2006, **232**(2):123-138.
37. O'Donovan N, Byrne AT, O'Connor AE, McGee S, Gallagher WM, Crown J: **Synergistic interaction between trastuzumab and EGFR/HER-2 tyrosine kinase inhibitors in HER-2 positive breast cancer cells**. *Investigational new drugs* 2011, **29**(5):752-759.
38. Baselga J, Cortes J, Kim SB, Im SA, Hegg R, Im YH, Roman L, Pedrini JL, Pienkowski T, Knott A *et al*: **Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer**. *N Engl J Med* 2012, **366**(2):109-119.
39. Blackwell KL, Burstein HJ, Storniolo AM, Rugo H, Sledge G, Koehler M, Ellis C, Casey M, Vukelja S, Bischoff J *et al*: **Randomized Study of Lapatinib Alone or in Combination With**

Trastuzumab in Women With ErbB2-Positive, Trastuzumab-Refractory Metastatic Breast Cancer. *Journal of Clinical Oncology* 2010, **28**(7):1124-1130.
40. Valachis A, Nearchou A, Polyzos NP, Lind P: **Cardiac toxicity in breast cancer patients treated with dual HER2 blockade**. *International journal of cancer Journal international du cancer* 2013, **133**(9):2245-2252.
41. Swaby R, Blackwell K, Jiang Z, Sun Y, Dieras V, Zaman K, Zacharchuk C, Powell C, Abbas R, Thakuria M: **Neratinib in combination with trastuzumab for the treatment of advanced breast cancer: A phase I/II study**. *Journal of Clinical Oncology* 2009, **27**(15).
42. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL: **Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene**. *Science* 1987, **235**(4785):177-182.
43. Andrulis IL, Bull SB, Blackstein ME, Sutherland D, Mak C, Sidlofsky S, Pritzker KP, Hartwick RW, Hanna W, Lickley L *et al*: **neu/erbB-2 amplification identifies a poor-prognosis group of women with node-negative breast cancer. Toronto Breast Cancer Study Group**. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 1998, **16**(4):1340-1349.
44. Sjogren S, Inganas M, Lindgren A, Holmberg L, Bergh J: **Prognostic and predictive value of c-erbB-2 overexpression in primary breast cancer, alone and in combination with other prognostic markers**. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 1998, **16**(2):462-469.
45. Lee JW, Soung YH, Seo SH, Kim SY, Park CH, Wang YP, Park K, Nam SW, Park WS, Kim SH *et al*: **Somatic mutations of ERBB2 kinase domain in gastric, colorectal, and breast carcinomas**. *Clin Cancer Res* 2006, **12**(1):57-61.
46. Shah SP, Morin RD, Khattra J, Prentice L, Pugh T, Burleigh A, Delaney A, Gelmon K, Guliany R, Senz J *et al*: **Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution**. *Nature* 2009, **461**(7265):809-813.
47. Shah SP, Roth A, Goya R, Oloumi A, Ha G, Zhao Y, Turashvili G, Ding J, Tse K, Haffari G *et al*: **The clonal and mutational evolution spectrum of primary triple-negative breast cancers**. *Nature* 2012:doi: 10.1038/nature10933.
48. Stephens PJ, Tarpey PS, Davies H, Van Loo P, Greenman C, Wedge DC, Zainal SN, al. E: **The landscape of cancer genes and mutational processes in breast cancer**. *Nature* 2012:doi:10.1038/nature11017.
49. Ellis MJ, Ding L, Shen D, Luo J, Suman VJ, Wallis JW, Van Tine BA, Hoog J, Goiffon RJ, Goldstein TC *et al*: **Whole Genome Sequencing to Characterise Breast Cancer Response to Aromatase Inhibition**. *Nature* 2012:doi: 10.1038/nature11143.
50. Cancer Genome Atlas Network T: **Comprehensive molecular portraits of human breast tumors.** *Nature* 2012:In Press.
51. Bose R, Kavuri SM, Searleman AC, Shen W, Shen D, Koboldt DC, Monsey J, Goel N, Aronson AB, Li S *et al*: **Activating HER2 mutations in HER2 gene amplification negative breast cancer**. *Cancer Discov* 2013, **3**(2):224-237.
52. Moy B, Kirkpatrick P, Kar S, Goss P: **Lapatinib**. *Nat Rev Drug Discov* 2007, **6**(6):431-432.
53. Kwak EL, Sordella R, Bell DW, Godin-Heymann N, Okimoto RA, Brannigan BW, Harris PL, Driscoll DR, Fidias P, Lynch TJ *et al*: **Irreversible inhibitors of the EGF receptor may circumvent acquired resistance to gefitinib**. *Proc Natl Acad Sci U S A* 2005, **102**(21):7665-7670.
54. Debnath J, Mills KR, Collins NL, Reginato MJ, Muthuswamy SK, Brugge JS: **The role of apoptosis in creating and maintaining luminal space within normal and oncogene-expressing mammary acini**. *Cell* 2002, **111**(1):29-40.

55. Debnath J, Muthuswamy SK, Brugge JS: **Morphogenesis and oncogenesis of MCF-10A mammary epithelial acini grown in three-dimensional basement membrane cultures**. *Methods* 2003, **30**(3):256-268.
56. Greulich H, Kaplan B, Mertins P, Chen TH, Tanaka KE, Yun CH, Zhang X, Lee SH, Cho J, Ambrogio L *et al*: **Functional analysis of receptor tyrosine kinase mutations in lung cancer identifies oncogenic extracellular domain mutations of ERBB2**. *Proc Natl Acad Sci U S A* 2012, **109**(36):14476-14481.
57. Bose R, Kavuri SM, Searleman AC, Shen W, Shen D, Koboldt DC, Monsey J, Goel N, Aronson AB, Li S *et al*: **Activating HER2 Mutations in HER2 Gene Amplification Negative Breast Cancer**. *Cancer discovery* 2013, **3**(2):224-237.
58. de Martino M, Zhuang D, Klatte T, Rieken M, Rouprêt M, Xylinas E, Clozel T, Krzywinski M, Elemento O, Shariat SF: **Impact of ERBB2 mutations on in vitro sensitivity of bladder cancer to lapatinib**. *Cancer biology & therapy* 2014, **15**(9):1239-1247.
59. Trowe T, Boukouvala S, Calkins K, Cutler RE, Jr., Fong R, Funke R, Gendreau SB, Kim YD, Miller N, Woolfrey JR *et al*: **EXEL-7647 inhibits mutant forms of ErbB2 associated with lapatinib resistance and neoplastic transformation**. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2008, **14**(8):2465-2475.
60. Kancha RK, von Bubnoff N, Bartosch N, Peschel C, Engh RA, Duyster J: **Differential sensitivity of ERBB2 kinase domain mutations towards lapatinib**. *PloS one* 2011, **6**(10):e26760.
61. Rexer BN, Ghosh R, Narasanna A, Estrada MV, Chakrabarty A, Song Y, Engelman JA, Arteaga CL: **Human breast cancer cells harboring a gatekeeper T798M mutation in HER2 overexpress EGFR ligands and are sensitive to dual inhibition of EGFR and HER2**. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2013, **19**(19):5390-5401.
62. Sun Z, Shi Y, Shen Y, Cao L, Zhang W, Guan X: **Analysis of different HER-2 mutations in breast cancer progression and drug resistance**. *Journal of Cellular and Molecular Medicine* 2015, **19**(12):2691-2701.
63. Gilmer TM, Cable L, Alligood K, Rusnak D, Spehar G, Gallagher KT, Woldu E, Carter HL, Truesdale AT, Shewchuk L *et al*: **Impact of common epidermal growth factor receptor and HER2 variants on receptor activity and inhibition by lapatinib**. *Cancer research* 2008, **68**(2):571-579.
64. Hu Z, Hu Y, Liu X, Xi R, Zhang A, Liu D, Xie Q, Chen L: **Tumor driven by gain-of-function HER2 H878Y mutant is highly sensitive to HER2 inhibitor**. *Oncotarget* 2015, **6**(31):31628-31639.
65. Fan YX, Wong L, Ding J, Spiridonov NA, Johnson RC, Johnson GR: **Mutational activation of ErbB2 reveals a new protein kinase autoinhibition mechanism**. *J Biol Chem* 2008, **283**(3):1588-1596.
66. Minami Y, Shimamura T, Shah K, LaFramboise T, Glatt KA, Liniker E, Borgman CL, Haringsma HJ, Feng W, Weir BA *et al*: **The major lung cancer-derived mutants of ERBB2 are oncogenic and are associated with sensitivity to the irreversible EGFR//ERBB2 inhibitor HKI-272**. *Oncogene* 2007, **26**(34):5023-5027.
67. Wang SE, Narasanna A, Perez-Torres M, Xiang B, Wu FY, Yang S, Carpenter G, Gazdar AF, Muthuswamy SK, Arteaga CL: **HER2 kinase domain mutation results in constitutive phosphorylation and activation of HER2 and EGFR and resistance to EGFR tyrosine kinase inhibitors**. *Cancer Cell* 2006, **10**(1):25-38.
68. Ma CX, Bose R, Gao F, Freedman RA, Telli ML, Kimmick G, Winer EP, Naughton MJ, Goetz MP, Russell C *et al*: **Neratinib Efficacy and Circulating Tumor DNA Detection of HER2**

Mutations in HER2 Non-amplified Metastatic Breast Cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2017.
69. De Mattos-Arruda L, Caldas C: **Cell-free circulating tumour DNA as a liquid biopsy in breast cancer**. *Mol Oncol* 2016, **10**(3):464-474.
70. Lanman RB, Mortimer SA, Zill OA, Sebisanovic D, Lopez R, Blau S, Collisson EA, Divers SG, Hoon DS, Kopetz ES *et al*: **Analytical and Clinical Validation of a Digital Sequencing Panel for Quantitative, Highly Accurate Evaluation of Cell-Free Circulating Tumor DNA**. *PloS one* 2015, **10**(10):e0140712.

We would like to thank the Alvin J. Siteman Cancer Center at Washington University School of Medicine and Barnes-Jewish Hospital in St. Louis, Missouri, for the use of the Clinical Trials Core which provided protocol development, study coordination, and regulatory coordination services. The Siteman Cancer Center is supported in part by an NCI Cancer Center Support Grant #P30 CA91842.

APPENDIX A: ECOG PERFORMANCE STATUS SCALE

Grade	Description
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: PATIENT'S MEDICATION DIARY

Today's Date: _____ Agent: Neratinib Study ID#: _____

Cycle: _____ Patient Name: _____

1. Complete one form for each month. Take _____mg (___tablets) of neratinib daily with food every morning.
2. Record the date, the number of capsules taken, and when you took them.
3. If you forget to take your neratinib dose within 12 hours of the usual time, do not take a dose that day. Restart taking the neratinib the next day.
4. If you have any questions or notice any side effects, please record them in the comments section. Record the time if you should vomit.
5. Please return the forms to your physician or your study coordinator when you go to your next appointment. Please bring your unused study medications and/or empty bottles with you to each clinic visit so that a pill count can be done.
6. Avoid St. John's wort, grapefruit, grapefruit juice, grapefruit hybrids, and star fruit from 7 days before you start taking neratinib and throughout the entire study.

Day	Date	What time was dose taken?	# of tablets taken	# of loose stools	Antidiarrheals taken? y/n	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						

APPENDIX C: PATIENT INSTRUCTIONS FOR THE MANAGEMENT OF DIARRHEA

Please review these instructions with your study doctor/team. Once all of your questions are answered, sign at the bottom and make sure you are given a copy of these instructions to take home.

Diarrhea is the most common side effect you may have while participating in this study. Diarrhea usually starts within a few hours to a few days of the first dose of study drug. In order to reduce or even prevent diarrhea as far as possible, you will be supplied with an anti-diarrheal medicine called loperamide to take at the start of the study. **Primary prophylactic use of antidiarrheal medication is mandatory for all enrolled subjects.** Loperamide will be dispensed directly by your study doctor/team on day 1 with the instruction to initiate treatment with loperamide at the same time as you take your first dose of neratinib.

Start taking the anti-diarrheal medication immediately with the first dose of neratinib as directed by your study doctor/team.

Your study doctor/team will call you 1 day, 2 days and 3 days after your first dose of study drug to find out if you are experiencing diarrhea and to provide further treatment instructions and advice if necessary. You will also have an easy to follow flow-chart to help you each day in the management of diarrhea if it is present.

If you are having new-onset diarrhea, persistent diarrhea or diarrhea with increase of 7 or more stools per day over usual, call your study doctor/team at 314-362-8903 to let them know so they can work with you to control the diarrhea. If you are dizzy or weak because of diarrhea, go to the study doctor's office or go to the hospital immediately.

Please record the number of stools and any anti-diarrheal treatment during the first two cycles along with the daily dose of study medication in your diary and return the completed diary at the next scheduled visit.

Information to provide when talking to your doctor

When talking to the study doctor/team I will provide as much of the information below as possible, in order to help my study doctor/team to assess my diarrhea and decide on the best treatment:

- Number of stools per day as compared to my normal bowel habits
- Presence of diarrhea during the night
- Presence of fever, dizziness, abdominal pain/cramping, or weakness
- What the stool looks like, that is, watery stools, blood, or mucus
- When I took my last study drug
- Any other information that could explain my diarrhea (food, recent travel, contact with other people with diarrhea).

Medications to treat diarrhea

My study doctor/team will provide me with loperamide on day 1 with the instruction to start treatment with loperamide along with the first dose of neratinib. I need to take the medications as directed by my study doctor/team.

✓ Loperamide:

- I will take 2 tablets/capsules (4 mg) with the first dose of neratinib
- After the first dose, I will take 4 mg (2 tablets/ capsules) three times daily for the first 14 days(not to exceed a total of 8 tablets/day; if I get constipated, I should contact my study doctor/team who will instruct me on how often and how much loperamide to take but I should not stop taking loperamide).
- After the first 14 days, I will take 2 tablets (4 mg) two times per day until the end of the second cycle of therapy regardless of whether I have diarrhea or not. If I get constipated, I should contact my study doctor/team who will instruct me on how often and how much loperamide to take but I should not stop taking loperamide).
- If I continue to have diarrhea while taking loperamide, I should contact my study doctor/team for additional anti-diarrheal medication.
- If I have diarrhea with increase of up to 6 stools per day over usual any time after completing the second cycle of therapy and I am not taking any anti-diarrheal medication, I will take 4mg (2 tablets/capsules) as needed (not to exceed 8 tablets capsules or 16 mg per day)

- □ Other medication (Study doctor/team to write in name of medication and instructions): _____

_____

_____

In case of more severe diarrhea and any diarrhea associated with fever, pain, infection, or dehydration, I may receive IV fluids, antibiotics and/or other medications.

Changes to my diet to treat diarrhea

If I have diarrhea, I will:

- Stop all lactose-containing products (milk, yogurt, cheese, etc)
- Drink 8 to 10 large glasses of clear liquids per day
- Eat frequent small meals
- Eat low fat foods such as the BRAT diet that includes **b**ananas, **r**ice, **a**pplesauce, and/or **t**oast:
  - The BRAT diet is a bland diet that is low in fat and fiber and will not irritate the stomach;
  - Bananas are high in potassium and can cause constipation which can help alleviate the diarrhea
  - Other similar foods are crackers, cooked cereals and pasta
  - This diet is not complete in nutrients and should only be taken for a short period of time and only upon the doctor's advice

My study doctor/team may have other suggestions for me. (Study doctor/team to write in any suggestions). _____

Study Medication adjustments

If I am experiencing loose stools or diarrhea and cannot reach my study doctor/team immediately, I will start taking anti-diarrheal medication per the instructions above until further advice is given by my study doctor/team. If I have more than 4-6 stools per day compared to normal despite taking anti-diarrheal medication for 24 hours, I will stop taking the study medication and wait for further instructions from my study doctor/team.

My signature below indicates I have reviewed this information and have received the following medications:

□ Neratinib Tablets
□ Loperamide

_____

Study participant name Study participant Signature and Date

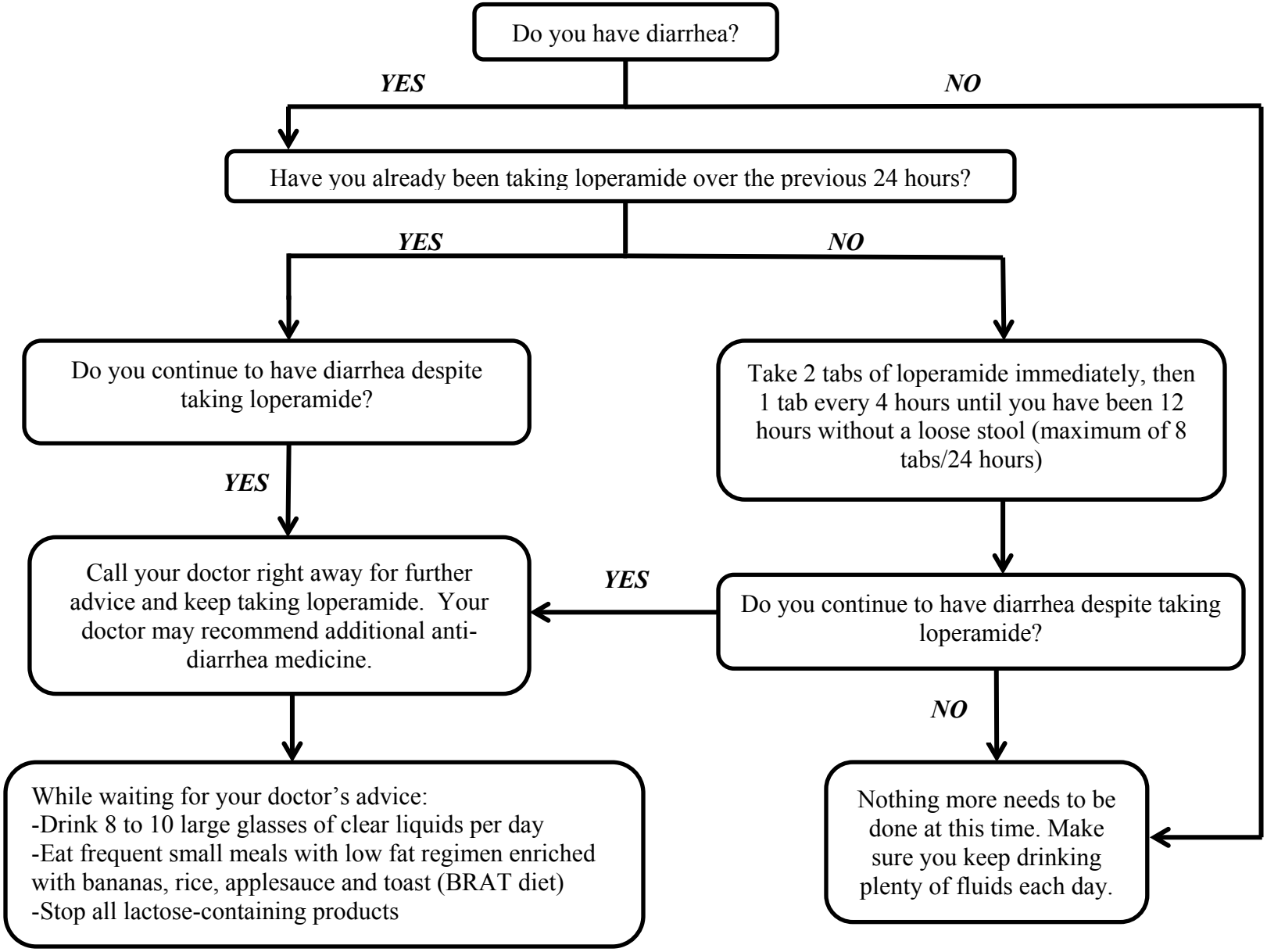
_____

Investigator delegate name Investigator delegate Signature and Date

OR

_____

Investigator name Investigator Signature and Date


Do you have diarrhea?
YES
NO
Have you already been taking loperamide over the previous 24 hours?
YES
NO
Do you continue to have diarrhea despite taking loperamide?
Take 2 tabs of loperamide immediately, then 1 tab every 4 hours until you have been 12 hours without a loose stool (maximum of 8 tabs/24 hours)
YES
Call your doctor right away for further advice and keep taking loperamide. Your doctor may recommend additional anti-diarrhea medicine.
YES
Do you continue to have diarrhea despite taking loperamide?
NO
While waiting for your doctor's advice:
-Drink 8 to 10 large glasses of clear liquids per day
-Eat frequent small meals with low fat regimen enriched with bananas, rice, applesauce and toast (BRAT diet)
-Stop all lactose-containing products
Nothing more needs to be done at this time. Make sure you keep drinking plenty of fluids each day.

APPENDIX D: RECIST 1.1 Tumor Evaluation Form

Patient Name DOB Study PI

Please record the following: * Description of Target, Non-Target, or New Lesions * Maximum of 5 Target lesions Version Date 10/30/2012

* Dimensional measurements of ***Lymph Nodes (LNs)*** are ***shortest*** axis and ***≥1.5 cm*** * All other lesions are ***longest*** axis.

* Follow Up Status of Non-Target Lesions: Increase/Decrease/Stable/Resolved

Date of Evaluation _____ _____ _____ _____

Target Lesions ***Baseline***

Site #/Description	*Modality*	*Image #*	*Dimension **cm***	*Modality*	*Image #*	*Dimension **cm***	*Modality*	*Image #*	*Dimension **cm***	*Modality*	*Image #*	*Dimension **cm***
#1	CT			CT			CT			CT		
#2	CT			CT			CT			CT		
#3	CT			CT			CT			CT		
#4	CT			CT			CT			CT		
#5	CT			CT			CT			CT		
Sum of Longest Dimensions			0.0			0.0			0.0			0.0

Non-Target Lesions

Site #/Description	*Modality*	*Image #*	*F/U Status*	*Modality*	*Image #*	*F/U Status*	*Modality*	*Image #*	*F/U Status*	*Modality*	*Image #*	*F/U Status*
#1	CT			CT			CT			CT		
#2	CT			CT			CT			CT		
#3	CT			CT			CT			CT		
#4	CT			CT			CT			CT		
#5	CT			CT			CT			CT		
#6	CT			CT			CT			CT		
#7	CT			CT			CT			CT		
#8	CT			CT			CT			CT		

New Lesions

Site #/Description	*Modality*	*Image #*	*Dimension **cm***	*Modality*	*Image #*	*Dimension **cm***	*Modality*	*Image #*	*Dimension **cm***	*Modality*	*Image #*	*Dimension **cm***
#1	CT			CT			CT			CT		
#2	CT			CT			CT			CT		
#3	CT			CT			CT			CT		
#4	CT			CT			CT			CT		
#5	CT			CT			CT			CT		

Comments

Initials/Date of Form Prep/Data Entry ***SignatureDate***

APPENDIX E: Definitions

Adverse Events (AEs)

Definition: any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug-related.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.03 can be downloaded from the CTEP website.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website: http://www.hhs.gov/ohrp/policy/advevntguid.html

Suspected Adverse Reaction (SAR)

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

Serious Adverse Event (SAE) or Serious Suspected Adverse Reaction

As defined in 21 CFR 312.32

Definition: an adverse event or suspected adverse reaction is considered "serious" if, in the view of the investigator, it results in any of the following outcomes:

- Death
- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Any other important medical event that does not fit the criteria above but, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

Unexpected Adverse Experience

Definition: any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure (or risk information, if an IB is not required or available).

Events that are both serious AND unexpected where there is a reasonable possibility of relationship to study drug must be reported to the FDA.

Life-Threatening Adverse Event / Life Threatening Suspected Adverse Reaction

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

Unanticipated Problems

Definition:

- unexpected (in terms of nature, severity, or frequency based on the adverse reaction table (Table 63) in the most current Investigator Brochure) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

Local IRB pre-approval of all protocol exceptions must be obtained prior to the event. For secondary sites, the Washington University PI will issue approval of the exception, but it must also be submitted to the local IRB with documentation of approval forwarded to Washington University. Washington University IRB approval is not required for protocol exceptions occurring at secondary sites.

APPENDIX F: Reporting Timelines

Expedited Reporting Timelines					
Event	**HRPO**	**QASMC**	**FDA**	**IBC** (Not Applicable)	**Puma Biotechnology, Inc.**
Serious AND unexpected suspected adverse reaction			Report no later than 15 calendar days after it is determined that the information qualifies for reporting		Report within fifteen (15) days
Unexpected fatal or life-threatening suspected adverse reaction			Report no later than 7 calendar days after initial receipt of the information		Report within seven (7) days
Unanticipated problem involving risk to participants or others	Report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day.	Report via email after IRB acknowledgment			
Major deviation	Report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day.				
A series of minor deviations that are being reported as a continuing noncompliance	Report within 10 working days.				
Protocol exception	Approval must be obtained prior to implementing the change				
Clinically important increase in the rate of a serious suspected adverse reaction of that list in the protocol or IB			Report no later than 15 calendar days after it is determined that the information qualifies for reporting		
Complaints	If the complaint reveals an unanticipated problem involving risks to participants or others OR noncompliance,				

Expedited Reporting Timelines					
Event	**HRPO**	**QASMC**	**FDA**	**IBC** (Not Applicable)	**Puma Biotechnology, Inc.**
	report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. Otherwise, report at the time of continuing review.				
Breach of confidentiality	Within 10 working days.				
Incarceration	If withdrawing the participant poses a safety issue, report within 10 working days. If withdrawing the participant does not represent a safety issue and the patient will be withdrawn, report at continuing review.				

Routine Reporting Timelines				
Event	**HRPO**	**QASMC**	**FDA**	**Puma Biotechnology, Inc.**
Adverse event or SAE that does not require expedited reporting	If they do not meet the definition of an unanticipated problem involving risks to participants or others, report summary information at the time of continuing review	Adverse events will be reported in the toxicity table in the DSM report which is typically due every 6 months.	The most current toxicity table from the DSM report is provided to the FDA with the IND's annual report.	Report within thirty (30) days (i.e., unrelated to study drugs or listed/expected event)
Minor deviation	Report summary information at the time of continuing review.			
Complaints	If the complaint reveals an unanticipated problem involving risks to participants or others OR noncompliance, report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. Otherwise, report at the time of continuing review.			
Incarceration	If withdrawing the participant poses a safety issue, report within 10 working days.			

Routine Reporting Timelines				
	If withdrawing the participant does not represent a safety issue and the patient will be withdrawn, report at continuing review.			

Expedited Reporting Timelines for Secondary Sites				
Event	**WU (Coordinating Center)**	**Local IRB**	**FDA**	**Puma Biotechnology, Inc.**
Serious AND unexpected suspected adverse reaction	Report no later than 11 calendar days after it is determined that the information qualifies for reporting.	Report all applicable events to local IRB according to local institutional guidelines.	The research team at Washington University is responsible for reporting all applicable events to the FDA as needed.	The research team at Washington University is responsible for reporting all applicable events to [MANUFACTURER] as needed.
Unexpected fatal or life-threatening suspected adverse reaction	Report no later than 4 calendar days after initial receipt of the information.			
Unanticipated problem involving risk to participants or others	Report no later than 4 calendar days after initial receipt of the information.			
Adverse event or SAE that does not require expedited reporting	As per routine data entry expectations			
Protocol exception	Approval must be obtained prior to implementing the change.			

APPENDIX G: Washington University Serious Adverse Event Reporting Cover Sheet

SAE COVER SHEET- Secondary Site Assessment

Washington University HRPO#:	Sponsor-Investigator:
Subject Initials:	Subject ID:
Treating MD:	Treating Site:
EVENT TERM:	Event Start Date:
EVENT GRADE:	Date of site's first notification:

Treating MD Event Assessment:

Is this event **possibly, probably, or definitely** related study treatment?

☐ yes ☐ no

If yes, please list which drug (if more than one)_____

Explain _____

_____

_____ _____ _____

Physician's Name **Physician's Signature** **Date**