Phase I/II Trial of Ruxolitinib in Combination with Trastuzumab in Metastatic HER2 Positive Breast Cancer

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Incyte-Supplied Agent(s): Ruxolitinib (Jakafi), INCB018424, INC424, Incyte
Other Agent(s): Trastuzumab (Herceptin), NSC 688097, Commercial

SYNOPSIS

Phase I/II Trial of Ruxolitinib in Combination with Trastuzumab in Metastatic HER2 Positive Breast Cancer (32-40 patients)

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Patient Selection (see section 3 for complete eligibility criteria)

1. HER2+ Metastatic Breast Cancer
2. Patients must have received at least two lines of HER2-directed therapy in the metastatic setting, and prior therapy for metastatic disease should include both pertuzumab and ado-trastuzumab unless contraindicated or declined by the patient.
3. ECOG performance status 0-2
4. Measurable or non-measurable disease
5. Adequate bone marrow and organ function
6. Left Ventricular Ejection Fraction ≥ 50%
7. No prior history of IL-6, JAK or STAT inhibitor, such as ruxolitinib
8. No HIV+ or active infection
9. No concurrent medications that are potent CYP3A4 inhibitors or inducers

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Phase I (n=10: Section 5): The dose of trastuzumab will be fixed at 6 mg/kg every 3 weeks (with 8 mg/kg initial re-load if no trastuzumab in > 28 days). The dose levels of ruxolinitib:

Dose Level	Ruxolitinib Dose
1	25 mg bid
0	20 mg bid
-1	15 mg bid
-2	10 mg bid

The maximum tolerated dose (MTD) combination is defined as the dose combination associated with a target probability of dose limiting toxicity of 0.25. The MTD will be estimated using the time-to-event continual reassessment method (TITE-CRM) as detailed in Section 13.

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Phase II (n=30 evaluable pts: Section 5): Recommended phase II dose of ruxolitinib (oral bid dosing) plus trastuzumab 6 mg/kg every 3 weeks (cycle = 21 days). If no trastuzumab > 28 days, patients will be initially re-loaded at 8 mg/kg, then 6 mg/kg. Pts treated in Phase I at recommended Phase II dose will be considered evaluable.

Design: Non-randomized, open-label trial

Stratification: Hormone Receptor Status (+/-)

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Evaluations

Disease Assessment:

CT chest, abdomen, and pelvis plus (OR) PET/CT will be obtained every 3 cycles (9 weeks of treatment +/- 4 days, allowing for protocol specified dose delays). Baseline bone scan required in all patients and will be performed every 3 cycles as clinically indicated. If patients have known brain metastases and are eligible for this trial, a head CT or brain MRI MUST be performed along with systemic imaging. If a patient does not have known brain metastases and is asymptomatic, no baseline brain imaging is required.

Blood: Samples will be obtained for biomarker analysis pre-treatment, on-treatment on cycle 2 day 1, and then at progression

Tumor Tissue: Pre-treatment biopsies from archival tissue or new biopsy, prior to cycle 2 day 1, and upon progression of disease will be discussed with pts with accessible disease (Optional)

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1. OBJECTIVES

Refer to Section 13: Statistical Considerations for definition of the endpoints below.

1.1 Primary Objectives

- To evaluate the safety and tolerability of ruxolitinib in combination with trastuzumab
- To determine the MTD of ruxolitinib with a fixed dose of trastuzumab (Phase 1)
- To estimate the efficacy end point of progression free survival (PFS) of the combination of ruxolitinib and trastuzumab in patients with HER2+ metastatic breast cancer who have progressed on a trastuzumab-containing regimen (Phase 2)

1.2 Secondary Objectives

- To assess the pharmacokinetics (PK) and pharmacodynamics (PD) of combination ruxolitinib and trastuzumab (Phase 1)
- To assess the objective response rate (ORR) and clinical benefit rate (CBR) in the study population (Phase 2)

1.3 Exploratory Objectives

- To explore differences in clinical outcome (PFS, ORR, and CBR) based on clinicopathologic features, such as hormone receptor status
- To explore the characteristics of the tumor and peripheral blood as potential pharmacodynamic or predictive biomarkers. Potential biomarkers will include tumor evaluation of pSTAT3 expression, serum IL-6, IL-8, and C-reactive protein.
- To conduct genomic profiling of serial tumor samples of subjects through RNA-Seq to explore mechanisms of acquired resistance and response

2. BACKGROUND

2.1 HER2 Overexpressed Breast Cancer

Breast cancer is the most common female cancer and the second most common cause of cancer death in women. Approximately 1,150,000 cases and 410,000 deaths from breast cancer occur annually worldwide (Parkin et al, 2005), and, in the U.S., there are an estimated 184,450 new cases and 40,480 deaths from breast cancer every year (Jemal et al, 2008). The vast majority of patients who die from breast cancer succumb to metastatic disease. The human epidermal growth factor receptor type 2 gene (HER2) is amplified in 20% to 30% of breast cancers (Revillion et al. 1998).

HER2+ breast cancers are associated with earlier recurrence and shorter overall survival and are associated with other adverse prognostic markers, such as high tumor grade, high rates of cell proliferation, increased nodal metastases, and relative resistance to certain types of chemotherapy (Slamon et al. 1987; Paik et al. 1990). The HER family of receptors, HER1, HER2, HER3, HER4 (also referred to as epidermal growth factor receptors ErbB-1, ErbB-2, ErbB-3, ErbB-4), is a group of related transmembrane receptor tyrosine kinases that regulate normal cell survival, proliferation, differentiation, and migration (Hudis, 2007).

2.2 Trastuzumab

Trastuzumab, a recombinant humanized monoclonal antibody against the extracellular domain of HER2, prevents cell proliferation and has greatly improved the treatment of HER2+ breast cancer. Clinical studies demonstrate that trastuzumab can dramatically improve disease-free and overall-survival in HER2+ patients in both the curable and advanced disease settings (Slamon et al. 2001; Romond et al. 2005).

A single arm, 222 patient, multinational study of trastuzumab monotherapy in HER2+ metastatic breast cancer patients who had progressed after one or two treatment regimens showed a 14% response rate (2% complete responses) and a median duration of response of 13 months (Cobleigh et al,1999; Herceptin (Trastuzumab) USPI, 2008). Although response rates of HER2+ metastatic breast cancer to trastuzumab monotherapy are low (11 - 34%), the combination of chemotherapy, including taxanes and vinorelbine, plus trastuzumab yield response rates which approach 70% in the first line setting (Slamon et al. 1987; Seidman et al. 2001; Esteva et al. 2002; Burstein et al. 2007).

In a pivotal trial in which women with previously untreated HER2+ MBC were randomized to chemotherapy versus chemotherapy with trastuzumab, the addition of trastuzumab increased time to progression from 4.6 months to 7.4 months (Slamon et al. 2005). Trastuzumab as a single agent is approved for treatment of HER2-overexpressing breast cancer in patients who have received one or more chemotherapy regimens (U.S. approval) or at least two chemotherapy regimens (European approval) for metastatic disease. Trastuzumab received approval in the U.S. for HER2+ metastatic breast cancer either as monotherapy or in combination with paclitaxel. Trastuzumab is also approved for the adjuvant treatment of HER2-overexpressing, node positive or node negative breast cancer. While the treatment effects of trastuzumab were seen in patients treated with anthracycline plus cyclophosphamide (AC) or paclitaxel, the size of the treatment effect was greater in the paclitaxel subgroup, with less cardiotoxicity. The incidence of New York Heart Association class III or IV cardiac dysfunction was 16% in patients treated with AC and trastuzumab compared with 2 % among those who had received paclitaxel and trastuzumab (Herceptin (Trastuzumab) USPI, 2008; Slamon et al, 2001).

2.3 Incyte-Supplied Investigational Drug

2.3.1 Ruxolitinib

a. Mechanism of Action

Ruxolitinib (INCB018424 phosphate, INC424, ruxolitinib phosphate) represents a potent, and selective inhibitor of JAK1 (Janus kinase 1) (inhibition concentration 50% [IC50]=3.3 ± 1.2 nM) and JAK2 (IC50=2.8 ± 1.2 nM) with modest to marked selectivity against TYK2 (tyrosine kinase 2) (IC50=19 ± 3.2 nM) and JAK3 (IC50=428 ± 243 nM), respectively. Ruxolitinib interferes with the signaling of a number of cytokines and growth factors that are important for hematopoiesis and immune function. JAK signaling involves recruitment of signal transducers and activators of transcription (STATs) to cytokine receptors, activation, and subsequent localization of STATs to the nucleus leading to modulation of gene expression. Dysregulation of the JAK-STAT pathway has been associated with several types of cancer and increased proliferation and survival of malignant cells.

b. Non-Clinical Pharmacology

Ruxolitinib has high aqueous solubility and high permeability *in vitro* based on Caco-2 cell culture study. In single oral dose PK studies, ruxolitinib was absorbed rapidly with mean Tmax values ranging from 0.5 to 2 h. In mice, dogs, minipigs and humans, there were minimal gender differences in the exposure of ruxolitinib and its metabolites. In rats, the Cmax and AUC (area under the concentration-time curve) values were several-fold higher in females compared to males, due to metabolism by male rat specific isozymes 2C11, 2C13 and CYP3A2. The terminal half-life, routes and rate of excretion for ruxolitinib-derived radioactivity and metabolites observed between male and female rats were similar. The oral bioavailability was variable across species, ranging from 22% in male monkeys to virtually

complete in female rats consistent with the species dependent clearance observed following IV dosing.

Following IV administration of ruxolitinib, the plasma clearance was species-dependent and ranged from moderate in male dogs (0.48 L/h/kg) to very high in male rats (9.4 L/h/kg). Based on the apparent steady-state volume of distribution, ruxolitinib was not distributed extensively beyond body water in dogs (1.1 L/kg) and monkeys (0.81 L/kg), but may be distributed to a greater degree in rats (1.6-3.8 L/kg) and minipigs (6.4 L/kg). The terminal elimination half-life ranged from 0.4 h (rat) to 2.5 h (dog). In monkeys, the PK parameters of ruxolitinib were comparable with and without the pretreatment of agents that increase gastric pH, indicating that elevated gastric pH is not likely to affect the oral exposure of ruxolitinib.

c. Animal Toxicity

Ruxolitinib was evaluated in several in animal based toxicology studies and did not exhibit significant toxicities at target concentrations. Cardiovascular evaluation in dogs demonstrated lowered blood pressure and increased heart rate at the highest dose of ruxolitinib (30 mg/kg). Effects noted in multiple-dose toxicity studies in mice (up to 4 weeks), rats (up to 6 months), and dogs (up to 12 months) were primarily those associated with the mechanism of action of ruxolitinib, a potent and reversible inhibitor of JAK-STAT signaling. Decreases in red blood cells, reticulocytes, eosinophils and lymphocytes have been observed along with lymphoid depletion in bone marrow and lymphoid organs. In addition, in dogs, demodectic mange, bacterial pneumonia and viral-induced papillomas, expected consequences of the pharmacology of JAK inhibition, were noted.

Embyro-fetal assessments in rat and rabbit, maternal toxicity and minimal embryo-fetal toxicity were noted at the highest doses. No teratogenicity was noted in rat or rabbit animal models. There were no reported effects on fertility or early embryonic development. In an evaluation of fertility and early embryonic development, no effects were noted on reproductive performance or fertility in male or female rats. Increases in post-implantation loss were noted at the higher doses. In a pre- and post-natal development and maternal function study in rats there were no adverse findings for fertility indices or for maternal and embryo-fetal survival, growth, and developmental parameters. Ruxolitinib passed into the milk of lactating rats with an exposure that was 13-fold higher than maternal plasma exposure.

d. Absorption, bioavailability, and pharmacokinetics

Ruxolitinib is a potent and selective inhibitor of the JAKs with selectivity for JAK1 and JAK2 (Quintás-Cardama et al, 2010). Ruxolitinib potently inhibits JAK1 and JAK2 (IC50<5nM), yet it does not significantly inhibit a broad panel of 28 kinases (<30% inhibition) when tested at 200 nM (approximately 100 × the average IC50 value for JAK1 and JAK2 enzyme inhibition). In cell-based assays relevant to the pathogenesis of MPNs (myeloproliferative neoplasms), such as JAK/STAT signaling and the growth of cytokine-dependent tumor cell lines, ruxolitinib demonstrates IC50 values of 80-300 nM. This effect is not due to general cytotoxicity, because ruxolitinib (up to 25 μM) had no significant effect on the growth of a cytokine-independent, BCR-ABL-driven cell line. In addition, ruxolitinib inhibited JAK/STAT signaling and growth of a cell line expressing the constitutively active JAK2 mutant (JAK2V617F) that has been implicated in the pathogenesis of the majority of Philadelphia chromosome negative MPNs. Ruxolitinib was also tested in cell-based assays relevant to the increased inflammatory cytokine levels observed in MPNs that contribute to MPN-related systemic symptoms. Ruxolitinib potently inhibited IL-23 stimulated IL-22

production in human T cells (IC50=50 nM), as well as IL-6, GM-CSF and TPO induced STAT3 phosphorylation in human peripheral blood mononuclear cells with IC50 values < 100 nM. Ruxolitinib also inhibited GCSF stimulated STAT3 phosphorylation in human neutrophils (IC50=28 ± 9 nM), as well as TPO induced STAT3 phosphorylation in human whole blood (IC50=281 ± 62 nM). Finally, ruxolitinib inhibited the production of IL-17 in response to IL-23 in T cells, and the production of monocyte chemotactic protein-1 in response to IL-6 in peripheral blood mononuclear cells with IC50 values of ≤120 nM (Fridman et al, 2011).

Ruxolitinib inhibited splenomegaly in mice resulting from intravenous (IV) inoculation of cells expressing the clinically relevant JAK2V617F mutation (Quintás-Cardama et al, 2010). After 3 weeks of treatment, more than 90% of vehicle-treated mice had succumbed to disease while more than 90% of ruxolitinib -treated mice survived. Treatment with ruxolitinib also reduced inflammatory cytokine levels in these mice. The effects of ruxolitinib were also tested in a mouse disease model of polycythemia vera-like disease, based on transplantation of a 1:1 ratio of JAK2 wild type green fluorescent protein (GFP)-expressing murine bone marrow cells with JAK2V617F-positive murine bone marrow cells, which have a repopulation advantage. In this model, oral ruxolitinib treatment at doses of 30 or 90 mg/kg twice a day for 21 consecutive days reduced levels of phosphorylated STAT5 (pSTAT5) in the spleen, as well as the spleen size. Ruxolitinib also effectively reduced the red cell parameters (RBC count, Hgb, Hct and reticulocyte count) and neutrophil count. The treatment was well tolerated as assessed by monitoring body weight, and histological assessments of spleen and bone marrow samples post-therapy revealed a decrease of hypercellularity (erythroid and myeloid lineages) in ruxolitinib treated groups as compared to vehicle treated animals. However, no significant decrease in the mutant allele burden surrogate readout (percentage of GFP-negative cells, i.e. cells expressing JAK2V617F) was observed, as assessed by flow cytometry. Treatment of mice with ruxolitinib in a cytokine-dependent multiple myeloma (MM) xenograft model resulted in a dose-dependent suppression of phosphorylated STAT3 (pSTAT3) and tumor growth. Efficacy was also observed in additional preclinical tumor models representing both hematologic and solid tumors. Taken together, these data indicate that ruxolitinib will be able to inhibit wild-type and mutant JAKs in the clinical setting and are consistent with its observed efficacy in patients with MPNs.

e. Clinical Trials

The clinical database (safety set) in solid tumor and hematologic malignancies consists of 787 patients treated in 6 studies evaluating patients with myelofibrosis (MF) (n=679), prostate cancer (n=22), MM (n=13), essential thrombocythemia (ET), and polycythemia vera (PV) (n=73), of whom 617 patients received ruxolitinib. Hematologic events are the most frequently reported adverse events (AEs) and include thrombocytopenia and anemia. The majority of these AEs are of Grades 1-2, seldom led to study drug discontinuation (<1% of patients), and can be usually managed through dose reduction or interruption. Increased rates of anemia resulted in an increase in packed red blood cell (PRBC) transfusion requirements for some ruxolitinib-treated patients. Platelet transfusions while on ruxolitinib were rare. Biochemistry laboratory abnormalities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and cholesterol were reported. The majority of these increases were Grade 1 or 2. No Grade 4 events were reported.

<u>Safety in clinical pharmacology studies</u>

The safety profile for ruxolitinib in the Phase I development program was assessed in over145 healthy subjects for single doses from 5 mg to 200 mg, and in 53 healthy subjects for repeat doses from 15 mg to 50 mg b.i.d. and 50 to 100 mg q.d. Ruxolitinib has also been administered to 32 subjects with various degrees of renal impairment, 24 subjects with various degrees of hepatic impairment, and 50 subjects with RA (rheumatoid arthritis). AEs were, in general, mild and resolved without interventions. In the first in human study one subject had hyponatremia after receiving 5 mg ruxolitinib. The hyponatremia was assessed as severe in intensity, unrelated to study medication, reversed within 5 days, and was reported as a serious adverse event (SAE). A Phase I dose escalation study and a study to evaluate food effect in Japanese healthy volunteers showed that administration of ruxolitinib with a high-fat meal led to results that were in line with results from the North American study. A Phase I dose escalation study in Chinese healthy volunteers showed similar overall results over a dose range of 10-50 mg. The pharmacokinetics of ruxolitinib was linear in the dose range studied, with a short half-life of 2-4 hours resulting in no notable drug accumulation following multiple doses.

In the repeat-dose study in healthy subjects, the intensity of an adverse event was graded according to the protocol-defined toxicity criteria based on Rheumatology Common Toxicity Criteria V 1.0. The dose-limiting AE was neutropenia, which occurred at a dose of 50 mg b.i.d. Neutropenia as an AE was noted in three subjects, all receiving the highest dose of ruxolitinib, 50 mg b.i.d. Neutropenia at the Grade 4 level, assessed as severe, led to study drug discontinuation on Day 5 in one subject, and was reported as a SAE. Neutrophil count returned to a normal level 12 days after the final dose of study medication. In two other subjects, neutropenia was Grade 1 or 2, and resolved with dose interruption or during continued dosing. The AE profile was similar for single- and multiple-dose studies, and no differences were observed between males and females. The most frequent (≥2 subjects) treatment-emergent AEs (TEAEs) occurring in the Phase I multiple-dose study were: neutropenia (4.2%), dizziness (2.8%), headache (2.8%) and nausea (2.8%). Overall, in healthy volunteer studies where frequent sampling of the neutrophil count was performed, a transient, reversible decrease in neutrophil count was frequently seen following dosing, which reversed after 12-24 hours off drug.

Two clinical pharmacology studies are ongoing to evaluate the effect of ruxolitinib on the pharmacokinetics of orally administered midazolam in healthy males subjects and of monophasic oral contraceptives in health female subjects.

The first Periodic Safety Update Report (PSUR) for ruxolitinib covered the reporting period from 16 Nov 2011 to 22 Feb 2013. A critical analysis of the efficacy and safety data revealed that the overall benefit-risk assessment of ruxolitinib remains favorable. Based on a single confirmed case of PML in an MF patient treated with ruxolitinib, this condition has been identified as an important potential risk. Reports of tuberculosis continue to be received in ruxolitinib-treated subjects in clinical trials. While the cumulative incidence of these tuberculosis reports appears to decline since the original Phase III trials, given the potential impact of JAK1/JAK2 inhibition upon immune defense, the risk tuberculosis will be monitored closely in subsequent PSURs.

Phase III studies in patients with myelofibrosis

The Controlled Myelofibrosis Study with Oral JAK Inhibitor Treatment I, (COMFORT-I) was a randomized, double-blind, placebo-controlled, multicenter study comparing the efficacy and safety of ruxolitinib tablets to a matched placebo in 309 patients with MF. The primary objective was to evaluate the efficacy, safety and tolerability of ruxolitinib given b.i.d. compared to placebo in patients with MF. Patients were randomized in a 1:1 ratio of ruxolitinib: placebo with no stratification. Ruxolitinib starting dose was based on baseline platelet count as follows: patients with baseline platelet count >200 x109/L started at 20 mg b.i.d. and patients with baseline platelet count >100 x 109/L to ≤200 x 109/L started at 15 mg b.i.d. The primary endpoint for this study was the percentage of patients with ≥35% reduction in spleen volume assessed by imaging (MRI or CT) from baseline, at Week 24. The Controlled Myelofibrosis Study with Oral JAK Inhibitor Treatment II (COMFORT II) was an open-label, randomized, active-comparator, multicenter study comparing the efficacy and safety of ruxolitinib tablets versus best available therapy as selected by the investigators in 219 adults patients with myelofibrosis. Key eligibility criteria were as follows: adult MF patients, not candidates for stem cell transplantation, with two or more risk factors identified by IPSS, palpable spleen length ≥5 cm below the left costal margin, peripheral blast count <10%. The primary endpoint for this study was the percentage of patients with ≥35% reduction in spleen volume assessed by imaging (MRI or CT) from baseline, at Week 48. These trials identified statistically significantly larger proportion of patients randomized to ruxolitinib achieved a ≥35% reduction from baseline in spleen volume compared to patients randomized to control. This effect was seen at both Week 24 (primary endpoint in COMFORT-I) and Week 48 (primary endpoint in COMFORT-II). For patients who remained on study, the median percent reduction from baseline in spleen volume in both ruxolitinib arms was approximately 30%, was reached as early as Week 12 and was maintained throughout the study. The control arms had a median percent increase from baseline in spleen volume throughout the study.

For additional information see the ruxolitinib Investigator's Brochure, Edition 12, dated 11 September, 2013.

Phase III study in patients with polycythemia vera

The RESPONSE Trial is a global, randomized, open label phase III pivotal study comparing ruxolitinib (starting dose of 10 mg b.i.d.) with best available therapy (HU, pipobroman, immunomodulatory drugs, pegylated interferon or interferon, anagrelide, observation only) in PV patients. The protocol is designed for patients with evidence of need for phlebotomy and splenomegaly, either resistant or intolerant to HU. The primary endpoint is a composite one with phlebotomy control (absence of phlebotomy from Week 8 to 32) and reduction in spleen volume by MRI (or CT if MRI is clinically contraindicated) of at least 35% measured at 32 weeks. Two key secondary endpoints include durability of the primary endpoint and complete hematologic remission. There are multiple, non-key secondary endpoints including modified clinico-hematologic response (overall response rate, durability and duration), as defined by the European Leukemia Net (ELN). The study is ongoing and 147 patients have been enrolled so far (as of 1 August 2012).

Phase I/II study in patients with multiple myeloma

Ruxolitinib is being evaluated in a phase II, multicenter, Simon-two-stage, open label, clinical trial to) in patients with relapsed or refractory MM. This was a multi-center, Simon 2-stage, open-label clinical trial. Patients were to receive ruxolitinib 25 mg orally b.i.d. on each day of each 28-day cycle. Tumor responses were assessed every cycle. Responding patients

could continue on ruxolitinib monotherapy. The patients who were assessed to have progressed at any time or were assessed to have stable disease after 3 cycles of ruxolitinib monotherapy had the addition of 40 mg oral dexamethasone daily on Days 1-4, 9-12, and 17-20 of each 28-day cycle (combination phase of the trial). Each cycle of dexamethasone was repeated every 28 days for 4 cycles. After the fourth cycle, 40 mg of dexamethasone was to be administered only on Days 1-4 of subsequent cycles. Treatment could be continued until occurrence of progressive disease or unacceptable toxicity. A total of 13 MM patients have been enrolled in Stage 1 to receive ruxolitinib as monotherapy (11 were evaluable for response). Seven of these subjects received ruxolitinib in combination with high dose dexamethasone after their disease progressed or after having had stable disease on ruxolitinib monotherapy. No MM patient has been enrolled in stage 2. None of the 11 MM evaluable subjects achieved a response according to the IRC (International uniform Response Criteria) when treated with ruxolitinib monotherapy. However, with combination therapy of high dose dexamethasone added to ruxolitinib, two out of seven subjects achieved a partial response and one subject had long-term stable disease.

Phase I/II study in patients with prostate cancer

A Phase II, open label study of ruxolitinib administered orally to patients with androgen independent metastatic prostate cancer (AIPC) was performed. The primary endpoint was PSA50 (prostate-specific antigen) response rate, a clinically validated endpoint associated with clinical benefit in patients with metastatic prostate cancer. This endpoint is defined as a PSA decline from baseline of 50% or greater, confirmed by measurement on two occasions at least 4 weeks apart. The study used a Simon 2-stage optimal design. The first stage of the trial (Part 1) was designed to enroll 21 patients. If fewer than two PSA50 responses were observed in the first
21 patients (no later than 10 weeks after the last patient in the first stage is enrolled), the trial would be closed and the agent will be declared inactive as a monotherapy. If two or more PSA50 responses are seen in the first stage, an additional 20 patients would be enrolled in the second stage (Part 2) for a total of 41 patients. The study in patients with advanced prostate cancer completed enrollment of 22 subjects in the first stage of the study. The study endpoint permitting advancement to a second stage of the study was not reached. Seventeen of 22 subjects enrolled experienced death or protocol-defined progressive disease while on study.

Given that all patients discontinued the study due to protocol-specified lack of efficacy, the primary efficacy endpoint of PSA50 response rate was not assessed. Similarly, the secondary endpoints of median time to progression and tumor response rates were not assessed. All patients provided samples for the secondary endpoints of evaluating population PK and plasma PD markers; however, the samples were not assessed as part of this analysis.

The primary endpoint of safety and tolerability was assessed in this analysis. All patients experienced at least one TEAE, and the majority of patients (16 patients; 72.7%) experienced treatment-related TEAEs. The events associated with ruxolitinib treatment included anemia, fatigue, diarrhea, nausea, leukopenia and peripheral edema, all of which were not unexpected events in this patient population of metastatic androgen AIPC, as were the most frequently reported severe or life-threatening TEAEs (anemia, thrombocytopenia, peripheral edema, increased blood alkaline phosphatase, bone pain).

Treatment with ruxolitinib was generally well-tolerated. Among the 11 SAEs reported by nine patients, the only SAE that was reported by more than one patient was anemia (two patients), and only two SAEs were considered to be possibly or definitely related to study

medication by the investigator (cardiac arrest and anemia, respectively). Further, only one patient discontinued treatment as a result of an AE (anemia). Among the four deaths, cardiac arrest was deemed by the investigator as possibly related to study medication in one patient, but the patient had other risk factors that may have also contributed to the event; the other three deaths were likely associated with medical history and metastatic AIPC.

Eight patients had laboratory abnormalities that resulted in serious, severe, or life-threatening AEs, which included the following events: anemia, increased blood alkaline phosphatase, increased γGT, thrombocytopenia, hyperuricemia, and leukopenia. There was no specific trend among patients with regards to laboratory abnormalities or ECG values out of the normal range. Overall, there were no specific safety concerns associated with ruxolitinib.

Phase IIa study in patients with rheumatoid arthritis (RA)
A total of 50 patients were enrolled to a double-blind, placebo-controlled study exploring the safety, tolerability, and efficacy of a 4-week course of ruxolitinib in subjects with active RA.This trial consisted of Cohort 1: 16 patients (12 received 15 mg b.i.d. of ruxolitinib, and four received placebo), Cohort 2: 34 patients (nine received 5 mg b.i.d. of ruxolitinib, 10 received 25 mg b.i.d. of ruxolitinib, 10 received 50 mg q.d. of ruxolitinib, and five received placebo). Efficacy demonstrated in this study has been notable. Efficacy greater than placebo was demonstrated in the top three doses in this study, with ACR20 (American College of Rheumatology 20% improvement response criteria) responses ranging from 50-83% of subjects, ACR50 responses in 40-50% of subjects, ACR70 responses in 25-30% of subjects, and ACR90 responses in 10-17% of subjects, while the placebo group and 5 mg b.i.d. group exhibited ACR20 responses of 33% and ACR50 responses of 11% with no ACR70 or 90 responses.

Ruxolitinib was well tolerated in RA patients dosed for 28 days with regimens including 5 mg b.i.d, 15 mg b.i.d., 25 mg b.i.d., and 50 mg q.d. AEs were seen in similar frequency in patients receiving ruxolitinib as in patients receiving placebo. The intensity of AEs was graded according to the protocol-defined toxicity criteria based on the Rheumatology Common Toxicity Criteria (Version 1.0). In general, AEs were mild and self-limited usually resolving with continued dosing. There were no clinically significant changes in mean Hgb or platelet counts, and mean neutrophil counts remained within the normal range. One patient dosed with 25 mg b.i.d. exhibited Grade 3 neutropenia, which improved with continued dosing, and one patient, who had a prior history of thrombocytopenia, probably immune-mediated thrombocytopenia, exhibited Grade 3 thrombocytopenia. SAEs of interstitial lung disease and congestive heart failure occurred with onset 20 days following the last dose of study medication (50 mg q.d.) in a patient taking methotrexate with coronary artery disease. The SAE resolved with treatment and was assessed as possibly related to study medication.

2.4 Rationale

In addition to trastuzumab, other HER2 therapies that specifically target upstream HER2 activity have been approved in HER2+ metastatic breast cancer, including pertuzumab, lapatinib, and T-DM1. Unfortunately, the control of tumor progression is only temporary and the majority of the patients' tumors eventually acquire resistance and progress (Hynes et al, 2005). Thus, there is the need to find alternative tumor targets to develop specific, more efficient, treatments for patients with ErbB2+ breast cancer. By combining RNAi loss-of-function screens with system biology interactome models, we completed a genome-wide screen searching for gene/pathways that are essential for the survival of ErbB2 overexpressing breast tumor cells but dispensable for normal cells (synthetic lethal

interactions). We identified that the JAK/STAT3 pathway is activated in HER2+ breast cancer and that blocking this pathway strongly reduces the viability of HER2+ cells.

In a tumor cell, by virtue of their accumulated alterations, multiple regulatory networks have been rearranged in order to adapt to specific genomic abnormalities. Consequently, tumor cells acquire specific vulnerabilities that create opportunities for therapeutic intervention. During the transformation mediated by overexpression of the oncogene ErbB2, we hypothesize that breast cancer cells become addicted to the activation of Stat3. Disruption of the pathway defined by the signaling 'IL6-JAK/STAT3-downstream targets' compromises the viability of ErbB2+ cancer cells without affecting non-transformed ones. Thus, key components of this pathway represent a novel strategy to select rational putative targets for the treatment of HER2+ breast cancer.

Computational biology algorithms have been successful in reverse engineering the regulatory networks of normal and neoplastic human cells from large gene expression profile (GEP) collections (Basso et al, 2006; Carro et al, 2009; Margolin et al, 2006; Wang et al, 2009). These methods have produced the first cell-context specific, genome-wide maps of transcriptional and post-translational interactions in human cells, "interactomes." By using two of these algorithms, ARACNe and MINDy, we have reconstructed the interactome of human mammary epithelial cells transformed with the ErbB2 oncogene. This has provided us with a list of gene functions that are differentially active between ErbB2-transformed and non-transformed mammary epithelial cells. We predict that preventing the activation of some of these functions will impact the transformation mediated by ErbB2. Although the value of the mentioned computational methods to study human cancer from an unbiased global perspective is unquestionable, these methods are hypothesis-generating strategies and must be validated. Importantly, the impact of these algorithms is severely compromised by the low through-put approaches necessary to functionally validate the in silica predictions. RNA interference (RNAi) has emerged as a powerful genetic strategy to interrogate gene function by loss-of-function studies (Silva et al, 2004). We have pioneered the design, construction and validation RNAi-based genetic approaches to perform high-through put (HTP) screens in mammalian systems (Silva et al, 2004; Silva et al, 2007; Silva et al, 2008; Silva et al, 2009; Paddison et al 2004). Using this technology, we have performed genome wide RNAi screens in human normal mammary epithelial MCF-10A cells and in an isogenic variant transformed with oncogenic ErbB2, MCF-10A/ErbB2. The combination of the computational predictions and the loss-of-function studies have allowed us to identify the activation of the JAK/STAT pathway as essential for ErbB2 mediated transformation (**Fig 1**).

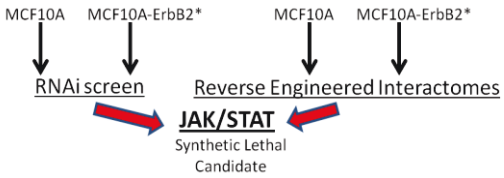

Fig 1: The genetic/ computational screen strategy

2.5 Preliminary Results

2.5.1 Target Validation

We observed that key members of the STAT family (Stat-1, 3 and 5) were phosphorylated upon ErbB2 overexpression (**Fig 2A**). The JAK/STAT pathway has been previously identified as a key component of the transformation of multiple tumor types, especially in lung cancer (Akca et al 2009, Byers et al 2009). Furthermore, an increasing amount of evidence points to this pathway as an essential hub of cancer cell homeostasis (Weerasinghe

et al 2007, Zhang et al 2007, Darnell et al 2005). Importantly, genetic (siRNA knock-down) and chemical (small molecule inhibitor, Stattic17) inhibition of Stat-3 reduced the viability of ErbB2+ cells without affecting non-transformed cells (**Fig 2B and 2C**). Annexin-V staining revealed that this effect was mediated by induction of apoptosis (**Fig 2C**). The dependency on Stat-3 had a profound effect in the growth of ErbB2+ cells not only *in vitro* but also *in vivo*, and knock-down of Stat-3 dramatically reduced the growth of ErbB2+ cells in mouse xenografts (**Fig 2D**). Once we validated that Stat-3 activation is essential to maintain the homeostasis of the ErbB2+ cells, we decided to investigate the mechanism that mediates its activation. By comparing the expression profile of parental and ErbB2 activated cells we found a strong upregulation of multiple interleukin molecules and, importantly, their receptors. The most upregulated interleukin (> 20 times) was intereukin-6 (IL-6), a well characterized bona-fide activator of Stat-3. Furthermore, the canonical IL-6 receptor (IL6R) was also found to be up-regulated. Importantly, the high levels of IL-6 mRNA detected were also associated with higher levels of secreted IL-6 into the culture media (**Fig 2E and 2F**).

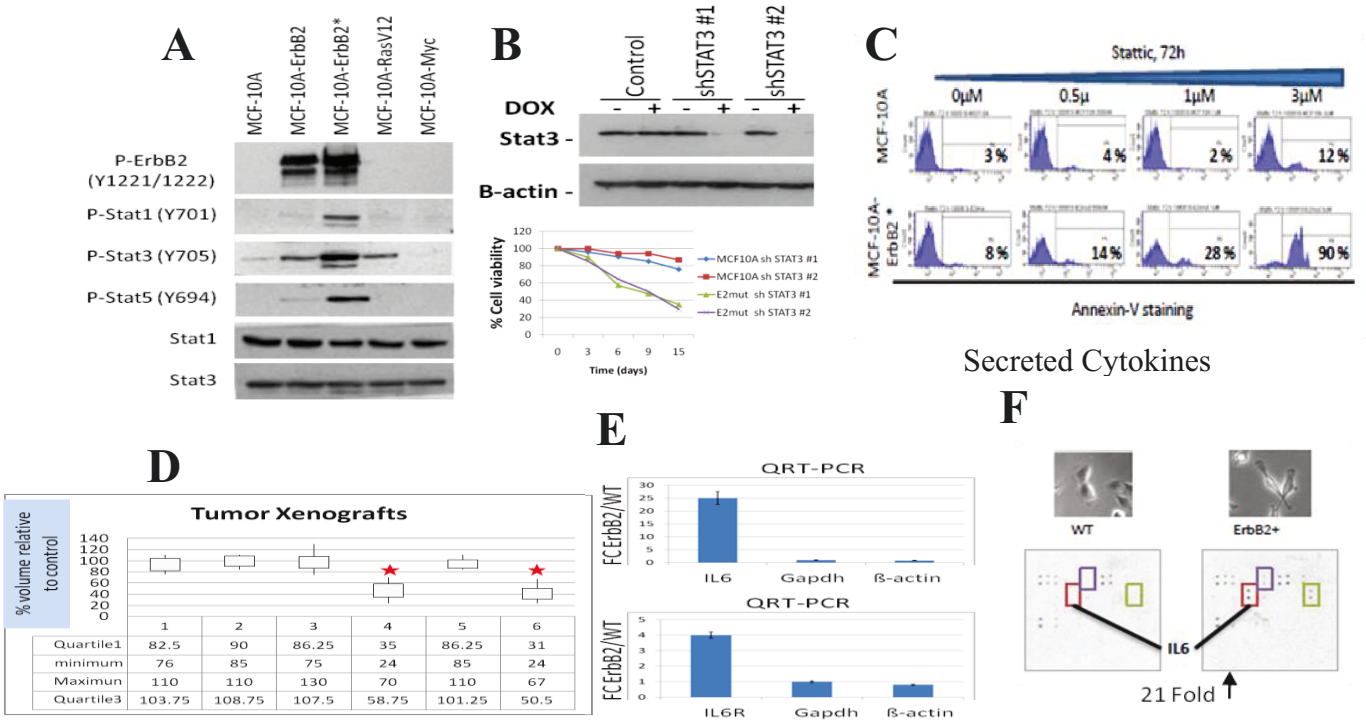

Fig 2: Activation of Stat3 is necessary for transformation of mammary epithelial cells induced by ErbB2. A) Phosphorylation levels of Stat members in parental and human mammary epithelial MCF-10A cells transformed with wild type ErbB2 or an activated form ErbB2*. B) The upper panel shows the efficiency of two shRNA against Stat3. The lower panel shows the impact that knock-down of Stat3 using the shRNAs from upper panel has on cell viability in parental and Erbb2-transform MCF-10A cells. C) Annexin-V staining showing that reduction in cell viability is associated to apoptosis. D-F) QRT-PCR data showing increased levels of IL6 and its receptor IL6R in ErbB2-transformed MCF-10A cells. F) Cytokine secretion of MCF10A and MCF10A-ErbB2 cells measured by Cytokine array.

2.5.2 Target Modulation

These data suggest a model where cells with activated ErbB2 produce high levels of IL-6 and upregulates its receptor. Secretion of this interleukin generates an autocrine loop that activates the Jak/Stat pathway, inducing the phosphorylation of Stat-3 that is necessary for the transformation process.

In order to translate our results to the clinical setting, we searched for compounds that could inhibit STAT3 activity *in vivo*. Because STAT3 mainly mediates its action through protein-

protein or protein-DNA interactions instead of an enzymatic activity, it has been considered difficult to target by classical mechanisms. Several approaches have been proposed to inhibit IL6/JAK/STAT pathway being the most used ones, based on the inhibition of the receptor activation via blocking antibodies such as Tocilizumab; STAT3 binding molecules, which show poor success; and inhibitors of JAK kinase activity (Sansone 2012).
First, we incubated MCF-10A/ErbB2 cells with a single dose of 10uM of ruxolitinib for increasing times and analyzed the effect on different components of the pathway by western blot (**Fig 3A**). Our results showed that ruxolitinib treatment decreases the phosphorylation of STAT3 in a time-dependent manner with the concomitant trigger of apoptosis in MCF-10A/ErbB2 as shown by the cleavage of PARP. These results confirmed the essential role of STAT3 activation in cells that overexpress ErbB2.

Second we studied by soft agar assays the effect of ruxolitinib on transformation driven by ErbB2-overexpression. As shown in **Fig 3B**, treatment with increasing doses impaired the anchorage-independent growth of MCF-10A/ErbB2 cells. Then, we followed the formation of acini in matrigel when treating with ruxolitinib. As shown, incubation with 5-10uM of ruxolitinib affects the growth of MCF-10A/ErbB2 cells in 3D, forming smaller structures with a normal-like shape, while MCF-10A cell fitness is almost not affected (**Fig 3C**).

In vivo, we injected MCF-10A/ErbB2 cells into the mammary fat pad of SCID mice and orally administered 90mg/kg of ruxolitinib twice a day. Treatment decreased tumor growth while was not toxic for the mice as indicated in no significant weight loss (**Fig 3D**). Finally, we tested if combining ruxolitinib with standard anti-ErbB2 therapy provided additional benefits. Importantly, we found that combination of ruxolitinib plus trastuzumab significantly inhibited tumor growth compared to either treatment alone (**Fig 3E**). In summary, we have demonstrated that the JAK/STAT pathway is a master regulator of ERBB2+ breast cancer and represents a novel orthogonal approach to treating these tumors. All together, these data provide a strong rationale for testing the efficacy of ruxolitinib treatment of ERBB2 amplified breast cancer in combination with trastuzumab.

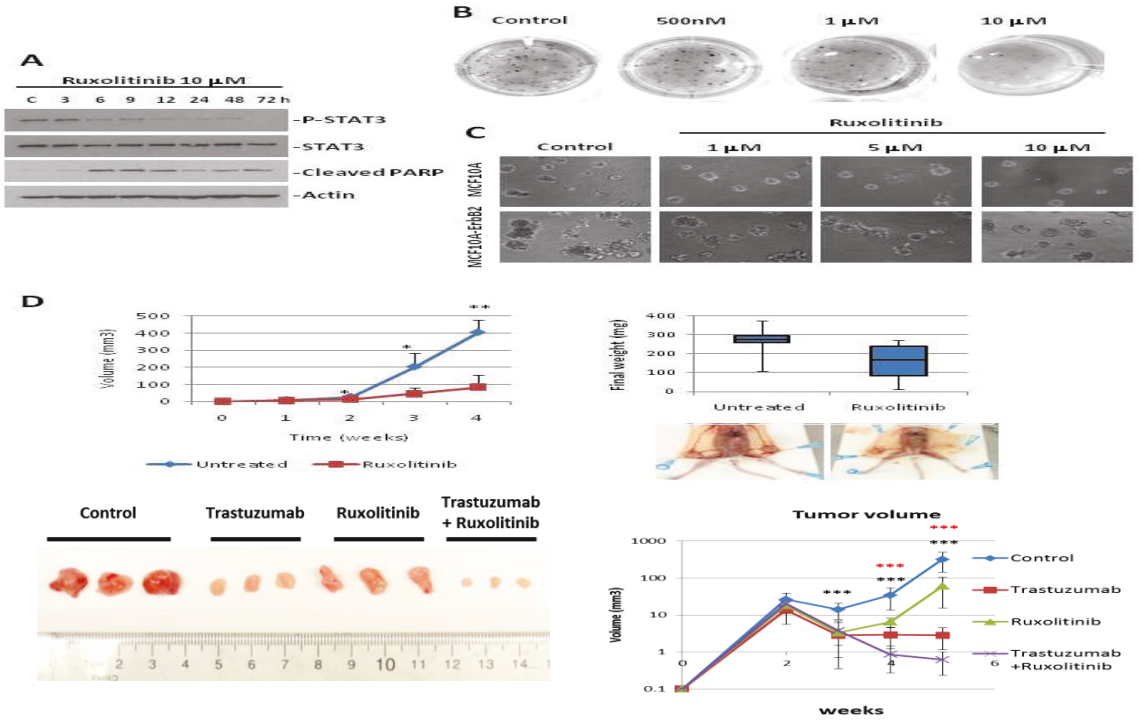

Fig 3. Ruxolitinib treatment impairs transformation of MCF-10A/ErbB2 cells. (A) Western blot showing the effect of incubation with ruxolitinib 10uM in phosphorylation of STAT3. Treatment with the JAK inhibitor affects the expression of S100A8 and S100A9 while promoting PARP and caspase3 cleavage. (B) and (C) Soft agar and matrigel assays in MCF-10A/ErbB2 cells treated with increasing doses of ruxolitinib. (D) Growth of MCF-10A/ErbB2 tumors in immunocompromised mice when treated with ruxolitinib. The right graph shows the final weight of the extracted tumors. (E) Growth of MCF-10A/ErbB2 tumors when mice were treated with different drug combinations.

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before treatment, C2D1, and after treatment metastatic biopsies. RNAseq will be performed on all tumor tissues to assess intrinsic molecular subtype, mutations, and interactome modeling for JAK/SAT pathway dependence. Metastatic biopsies will also be sent for 150 protein reverse phase protein analysis (RPPA). These unbiased approaches will allow for integrated exploration of candidate resistance and sensitivity biomarkers. In addition, specific candidate biomarkers will be assessed including IHC for pSTAT3 (Cell Signaling), serum IL-6 and IL-8 (Immulite 1000, Siemens Healthcare Diagnostics), and serum C-reactive protein (Cobas Integra 400 Plus, Roche Dx).

2.6.1 Transcriptional Profiling

RNA sequencing [RNA-seq] has emerged as the single most powerful genomic profiling technology. It can be performed from less tissue [requires only 100-200ng RNA] and at lower cost than whole genome sequencing and delivers identification of both mutational events and gene expression. Unlike traditional RNA microarray analysis, RNA-seq is unbiased with respect to potential content, so it can detect previously unidentified genes or transcripts which may be involved in the phenotype of interest. One of the biggest advantages of RNA-seq over microarray analysis is the ability to detect single nucleotide variants, small insertions and deletions (InDels) and transcript isoforms. RNA-seq also offers improved specificity than microarrays, so it can detect different isoforms of a transcript, and offers increased dynamic range of expression over microarray analysis. Given the power of RNA-seq it is our chosen method of transcriptional profiling. The quality of RNA isolated from FFPE samples is potentially compromised by loss in poly-A tails, fragmentized, and chemical modification, with possible additional deterioration from prolonged storage times. However, even with these limitations, RNAseq from FFPE breast cancers has been successful [PMID: 22808097]. In addition, the Columbia University genome center has compared RNA sequencing from the same samples, fresh frozen and PPFE, and found highly concordant sequencing.

2.6.2 Reverse Phase Proteomic Array (RPPA)

Reverse phase protein array (RPPA) is a high-throughput antibody-based technique developed for functional proteomics studies to evaluate protein activities in signaling networks. RPPA has been developed and validated as a quantitative, sensitive, and reproducible proteomic technology. It is inexpensive, automated, and only requires 30 micrograms of sample. Over 150 total and phosphospecific antibodies can be assessed. RPPA study has major strengths in identification and validation of cellular targets, characterization of signaling pathways and networks, as well as determination of on and off target activity of novel drugs. Among the cancer pathways represented in the RPPA data are the pathways most important for analysis of the combined treatment with trastuzumab and ruxolitinib, including the JAK/STAT, PI3K, and MAPK pathways. Each sample is analyzed for cell cycle progression, apoptosis, functional proteomics, and signaling network activity. RPPA is the current technology being used for proteomic profiling in the cancer genome atlas (TCGA) project. Importantly, RPPA has recently been validated using FFPE tissues (Guo, Wang et al. 2007, Guo, Liu et al. 2012). In addition, the molecular pathology core at Columbia University Medical Center has experience preparing RPPA samples and analyzing the results [unpublished results].

3. PATIENT SELECTION

3.1 Inclusion Criteria

A subject must meet all of the following criteria to be eligible for the study.

1. Subjects must have histologically or cytologically confirmed adenocarcinoma of the breast with locally recurrent or metastatic disease. Locally recurrent disease must not be amenable to any local treatment with curative intent. Metastatic disease must be demonstrated either radiographically or histologically.
2. Primary tumors and/or metastatic lesions must demonstrate HER2-neu overexpression, per the 2013 recommendations (Wolff, Hammond et al. JCO 2013), i.e. immunohistochemistry (IHC 3+) or amplification by in situ hybridization based on the following:
   a. Single-probe average *HER2* copy number ≥6.0 signals/cell
   b. Dual-probe HER2/CEP17 ratio ≥2.0 with an average HER2 copy number ≥4.0 signals/cell
   C. Dual-probe HER2/CEP17 ratio ≥2.0 with an average HER2 copy number <4.0 signals/cell
   d. Dual-probe HER2/CEP17 ratio < 2.0 with an average HER2 copy number > 6.0 signals/cell

   Patients may be estrogen and/or progesterone positive (≥1%) or negative (<1%). Hormone receptor status will be a stratification factor.

3. Patients must have received at least two lines of HER2-directed therapy in the inoperable locally advanced and/or metastatic setting. Prior therapy for inoperable locally advanced/metastatic disease should include trastuzumab plus pertuzumab as well as ado-trastuzumab. Pertuzumab and ado-trastuzumab should have been previously used, unless for reasons that include, but are not limited, to the following: intolerance to pertuzumab and/or ado-trastuzumab, medical contraindication, regimen declined by patient, treating investigator discretion, or medical insurance coverage issues which prevented administration of pertuzumab or ado-trastuzumab. These reasons must be reviewed with the study chairs and documented in the medical record and care report form. Patients who relapse within 12 months of completing neoadjuvant/adjuvant pertuzumab or ado-trastuzumab would be considered as having progressed on that regimen.

Patients may have measurable disease only, non-measurable disease only, or both (RECIST 1.1). Concomitant treatment with bone-targeted therapies such as RANKL inhibitors or bisphosphonates is allowed. It is anticipated that most patients will have measurable disease, given the behavior of HER2+ metastatic breast cancer.

4. Because no dosing or adverse event data are currently available on the use of ruxolitinib in combination with trastuzumab in patients <18 years of age, children are excluded from this study.

5. Women and men of all races and ethnic groups are eligible for this trial.

6. ECOG performance status 0-2 (Karnofsky ≥60%, see Appendix A).

7. Left ventricular ejection fraction ≥ 50% by transthoracic echocardiography or multi-gated acquisition scan (MUGA) within 28 days prior to the first dose of the study drug.

8. The subject has a baseline corrected QT interval ≤ 480ms.

9. Patients must have normal organ and marrow function as defined below:
   - leukocytes ≥ 3,000/mcL
   - absolute neutrophil count ≥ 1,500/mcL
   - platelets ≥ 100,000/mcL
   - hemoglobin ≥ 9 g/dL
   - total bilirubin ≤ 1.5 × the upper limit of normal
   - AST(SGOT)/ALT(SGPT) ≤ 2.5 × institutional upper limit of normal
   - Serum creatinine ≤ 1.5 × the upper limit of normal or calculated creatinine clearance ≥ 60 mL/min

10. Women of childbearing potential and men must use adequate contraception prior to study entry and for the duration of study participation. Contraception should continue to be used for a minimum of 5 mean half-lives after the last dose of study drugs (mean Trastuzumab half-life at 6 mg/kg 16 days; mean half-life Ruxolitinib: 3 hours)

11. Patient is able to swallow, retain, and absorb oral medication.

12. Life expectancy of at least 12 weeks

13. Informed Consent. Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

A subject who meets any of the following criteria is ineligible for the study:

1. Patients who have had chemotherapy, hormonal therapy, or radiotherapy within 2 weeks prior to entering the study or those who have not recovered from adverse events due to agents administered more than 2 weeks earlier.

2. Patients who are receiving any other investigational agents or have received other investigational agents within 2 weeks or 5 half-lives of the compound or active metabolites, whichever is longer before the first dose of the study treatment.

3. Patients who have previously been treated with an IL-6, JAK or STAT inhibitor for any indication, such as ruxolitinib or tocilizumab.

4. Symptomatic or unstable brain metastases. (Note: Asymptomatic patients with metastatic brain disease who have been on a stable dose of corticosteroids for treatment of brain metastases for at least 14 days prior to randomization are eligible to participate in the study).

5. History of allergic reactions attributed to compounds of similar chemical or biologic composition to ruxolitinib or trastuzumab.

6. The effects of ruxolitinib on the developing human fetus are unknown. For this reason and because JAK2 inhibitor agents as well as other therapeutic agents used in this trial are known to be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform the principal investigator immediately.

7. Patients receiving any medications or substances that are strong inhibitors of CYP450 3A4 isoenzyme are ineligible. Please refer to **Appendix C** for list of common strong inhibitors of the CYP 3A4 isoenzyme. This list is not meant to be comprehensive, but provides an example of medications. If there it remains unclear as whether the medication is a strong inhibitor, check the prescribing information of the medication for accurate details. Patients must be off the strong inhibitor for at least 1 week prior to being deemed eligible.

8. Patients may not have an uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness that would limit compliance with study requirements.

9. Patients must not have clinically significant cardiovascular disease (New York Heart Association Class III or IV heart failure), uncontrolled clinically significant atrial or ventricular cardiac arrhythmias, or any of the following within the past 6 months: myocardial infarction, new evidence of transmural infarction on ECG, unstable angina, coronary angioplasty.

10. Pregnant women are excluded from this study because ruxolitinib is a Class C agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with ruxolitinib, breastfeeding must be discontinued if the mother is treated with ruxolitinib. These potential risks also apply to trastuzumab, which can cause fetal harm when administered to a pregnant woman.

11. Active Infections. Patients with known active infections with human immunodeficiency virus (HIV), hepatitis A virus (HAV), hepatitis B (HBV), and hepatitis C virus (HCV) infections will not be considered for this trial. HIV+ patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with ruxolitinib. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy. Testing for HIV or hepatitis is not required.

4. REGISTRATION PROCEDURES

CUMC Research Participant Registration
Confirm eligibility as defined in the section entitled Criteria for Subject Eligibility.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures, along with applicable institutional policies and federal regulations.

Only Investigators/Research personnel properly trained and delegated to consent subjects for this protocol will participate in the consenting process. Furthermore, properly delegated/trained Physician Investigators (i.e., MD, MD PhD) are required to sign/verify a protocol specific Eligibility Checklist for each subject enrolled on the study, in addition to providing the relevant source documentation to confirm subject eligibility.

All participants must be centrally registered through the Central Registration Office within Herbert Irving Comprehensive Cancer Center at CUMC prior to initiation of study treatment.

Registration hours are available Monday through Friday from 9:00am – 5:00pm EST (excluding holidays and weekends). Same day patient registrations (and after hour registrations) will be accommodated on a case-by-case basis provided that the study team has expressed all time sensitive registration concerns/cases in a timely manner to the Central Registration Office.

CPDM Central Registration Procedures:

Within 48 hours of obtaining consent (excluding holidays and weekends), a completed/signed IRB approved informed consent HIPAA form, and demographics forms must be submitted to the CPDM Central Registration Office via an email to CPDMRegistration@columbia.edu or fax to 212.305.5292, with the subject line "AAAM1906 Pending Subject Registration Request (PHI)". Upon receipt, applicable subject information as well as a "pending eligibility" status will be entered into HICCC's institutional database. This status will remain until further source documentation is made available to confirm overall patient eligibility. Required materials for all pending registration submissions are as follows:

- Completed/signed IRB approved/stamped Informed Consent Forms, including additional study ICFs (i.e. tissue, DNA, etc.) as applicable
- The completed/signed IRB approved HIPAA Authorization form
- Completed/signed CPDM ICF checklist
- Completed/signed HICCC personal census form
- Completed/signed CPDM Demographics Note to File

In order to confirm eligibility status, Investigators/designees (i.e., study specific Clinical Research Coordinator/Research Nurse, etc.) must submit the following documentation to the Central Registration Office via email or fax:

- The completed/signed study specific Eligibility Checklist (signed by a Physician level Investigator)
- Copies of source documentation necessary for each item to be verified on the CUMC specific Eligibility Checklist, including but not limited to:
    - Copy of required laboratory test and procedure reports (i.e., hematology, serum chemistry, pregnancy test when applicable, MRI reports, CT/bone scans or PET/CT, etc.)
    - Copy of pathology and surgical reports
    - Copy of clinic note(s) or other appropriate medical records capturing the consent process information, along with providing source documentation of

any other items needed for screening/eligibility that are not captured in other source document forms (e.g., positive investigator statements of unique eligibility items not captured via other direct source documentation, concomitant medication lists, etc)
    - Protocol deviation/waiver approvals (if applicable)
- **Please note**: subject line of email or fax should include the following: "AAAM1906 Complete Subject Registration Request (PHI)."

Upon Receipt of the above-mentioned documentation, participant eligibility information will be verified by a qualified Central Registration Registrar. If any questions arise during the review process, queries in the form of emails will be addressed to the applicable study team personnel for clarification prior to enrollment. All applicable finalized registration/eligibility information will then be entered into HICCC's institutional database by the Central Registration Registrar. Upon completion, an official subject registration notification email will be sent to the PI/research team, which will include eligibility/enrollment status, as well as subject ID information. Protocol therapy may not be initiated prior to receipt of this notification from the Central Registration Office.

All screenfail/ineligible subjects, as well as subjects who withdraw consent prior to enrollment/initiation of protocol therapy must be submitted to the Central Registration office in a manner analogous to the procedures noted above. Applicable source documentation will be required within the corresponding submissions.

Central Registration Procedures- Affiliate Institution Research Participant Registration Process:

All Affiliate Institutions **must** register subjects with the coordinating center (CUMC) **prior** to any administration of study drug/intervention/local institution registration. Please see instructions below:

Within 48 hours of obtaining consent (excluding holidays and weekends), **the Affiliate Institution CRN and/or CRC is required to submit the following documents to the coordinating center's designee (CUMC's Multicenter Core) via the study email at m1906@columbia.edu**. The coordinating center's designee will review the documents for accurateness, and subsequently submit the documents to the CPDM Central Registration Office via email at CPDMRegistration@columbia.edu (or via fax at 212.305.5292), with a request to register the patient "pending eligibility." The title of the email should read, "AAAM1906 Pending Subject Registration Request (PHI)". The following documents should be submitted with the pending registration request:

- Redacted Completed/signed IRB approved/stamped Informed Consent Forms, including additional study ICFs (i.e. tissue, DNA, etc.) as applicable
- Redacted Signed HIPAA (or institutional equivalent)
- MCT CPDM Demographics Note to File form

The Affiliate Institution's investigator/research nurse/data manager/coordinator must contact the coordinating center's designee (CUMCMulticenter Core) via telephone or email at m1906@columbia.edu to communicate the following:

- Notify of pending registration request
- Confirm method of registration request submission (email or fax)
- Communicate expected time-line of registration request submission (i.e., same day, next day, within the hour, etc.)

To complete registration, the Affiliate Institution's investigator/research nurse/data manager/coordinator should then submit the following documents to the CUMC study specific designee:

- A signed Affiliate Site Eligibility Checklist (signed by the investigator)
- Copies of redacted source documentation necessary for each item to be verified on the CUMC specific Eligibility Checklist, including but not limited to:
  - Copy of required laboratory test and procedure reports (i.e., hematology, serum chemistry, pregnancy test when applicable, MRI reports, CT/bone scans, etc.)
  - Copy of pathology and surgical reports
  - Copy of clinic note(s) capturing the consent process information, along with providing source documentation of any other items needed for screening/eligibility that are not captured in other source document forms. (e.g., positive investigator statements of unique eligibility items not captured via other direct source documentation, concomitant medication lists, etc.)
  - Protocol deviation/waiver approvals (if applicable)

Please note: subject line of email or fax should include the following: "AAAM1906 Complete Subject Registration Request (PHI)".

Upon receipt of the above mentioned documents, the designated study specific Clinical Research Coordinator will review all documents and verify patient eligibility. If any questions arise during the review process, queries in the form of emails will be addressed to the applicable affiliate site study team personnel for clarification prior to enrollment. Upon verification, the CUMC study specific designee will then forward all documents to the CPDM Central Registration Office for central registration (as described above). The CPDM Central Registration Registrar will review all applicable documents and communicate to the CUMC study specific designee in order to clarify any items. The CUMC study specific designee will communicate with the applicable site study team personnel for additional clarifications necessary prior to enrollment.

Upon receipt of the subject registration notification email, the CUMC study specific designee will forward the notification email (which will include the study specific patient ID) to the affiliate site's Principal Investigator, Consenting Professional, and applicable research personnel. This notification should be filed in the patient research binder accordingly. Protocol therapy **may not** be initiated prior to receipt of this notification from the coordinating center.

All screenfail/ineligible subjects, as well as subject's who withdraw consent prior to enrollment/initiation of protocol therapy must be submitted to the Central Registration office in a manner analogous to the procedures noted above. Applicable source documentation will be required within the corresponding submissions.

5. TREATMENT PLAN

5.1 Agent Administration

Treatment will be administered on an outpatient basis. Expected adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Table 1: Regimen Description

Agent	Premedications; Precautions	Dose	Route	Schedule	Cycle Length
Ruxolitinib	None	Phase I (assigned): BID dosing (10 mg, 15 mg, 20 mg, or 25 mg) Phase II: Based upon MTD from phase I	PO	Days 1-21	21 days (3 weeks)
Trastuzumab	None (unless prior reaction)	6 mg/kg in 250 cc NS (8 mg/kg initial re-load if no trastuzumab > 28 days)	IV over 90 minutes for initial dose. IV over 30-90 minutes for subsequent doses	Day 1, Week 1	

Treatment Cycle

The duration of a treatment cycle will be 21 days. Ruxolitinib will be taken orally twice daily, every day for 21 days. Trastuzumab will be administered IV on Day 1 out of a 21-day cycle. The initial dose of trastuzumab will be 8 mg/kg (loading dose) if the patient has not received trastuzumab in > 28 days. If the patient has received a dose of trastuzumab within the prior 28 days then the initial dose will be 6 mg/kg. Subsequent doses will be 6 mg/kg (maintenance dose).

If the patient has a history of infusion reaction to trastuzumab, the pre-medications will be administered 30 minutes prior to administration of trastuzumab. Pre-medications are allowed and should be followed by institutional standards. These can include acetaminophen 650mg po once, diphenhydramine 25-50mg po/IV once 30 minutes prior to administration of drug, and/or a H2 receptor antagonist (such as famotidine 20 mg po/IV).

Patient compliance with ruxolitinib will be assessed by medication diary. The patient will be requested to record each dose of medication. The medication diary will be returned to research staff at the end of each cycle.

Ruxolitinib
Ruxolitinib is an FDA-approved treatment for intermediate to high-risk myelofibrosis, including primary myelofibrosis, post-polycythemia vera myelofibrosis and post-essential thrombocythemia myelofibrosis. It does not require specific prophylactic or supportive regimens. It is an oral therapy and can be administered with or without food according to manufacturer's recommendations. Subjects in the phase I study will be given a 3-week supply of medication. Dosing will start at 20 mg b.i.d. for the phase I.

Trastuzumab
Trastuzumab is FDA-approved for HER2+ breast cancer and gastric cancer. It does not require specific prophylactic or supportive regimens. It is an intravenous therapy that should be prepared according to manufacturer's recommendations. It can be administered with or without food.

5.2 General Concomitant Medication and Supportive Care Guidelines

Ruxolitinib is an FDA approved treatment for myelofibrosis and is commercially available. Per manufacturer Prescribing Information (PI), there are no concurrent supportive or prophylactic regimens recommended.

Ruxolitinib is predominantly metabolized by CYP3A4. When administering ruxolitinib with strong CYP3A4 inhibitors, a dose reduction is recommended. Thus, co-administration of ruxolitinib with strong CYP3A4 inhibitors will not be allowed. Patients should be closely monitored and the dose titrated based on safety and efficacy. No dose adjustment is recommended when ruxolitinib is co-administered with mild or moderate CYP3A4 inhibitors (i.e. erythromycin) or a CYP3A4 inducer – and patients on these medications will not be excluded. **Appendix C** presents guidelines for identifying medications/substances that could potentially interact with the study agent(s).

If a subject requires additional anti-cancer therapy, the subject must be withdrawn from study treatment, with the exception of palliative radiotherapy (ie, to bone metastasis or for subjects who have disease progression limited to the CNS but who are otherwise benefiting from study treatment), which may be allowed during the study but must be discussed with the overall principal investigator. Study treatment should be withheld until palliative radiotherapy is terminated. This treatment break should not be considered as treatment interruption in determining the DLT. Palliative radiotherapy to metastatic disease sites is allowed provided there are other sites of disease and ≥ 1 week has elapsed since the completion of radiotherapy and all treatment-related toxicities have resolved or are at a new stable baseline. Subjects who have received radiation to the spine, pelvis, ribs, or femur should be discussed and approved by one of the overall principal investigator prior to study re-entry. If anti-cancer treatment follows discontinuation of study treatment due to clinical progression determined by the investigator, the basis for this determination should be documented.

5.3 Duration of Therapy

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

- Disease progression

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

In the absence of one of the events listed above, there is no pre-defined limit on the number of potential cycles.

5.4 Duration of Follow Up

Patients will no longer be followed after removal unless adverse event(s) occur. All adverse events, both serious and non-serious, and deaths that are encountered during the study and within 30 days of the last study intervention should be followed. See Section **7.4.7.**

5.5 Criteria for Removal from Study

Patients will be removed from study when any of the criteria listed in **Section 5.3** applies. The reason for study removal and the date the patient was removed must be documented in source documentation and the Case Report Form.

6. DOSING DELAYS/DOSE MODIFICATIONS

The most common adverse events are listed below. Definitions of grading of each Adverse Events are defined per CTCAE 4.0. Doses of ruxolitinib that are missed will not be made up, and upon resumption of dosing, the cycle will stay on schedule. If the dose is reduced for hematologic toxicities, a repeat complete blood count with differential should be performed within 7 days (+/- 2 days) of dose reduction. Management for each grade of AE is outlined below.

Dosing delays/ dose modifications should occur only if adverse event is deemed possibly, probably, or definitely related to study drug.

Table 2: Dose Levels

Dose Level	Ruxolitinib Dose
1	25 mg bid
0	20 mg bid
-1	15 mg bid
-2	10 mg bid

Table 3: Neutropenia

Neutrophil Count Decreased	Management/Next Dose for Ruxolitinib	Management/Next Dose for Trastuzumab
Grade 1 <LLN – 1500	No change in dose	No change in dose
Grade 2 <1500 – 1000	No change in dose	No change in dose
Grade 3 <1000 – 500	Hold* until ≤ Grade 2. Resume at one dose level lower, per investigator discretion**	No change in dose
Grade 4	Hold* until ≤ Grade 2. Resume at	No change in dose

Neutrophil Count Decreased	**Management/Next Dose for Ruxolitinib**	**Management/Next Dose for Trastuzumab**
<500	one dose level lower**	
*Patients requiring a ruxolitinib delay of > 28 days should go off protocol therapy. Growth factor support is allowed, per investigator discretion. **Patients requiring > 2 dose reductions of ruxolitinib should go off protocol therapy.		

Table 4: Thrombocytopenia

Platelet Count Decreased	**Management/Next Dose for Ruxolitinib**	**Management/Next Dose for Trastuzumab**
Grade 1 <LLN - 75,000	No change in dose	No change in dose
Grade 2 <75,000 – 50,000	No change in dose	No change in dose
Grade 3 <50,000 - 25,000	Hold* until ≤ Grade 2. Resume at one dose level lower, per investigator discretion**	No change in dose
Grade 4 <25,000	Hold* until ≤ Grade 2. Resume at one dose level lower**	No change in dose
*Patients requiring a ruxolitinib delay of > 28 days should go off protocol therapy. **Patients requiring > 2 dose reductions of ruxolitinib should go off protocol therapy.		

Table 5: Anemia

Hemoglobin Decreased	**Management/Next Dose for Ruxolitinib**	**Management/Next Dose for Trastuzumab**
Grade 1 <LLN – 10	No change in dose	No change in dose
Grade 2 <10 – 8	No change in dose	No change in dose
Grade 3 <8	Transfuse PRBC as clinically indicated. Hold* until ≤ Grade 2. Resume at one dose level lower, per investigator discretion**	No change in dose
Grade 4	Transfuse PRBC as clinically indicated. Hold* until ≤ Grade 2. Work up for alternative causes of blood loss. First occurrence: Resume at one dose level lower. Second occurrence: off therapy.	No change in dose
*Patients requiring a ruxolitinib delay of > 28 days should go off protocol therapy. Growth factor support is allowed, per investigator discretion. **Patients requiring > 2 dose reductions of ruxolitinib should go off protocol therapy.		

Table 6: Febrile Neutropenia

Febrile Neutropenia	**Management/Next Dose for Ruxolitinib**	**Management/Next Dose for Trastuzumab**
Grade 1	N/A	N/A
Grade 2	N/A	N/A
Grade 3	Hold* until ≤ Grade 2. Resume at	No change in dose

Febrile Neutropenia	**Management/Next Dose for Ruxolitinib**	**Management/Next Dose for Trastuzumab**
	one dose level lower **	
Grade 4	Hold* until ≤ Grade 2. First occurrence: Resume at one dose level lower. Second occurrence: off therapy.	No change in dose
*Patients requiring a ruxolitinib delay of > 28 weeks should go off protocol therapy. Growth factors support is allowed, per investigator discretion. **Patients requiring > 2 dose reductions should go off protocol therapy.		

Table 7: Non-Hematologic Toxicities

Non-Hematologic Toxicities (non-Cardiac)	**Management/Next Dose for Ruxolitinib**	**Management/Next Dose for Trastuzumab**
Grade 1	No change in dose	No change in dose
Grade 2	Hold* until ≤ Grade 1. Recommend resume at same dose level, but per investigator discretion. If the toxicity is deemed possibly, probably, or definitely related to ruxolitinib – and not reduced after the first occurrence - a dose reduction should be considered after a second recurrence, if still deemed possibly, probably, or definitely related.	No change in dose
Grade 3	Hold* until ≤ Grade 1. Resume at one dose level lower**	No change in dose
Grade 4	Off protocol therapy	Off protocol therapy
*Patients requiring a delay of >2 weeks should go off protocol therapy. **Patients requiring > two dose reductions should go off protocol therapy.		
Recommended management if diarrhea: loperamide antidiarrheal therapy Dosage schedule: 4 mg at first onset, followed by 2 mg with each loose motion until diarrhea-free for 12 hours (maximum dosage: 16 mg/24 hours) Adjunct anti-diarrheal therapy is permitted and should be recorded when used.		
Recommended management if nausea/vomiting: anti-emetics		

6.1 Management of Cardiac Toxicity

Frequent cardiac evaluations will be performed in this study because cardiotoxicity has been reported in patients treated with single-agent trastuzumab and in combination with targeted therapies. Initiation of trastuzumab will depend on review of the initial multiple-gated acquisition (MUGA) scan/echocardiogram (ECHO) results. It is recommended patients have their MUGA scans/ECHOs performed at the same radiology facility to eliminate variability between facilities. The same method for assessment should be used throughout the study. A MUGA/ECHO should be performed at baseline, prior to cycle 2 (during phase I), and then every 3 cycles (~9 weeks+/- 1 week) from the last MUGA/ECHO while on study treatment.

The cardioprotective drug dexrazoxane may not be taken by subjects participating in this trial.

6.2 Asymptomatic Decrease in LVEF (Left Ventricular Ejection Fraction)

Decision to continue or stop trastuzumab treatment is based on the measured ejection fraction as it relates to the radiology facility's lower limit of normal (LLN) and change in ejection fraction from baseline. Guidelines for performing MUGA scan/ECHO and management of patients who have an asymptomatic decrease in LVEF from baseline are provided below:

Table 8: Guidelines for Trastuzumab Suspension

Relationship of LVEF to Radiology Facility's LLN	Decrease of < 10 Percentage Points From Baseline	Decrease of 10 to 15 Percentage Points From Baseline	Decrease of ≥ 16 Percentage Points From Baseline
Within normal limits	Continue Trastuzumab	Continue Trastuzumab	Hold Trastuzumab[a,b]
1-5 percentage points below LLN	Continue Trastuzumab	Hold Trastuzumab[a,b]	Hold Trastuzumab[a,b]
≥ 6 percentage points below LLN	Continue Trastuzumab[a]	Hold Trastuzumab[a,b]	Hold Trastuzumab[a,b]
LLN, lower limit of normal; LVEF, left ventricular ejection fraction; MUGA, multiple gated acquisition scan. Source: Cardiac management during adjuvant trastuzumab therapy (Mackey et al. 2008).			
[a] Repeat MUGA/ECHO after 4 weeks.			
[b] After two consecutive holds permanently discontinue trastuzumab.			

If trastuzumab is held or discontinued during therapy, treatment with ruxolitinib may be continued, per the investigator. Trastuzumab must be permanently discontinued if withheld for more than 9 weeks or earlier per discretion of the investigator. If LVEF is maintained at a "continue and repeat MUGA/ECHO" or improves from a "hold" to a "continue and repeat MUGA/ECHO" category, additional MUGA scans/ECHO prior to the next scheduled MUGA scan/ECHO will be at the discretion of the investigator.

6.3 Symptomatic Decrease in LVEF

For symptomatic Grade 3 and Grade 4 congestive heart failure (CHF), hold treatment with study drugs. A cardiology consult should be obtained and treatment per institutional standards should be initiated. Confirm diagnosis of CHF with either a MUGA scan/ECHO. A chest x-ray is also required. Once a diagnosis of CHF is confirmed, treatment with study drugs must be permanently discontinued and reported as an AE.

6.4 Cardiac Ischemia/ Infarction

Grade 1: Continue treatment with frequent monitoring.

Grade 2: Hold treatment with study drugs and conduct cardiac evaluation. Based on this evaluation, treatment may be continued at the discretion of the investigator.

Grade 3 or 4: Discontinue treatment with study drugs.

6.5 Arrhythmia

Grade 1: Continue treatment with study drugs with careful monitoring OR hold treatment and conduct cardiac evaluation. Based on cardiac evaluation, treatment with study drugs may continue or discontinue at the discretion of the investigator.

Grade 2: Hold treatment with study drugs and conduct cardiac evaluation. Based on cardiac evaluation, treatment with study drugs may continue or discontinue at the discretion of the investigator.

Grade 3 or 4: Discontinue treatment with study drugs.

6.6 Management of Corrected QTc Prolongation

At any time point, if there is an increase in the QTc intervals (using Bazett or Fridericia correction formula) to > 500 msec (ie, Grade 3 QTc prolongation), an additional ECG should be performed. If the average QTc is > 500 msec, study treatment should be withheld. If the subject is asymptomatic (ie, does not have palpitations, dizziness, syncope, orthostatic hypotension, a significant ventricular arrhythmia on ECG, or a change in vital signs), the following actions should be taken:

- Hold ruxolitinib. Can continue trastuzumab.
- Check electrolytes, especially magnesium and potassium; correct abnormalities as clinically indicated
- Modify the dose as a Grade 3, Non-hematologic AE as described in Table 7 after the QTc elevation has resolved.

Subjects with QTc prolongation and symptoms must be monitored closely. Cardiology consultation is recommended for evaluation and subject management. No additional study treatment will be given to the subject until after the event has resolved.

6.7 Management of Trastuzumab Infusion Reaction

Infusion reactions consist of a symptom complex, such as fever and chills, and on occasion nausea, vomiting, pain (in some cases at tumor sites), headache, dizziness, dyspnea, hypotension, rash, and asthenia. Infusion reactions should be managed by standard clinical practice. Stop infusion and give antipyretics if fever occurs within 24 hours of trastuzumab infusion. Once the temperature is < 38°C, resume infusion at a slower rate and monitor the patient according to institutional standard practice. Chills triggered by trastuzumab infusion can be treated with acetaminophen and/or diphenhydramine hydrochloride. Meperidine may be given at the investigator's discretion.

Pulmonary toxicity including dyspnea, interstitial pneumonitis, pulmonary infiltrates, pleural effusions, non-cardiogenic pulmonary edema, pulmonary insufficiency and hypoxia, acute respiratory distress syndrome, and pulmonary fibrosis can occur as sequelae of infusion reactions. Management of pulmonary toxicity should follow clinical standard practice.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

An Adverse Event (AE) is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of study treatment or any procedure specified in the protocol, even if the event is not considered to be related to the study.

Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms or require treatment or further diagnostic tests.

Adverse events will be monitored from the time the subject signs informed consent. Subjects will be instructed to report all AEs during the study and subjects will be assessed for the occurrence of AEs throughout the study. All AEs (serious and nonserious) must be recorded on the source documents and case report forms regardless of the assumption of a causal relationship with the study drug.

Adverse Events that begin or worsen after informed consent should be recorded in the Adverse Events section of the case report form (CRF). Conditions that were already present at the time of informed consent should be recorded in the baseline symptoms section of the CRF. Adverse Event monitoring should be continued for at least 30 days following the last dose of study treatment. Adverse Events (including lab abnormalities that constitute AEs) should be described using a diagnosis whenever possible, rather than individual underlying signs and symptoms. When a clear diagnosis cannot be identified, each sign or symptom should be reported as a separate AE.

The following list of AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

7.1 Comprehensive Adverse Events and Potential Risks List

Adverse Event List for Ruxolitinib

Adverse Events with Possible Relationship to Ruxolitinib (CTCAE 4.0 Term)		
Likely (> 20%)	**Less Likely (≤ 20%)**	**Rare but Serious (<3%)**
BLOOD AND LYMPHATIC SYSTEM DISORDERS		
1. Anemia	1. Febrile neutropenia	
CARDIAC DISORDERS		
	1. Atrial Flutter 2. Atrial Fibrillation	Cardiac Failure
GASTROINTESTINAL DISORDERS		
1. Diarrhea	1. Abdominal pain 2. Abd Distension 3. Constipation 4. Dyspepsia 5. Flatulence 6. Nausea	1. Colitis 2. Gastrointestinal hemorrhage
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS		
	1. Fatigue 2. Fever 3. Pain 4. Edema limb	
INFECTIONS AND INFESTATIONS		

	1. Infection	1. Posterior multifocal leukoencephalopathy
INJURY, POISONING, AND PROCEDURAL COMPLICATIONS		
1. Bruising		
INVESTIGATIONS		
1. Platelet count decreased	1. Aspartate aminotransferase increased 2. Bilirubin increased 3. GGT increased 4. Neutrophil count decreased2 5. Weight Gain	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS		
	1. Arthralgia 2. Back pain	
NERVOUS SYSTEM DISORDERS		
	1. Headache 2. Dizziness	
PSYCHIATRIC DISORDERS		
	1. Confusion 2. Insomnia	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS		
	1. Cough 2. Dyspnea 3. Epistaxis	1. Pleural Effusion
SKIN AND SUBCUTANEOUS TISSUE DISORDERS		
	1. Rash	***Rash acneif***
VASCULAR DISORDERS		
		1. Hematoma

The comparison of the control groups to the ruxolitinib patients showed that headache was more frequent in ruxolitinib-treated patients (13.6% vs. 6.0% on placebo and 5.5% on best available therapy: BAT). Most AEs of headache were Grade 1 or 2. Similarly, dizziness (12.0% vs. 6.6% on placebo and 6.8% on BAT) was more frequent in ruxolitinib-treated patients, again mostly Grade 1 or 2. When adjusted for patient-year exposure, the differences are still present for headache and dizziness.

Weight increase was also more frequent in ruxolitinib-treated patients than in the control groups (9.6% vs. 1.3% on placebo and 1.4% on BAT). Although some of these patients had co-reported AEs of edema, many had a past medical history of weight loss and the weight gain usually gradually accumulated over the course of one year of treatment. The majority of weight gain AEs were Grade 1 and 2. It is worth noting that weight gain may be a beneficial effect in patients with MF, given the catabolic nature of the disease and the frequency of weight loss reported as a constitutional symptom. Other preferred terms with increased frequency in the ruxolitinib arms included bruising (2.6% vs. 1.3% on placebo), contusion (8.6% vs. 5.3% on placebo and 1.4% on BAT), urinary tract infection (7.3% vs. 4.6% on placebo and 2.7% on BAT), herpes zoster (4.0% vs. 0.7% on placebo and 0% on BAT) and flatulence (3.3% vs. 1.3% on placebo and 0% on BAT).

Abdominal pain was more frequent in the control groups than in the ruxolitinib group (43% on placebo and 13.7% on BAT vs. 12% on ruxolitinib), as were weight decrease (8.6% on placebo and 8.2% on BAT vs. 1% on ruxolitinib), early satiety (8.6% on placebo and 0% on BAT vs. 0.3% on ruxolitinib) and splenic infarction (6.0% on placebo and 0% on BAT vs. 1.0% on ruxolitinib).

A total of 13 reports of tuberculosis (TB) during ruxolitinib treatment had been received from all clinical trials, as of February 2013. The cumulative incidence of TB reported in subjects treated with ruxolitinib (13/4755; 0.27%) is less than that originally observed in the Phase III clinical trials (3/301; 1.0%), possibly due to differences in time of exposure to ruxolitinib and a more heterogeneous study population. The Phase III clinical trial sample size did not allow detection of a statistically significant difference in the incidence of tuberculosis between the treatment and control arms. The cumulative incidence of these TB reports appears to have declined since the original Phase III trials.

Progressive multifocal leukoencephalopathy (PML) was reported in a myelofibrosis patient receiving ruxolitinib, with an atypical feature of a short time to onset (approximately 1 month) after initiation of ruxolitinib treatment. Because of its mechanism of action, ruxolitinib may be associated with a higher risk of infections. However, infections are also well known complications of myelofibrosis and the occurrence of PML has been described in myeloproliferative diseases in the absence of ruxolitinib treatment. Whereas a contribution of ruxolitinib therapy cannot be formally ruled out, the available information with the observation of a single case of PML is currently insufficient to formally establish a causal association of PML with the drug therapy. Based on this report and provided the potentially disabling outcome of this condition, the potential risk of PML is closely monitored.

Non-melanoma skin cancers (NMSC) have been reported in patients treated with ruxolitinib. These are highly uncommon. Most of these patients had histories of extended treatment with hydroxyurea (a chemotherapy associated with skin cancer) and prior NMSC or pre-malignant skin lesions. Hydroxyurea is not a standard chemotherapy given to patients with breast cancer. A causal relationship to ruxolitinib has not been established.

Updated Risk Section, including update on NMSC and the following statement: “Ruxolitinib is currently under clinical development for the treatment of solid tumors in combination with cytotoxic chemotherapy. To date there have been no specific adverse drug reactions have been identified that are unique to ruxolitinib combination therapies under study, and the relative strength index derived primarily from treatment of myeloproliferative neoplasms is appropriate for these ongoing studies in solid tumors.

Adverse Event List for Trastuzumab

Adverse Events with Possible Relationship to Trastuzumab (CTCAE 4.0 Term)		
Likely (> 20%)	**Less Likely (≤ 20%)**	**Rare but Serious (<3%)**
BLOOD AND LYMPHATIC SYSTEM DISORDERS		
	1. Anemia 2. Febrile neutropenia[2]	
CARDIAC DISORDERS		
	1. Cardiomyopathy 2. Left ventricular systolic dysfunction 3. Pericardial effusion 4. Pericarditis 5. Sinus tachycardia 6. Supraventricular tachycardia	
GASTROINTESTINAL DISORDERS		
	1. Abdominal pain 2. Diarrhea 3. Mucositis oral 4. Nausea 5. Vomiting	
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS		
	1. Chills 2. Fatigue 3. Fever 4. Flu like symptoms 5. Infusion related reaction 6. Non-cardiac chest pain 7. Pain	
IMMUNE SYSTEM DISORDERS		
	1. Allergic reaction[3] 2. Anaphylaxis	
INFECTIONS AND INFESTATIONS		
	1. Infection[4]	
INVESTIGATIONS		
	1. Alkaline phosphatase increased 2. Aspartate aminotransferase increased 3. GGT increased 4. Neutrophil count decreased[2]	
METABOLISM AND NUTRITION DISORDERS		
	1. Anorexia	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS		
	1. Arthralgia 2. Back pain 3. Bone pain 4. Myalgia	
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS)		
	1. Tumor pain	
NERVOUS SYSTEM DISORDERS		
	1. Headache 2. Peripheral sensory neuropathy	

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS		
	1. Allergic rhinitis 2. Cough 3. Dyspnea[3] 4. Hypoxia[3] 5. Pneumonitis[3] 6. Pulmonary edema 7. Pulmonary fibrosis	1. Adult respiratory distress syndrome[3] 2. Bronchospasm[3] 3. Pneumonitis[3] 4. Pulmonary edema 5. Pulmonary fibrosis
SKIN AND SUBCUTANEOUS TISSUE DISORDERS		
	1. Rash acneiform 2. Rash maculo-papular 3. Urticaria	***Rash acneiform (Gr. 3)*** ***Rash maculo-papular (Gr. 3)*** ***Urticaria (Gr. 3)***
VASCULAR DISORDERS		
	1. Hypertension[5] 2. Hypotension[5]	

[1]This table will be updated as the toxicity profile of the agent is revised.
[2]Fatal event when given in combination with Xeloda® (capecitabine) and Taxotere® (docetaxel).
[3]Severe hypersensitivity reactions including angioedema and pulmonary adverse events (e.g., hypoxia, dyspnea, pulmonary infiltrates, pleural effusion, and acute respiratory distress syndrome) have been reported.
[4]Infection may include any of the 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.
[5]Associated with infusion reactions.

Additional adverse events:
The following are adverse events which have been reported on trastuzumab trials; however, the relationship to trastuzumab is currently undetermined.

- *Cardiac Disorders* – acute coronary syndrome, atrial fibrillation, cardiac arrest, myocardial infarction; ventricular arrhythmia; ventricular fibrillation; ventricular tachycardia
- *Ear and Labyrinth Disorders* - hearing impaired
- *Endocrine Disorders* – hypothyroidism
- *Eye Disorders* - blurred vision; extraocular muscle paresis
- *Gastrointestinal Disorders* - colitis; dyspepsia; enterocolitis; esophageal ulcer; gastritis; pancreatitis; upper gastrointestinal hemorrhage
- *General Disorders and Administration Site Conditions* - sudden death NOS
- *Immune System Disorders* - immune system disorders - Other (autoimmune thyroiditis)
- *Investigations* - alanine aminotransferase increased; blood bilirubin increased; cardiac troponin-I; creatinine increased; platelet count decreased
- *Metabolism and Nutrition Disorders* - hypomagnesemia; hyponatremia
- *Musculoskeletal and Connective Tissue Disorders* - avascular necrosis; generalized muscle weakness; muscle weakness left-sided; muscle weakness lower limb; muscle weakness right-sided; muscle weakness trunk; muscle weakness upper limb; musculoskeletal and connective tissue disorder - Other (myopathy)
- *Nervous System Disorders* - ataxia; cognitive disturbance; depressed level of consciousness; dizziness; hydrocephalus; ischemia cerebrovascular; neuralgia; seizure; syncope
- *Psychiatric Disorders* - anxiety; confusion; depression; psychosis
- *Renal and Urinary Disorders* - acute kidney injury; hematuria; proteinuria; urinary tract obstruction
- *Reproductive System and Breast Disorders* - fallopian tube obstruction; prostatic obstruction; spermatic cord obstruction; uterine obstruction; vaginal obstruction

- *Respiratory, Thoracic and Mediastinal Disorders* - apnea; laryngeal edema; pharyngolaryngeal pain; pleural effusion; pneumothorax; pulmonary hypertension; voice alteration
- *Skin and Subcutaneous Tissue Disorders* - nail loss; pruritus; skin ulceration
- *Vascular Disorders* - thromboembolic event

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

Additional adverse events from the FDA-approved October 2010 Prescribing Information for Herceptin® (Trastuzumab)

General Disorders – edema limbs, edema trunk
Infections and Infestations – pharyngitis
Nervous System Disorders – dizziness
Psychiatric Disorders – depression, insomnia
Renal and Urinary Disorders – other (glomerulopathy)

7.2 Definitions and Reporting

7.2.1 Dose Limiting Toxicity (DLT)

A dose-limiting toxicity is defined as any grade 3 non-hematologic toxicities despite maximal supportive care or any grade 4 hematologic toxicity directly related to study drug. In order for a patient to be completely evaluable for DLT, the patient must incur a DLT during the first 21 days of actively taking ruxolitinib plus trastuzumab. If, for instance, the patient temporarily stops ruxolitinib due to uncomplicated hematologic toxicities, the patient can resume ruxolitinib at the same dose and the DLT period will remain defined as the first 21 days of actively taking the study medication. In order to be considered completely followed, the DLT period (i.e. 21 days of actively taking ruxolitinib plus trastuzumab) must be completed within 30 days of the first dose of ruxolitinib. If a patient is not completely followed due to dropout, treatment discontinuation, or death which is unrelated to study drug, we will replace that patient so that the sample size refers to those with complete DLT follow-up. However, patients with partial follow-up will be included up to their last assessment while on treatment.

7.2.2 Adverse Event (AE)

AEs will be assessed according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. The occurrence of AEs should be sought by non-directive questioning of the subject during the screening process after signing informed consent and at each visit during the study. As far as possible, each AE should be evaluated to determine:
• The severity grade (CTCAE Grade 1-5)
• Reasonable possibility that AE is related to the study treatment: Definite, Probable, Possible, Unlikely, Unrelated (Section **7.3.4**)
• Start and end dates, unless unresolved at final exam

Action taken with respect to study drug (i.e., none, dose adjusted, temporarily interrupted, permanently discontinued, unknown, not applicable)
• Outcome (eg, not recovered/not resolved, recovered/resolved, recovering/resolving, recovered/resolved with sequelae, fatal, unknown)
• Whether it is serious, as per Serious Adverse Event (SAE) definition provided in Section **7.2.2**.

Unlike routine safety assessments, SAEs are monitored continuously and have special reporting requirements, see Section **7.2.2**.

All AEs should be treated appropriately. If a concomitant medication or non-drug therapy is given, this action should be recorded on the AE CRF as well as the Prior/Concomitant medications CRF.

Once an AE is detected, it should be followed until its resolution or until it is judged to be permanent; assessment should be made at each visit (or more frequently if necessary) of any changes in severity, the suspected relationship to the study drug, the interventions required to treat it, and the outcome.

Disease progression should not be regarded or reported as an AE itself, unless it is associated with a separate AE.

Laboratory abnormalities that constitute an AE in its own right (are considered clinically meaningful, induce clinical signs or symptoms, require concomitant therapy or require changes in study treatment), should be recorded on the AE CRF. Whenever possible, a diagnosis, rather than a symptom should be provided (i.e., anemia instead of low hemoglobin). Laboratory abnormalities that meet the criteria for AEs should be followed until they have returned to normal or an adequate explanation of the abnormality is found.

When an abnormal laboratory or test result corresponds to a sign or symptom of a previously reported AE, it is not necessary to separately record the lab/test result as an additional event.

Laboratory abnormalities that do not meet the definition of an AE should not be reported as AEs. A Grade 3 or 4 (severe) event, as per CTCAE, does not automatically indicate a SAE unless it meets the definition of serious, as defined below, and/or as per the investigator's discretion.

7.2.3 Serious adverse event (SAE)

A serious adverse event (SAE) is any adverse event, occurring at any dose and Regardless of causality that:

• Results in death.

• Is life-threatening. Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.

• Requires or prolongs inpatient hospitalization (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).

• Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.

• Is a congenital anomaly or birth defect; or

• Is an important medical event when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Events **not** considered to be serious adverse events are hospitalizations for:

• Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures.

• Elective or pre-planned treatment for a pre-existing condition that did not worsen.

• Emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission.

• Respite care

7.2.4 Unanticipated Problem

An Unanticipated Problem (UP) is any incident, experience or outcome involving risk to subjects or others in any human subjects research that meets all of the following criteria:

- Unexpected (in terms of nature, severity or frequency) given (a) the research procedures that are described in the IRB-approval protocol and informed consent document, and (b) the characteristics of the subject population being studied;
- Related or possibly related to participation in such research (i.e., there is a reasonable possibility that the incident, experience or outcome may have been caused by the procedures involved in such 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.

7.2.5 Suspected Adverse Reaction

A Suspected Adverse Reaction (SAR) is any AE for which there is a reasonable possibility that it was caused by the drug.

Reasonable possibility means that there is evidence to suggest a causal relationship between the drug and the AE. Examples of reasonable possibility are:

- 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 that those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

7.2.6 Expectedness
Adverse events can be 'Expected' or 'Unexpected.'

Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.

For the purposes of this study, an adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk.

7.2.7 Attribution
Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

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

7.2.8 Expedited Adverse Event Reporting
Participating investigators will assess the occurrence of AEs and SAEs at all participant evaluation time points during the study.

All AEs and SAEs whether reported by the participant, discovered during questioning, directly observed, or detected by physical examination, laboratory test or other means, will be recorded in the participant's medical record and on the appropriate study specific case report forms.

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

For multi-site trials where a Columbia University Medical Center investigator is serving as the principal investigator, each participating investigator is required to abide by the reporting requirements set by Columbia University Medical Center. The study must be conducted in compliance with FDA regulations, local safety reporting requirements, and reporting requirements of the principal investigator.

Each investigative site will be responsible to report SAEs that occur at that institution to their respective IRB. It is the responsibility of each participating investigator to report serious adverse events to the study sponsor (i.e. Kevin Kalinsky, MD, MS and Incyte representative) as described below.

In addition to the reporting requirements for SAEs, a separate case report form will be made for reporting of any grade adverse events attributable to research biopsies. These events will

not require expedited reporting unless they also meet the requirements for SAE reporting, as detailed below.

Copies of all IND safety reports submitted to the FDA and/or institutional IRB by the institution under the institution's IND will be shared with Incyte Pharmacovigilance representative, so that these reports can be evaluated and included in the Investigator Brochure and future Incyte IND safety submissions per regulations.

7.2.9 Serious Adverse Event Reporting

All serious adverse events that occur after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment must be reported to Columbia University Medical Center Overall Principal Investigator on the local institutional SAE form. This includes events meeting the criteria outlined in Section **7.2.2**, as well as the following:

- All Grade 4 (life-threatening or disabling) Events – Unless expected AND specifically listed in the protocol as not requiring reporting.

- All Grade 5 (fatal) Events – When the participant is enrolled and actively participating in the trial OR when the event occurs within 30 days of the last study intervention.

Note: If the participant is in long term follow up, report the death at the time of continuing review.

Participating investigators must report each serious adverse event to the Columbia University Medical Center Overall Principal Investigator within 24 hours of learning of the occurrence. In the event that the participating investigator does not become aware of the serious adverse event **immediately** (e.g., participant sought treatment elsewhere), the participating investigator is to report the event within 24 hours after learning of it and document the time of his or her first awareness of the adverse event. Report serious adverse events by telephone, email or facsimile to:

Name of Contact:	Kevin Kalinsky, MD
Address:	Columbia University Medical Center Herbert Irving Comprehensive Cancer Center 161 Fort Washington Avenue New York, NY 10032
Email:	M1906@columbia.edu
Phone:	212-305-1945
Fax:	212-305-0178

Within the following 24-48 hours, the participating investigator must provide follow-up information on the serious adverse event. Follow-up information should describe whether the event has resolved or continues, if and how the event was treated, and whether the participant will continue or discontinue study participation.

Follow-up information is sent to the same person to whom the original SAE Report Form was sent, using a new SAE Report Form stating that this is a follow- up to the previously reported SAE and giving the date of the original report. Each re-occurrence, complication, or progression of the original event should be reported as a follow-up to that

event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, and whether the subject **continued** or withdrew from study participation or if study drug was interrupted or discontinued.

If the SAE is not previously documented in the Investigator's Brochure for the study drug (new occurrence) and is thought to be related to the Sponsor's study drug, a Sponsor's associate may urgently require further information from the Investigator for reporting to Health Authorities.

The Sponsor may need to issue an Investigator Notification (IN) to inform all Investigators involved in any study with the same drug that this SAE has been reported. Suspected Unexpected Serious Adverse Reactions (SUSARs) will be collected and reported to the competent authorities and relevant ethics committees in accordance with Directive 2001/20/EC or as per national regulatory requirements in participating countries. If the study is open-label, no special unblinding procedures are needed for exceptional circumstances or medical emergencies.

7.2.10 Pregnancy

Pregnancy, in and of itself, is not regarded as an AE, unless there is suspicion that study drug may have interfered with the effectiveness of a contraceptive medication or method. The procedures that will be followed based on whether a pregnancy is confirmed by a positive serum or urine test result are listed below:

- Investigator and subject must notify each other immediately.
    - Investigator must notify the Principal Investigator and Incyte within 24 hours of learning of the occurrence
- Study drug must be discontinued immediately.
- Subject must be withdrawn from the study.
- Investigator must complete and submit the Pregnancy Initial and **Follow-up report** forms to the Principal Investigator and to Incyte.
- A serum pregnancy test must be performed to confirm the urine test result. (The serum test should be performed at the investigative site to ensure the test will be performed promptly and the result available immediately for review.)

If a negative serum test does not confirm the urine test result, then:

- The Investigator will use his/her expert judgment, based on an assessment of the potential benefit/risk to the subject, to determine if it is in the subject's best interest to resume study drug and continue participation in the study.

To ensure subject safety, each pregnancy in a subject during maternal or paternal exposures to study drug must be reported within 24 hours of learning of its occurrence. Data on fetal outcome and breast-feeding are collected for regulatory reporting and drug safety evaluation. The pregnancy should be followed- up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications by following until the first well-baby visit. Pregnancy should be recorded on a Clinical Study Pregnancy Form and reported by the Investigator to the Incyte. Pregnancy

follow-up should be recorded on the same form and should include an assessment of the possible relationship to the study drug of any pregnancy outcome and follow-up to the first well-baby visit. **Any SAE experienced during pregnancy must be reported on the SAE Report Form and to Incyte Corporation.**

7.2.11 Non-Serious Adverse Event Reporting

Non-serious adverse events will be reported to the Columbia University Medical Center Overall Principal Investigator on the toxicity Case Report Forms.

7.2.12 Reporting to the Institutional Review Board (IRB)

Unanticipated Problems (Ups) are to be reported to the IRB. SAEs not constituting UPs are to be reported to the HICCC DSMC.

Other investigative sites should report serious adverse events to their respective IRB according to the local IRB's policies and procedures in reporting adverse events. A copy of the submitted institutional SAE form should be forwarded to:

Name of Contact:	Kevin Kalinsky, MD
Address:	Columbia University Medical Center Herbert Irving Comprehensive Cancer Center 161 Fort Washington Avenue New York, NY 10032
Email:	M1906@columbia.edu
Phone:	212-305-1945
Fax:	212-305-0178

SAEs are to be reported to the HICCC DSMC, if they are considered UPs, then they must be submitted to CUMC IRB per institutional policies.

Unanticipated Problems must be reported promptly, but not later than 7 calendar days following the occurrence of the UP or the Principal's Investigator's acquiring knowledge of the UP.

Expected AEs must be reported at the time of continuing review of a protocol.

7.2.13 Reporting to the Food and Drug Administration (FDA)

Columbia University Medical Center Overall Principal Investigator (Sponsor-Investigator), as holder of the IND, will be responsible for all communication with the FDA. Columbia University Medical Center Principal Investigator will report to the FDA, regardless of the site of occurrence, any adverse event that is serious, unexpected and reasonably related (i.e., possible, probable, definite) to the study treatment.

The Columbia University Medical Center Sponsor Investigator must report the following SARs:

- To the FDA, as soon as possible, but no later than 7 calendar days after the S-I's initial receipt of the information, any unexpected fatal or life-threatening SAR.
- To the FDA and all participating investigators, as soon as possible but no later than 15 calendar days after the S-I determines that information qualifies for

reporting, in an IND safety report, any SAR that is both serious and unexpected.

- To the FDA and all participating investigators, as soon as possible but no later than 15 calendar days after the S-I determines that the information qualifies for reporting, any findings from epidemiological studies, pooled analysis of multiple studies or clinical studies, whether or not conducted under an IND or by the S-I, that suggest a significant risk in humans exposed to the drug.
- To the FDA and all participating investigators, as soon as possible, but no later than 15 calendar days after the S-I determines that the information qualifies for reporting, any findings from animal or in vitro testing, whether or not conducted by the S-I, that suggest a significant risk in humans exposed to the drug.
- To the FDA and all participating investigators, as soon as possible, but no later than 15 calendar days after the S-I determines that the information qualifies for reporting, any clinically important increase in the rate of a Serious SAR over that listed in the protocol or Investigator Brochure.
- Expected SAEs and AEs should be included in the IND Annual Reports.

Follow-up information to a safety report should be submitted as soon as the relevant information is available. However, if the results of a sponsor's investigation show that an adverse drug experience not initially determined to be reportable are so reportable, the sponsor must report such experience as soon as possible, but no later than 15 calendar days after the determination is made.

All other serious unexpected experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 15 calendar days after initial receipt of the information.

Events will be reported to the FDA by telephone (1-800-FDA-1088) or by fax (1-800-FDA-0178) using Form FDA 3500A (Mandatory Reporting Form for investigational agents) or FDA Form 3500 (Voluntary Reporting Form for commercial agents). Forms are available at http://www.fda.gov/medwatch/getforms.htm.

7.2.14 Reporting to Incyte Corporation

The Overall Principal Investigator will report to Incyte Corporation any serious adverse events within 24 hours of learning of the event. Reporting will occur by emailing the completed Serious Adverse Event Reporting Form to IncytePhVOpsIST@incyte.com.

The Overall Principal Investigator send copies of any IND Safety reports submitted to the FDA will be sent to Incyte so that these reports can be evaluated and included in the Investigator's Brochure or Incye IND safety submissions. These will be sent via email to IncytePhVOpsIST@incyte.com.

,Every six months, the overall Principal Investigator will submit copies of IND safety reports in the format of a lie listing with details regarding the reports that were submitted to the FDA. These will be inclusive of all sites to they will be sent via email to IncytePhVOpsIST@incyte.com.

The Overall Principal investigator will provide complete data transfers for AEs listings, including AEs, on labs, on a quarterly basis for the duration of the study. The data transfer will not include any Protected Health Information as defined by HIPAA.

7.2.15 **Guidelines for Processing IND Safety Reports**

The U.S. Food and Drug Administration (FDA) regulations require sponsors of clinical studies to notify the FDA and all participating investigators of any serious and unexpected adverse experiences that are possibly related to the investigational agent. The CUMC Principal Investigator will review all applicable IND Safety Reports and has the responsibly for forwarding the IND Safety Reports to the Affiliate Institutions. The Affiliate Institution investigators are to review, send a copy to their IRB according to their local IRB's policies and procedures, and file a copy with their regulatory documents. All Affiliate site INDSR submissions, along with IRB acknowledgment (per local policies and procedures) are to be forwarded to CUMC for placement within the trial master file.

7.2.16 Reporting to Hospital Risk Management

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

7.2.17 Monitoring of Adverse Events and Period of Observation

All adverse events, both serious and non-serious, and deaths that are encountered from initiation of study intervention, throughout the study, and within 30 days of the last study intervention should be followed to their resolution, or until the participating investigator assesses them as stable, or the participating investigator determines the event to be irreversible, or the participant is lost to follow-up. The presence and resolution of AEs and SAEs (with dates) should be documented on the appropriate case report form and recorded in the participant's medical record and subject binder to facilitate source data verification.

For some SAEs, the study sponsor or designee may follow-up by telephone, fax, and/or monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. Participating investigators should notify Columbia University Medical Center Overall Principal Investigator and their respective IRB of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

7.3 Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm. Second malignancies require ONLY routine reporting unless otherwise specified.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agents administered in this study can be found in Section 7.

8.1 Investigational Agent(s)

This study will evaluate the effects of a novel combination of two commercially available,

FDA-available medications. Ruxolitinib has been approved by the FDA for treatment of intermediate and high risk myelofibrosis. Trastuzumab has been approved by the FDA for the treatment of HER2+ breast cancer. Both medications have been widely used and have considerable safety data. To our knowledge, this combination has been not been evaluated in patients with breast cancer.

8.1.1 Ruxolitinib

8.1.1.1 Product Description

Ruxolitinib (INCB018424 phosphate, INC424, ruxolitinib phosphate) represents a novel, potent, and selective inhibitor of JAK1 (Janus kinase 1) (inhibition concentration 50% [IC50]=3.3 ± 1.2 nM) and JAK2 (IC50=2.8 ± 1.2 nM) with modest to marked selectivity against TYK2 (tyrosine kinase 2) (IC50=19 ± 3.2 nM) and JAK3 (IC50=428 ± 243 nM), respectively. Ruxolitinib interferes with the signaling of a number of cytokines and growth factors that are important for hematopoiesis and immune function.

Ruxolitinib phosphate is a kinase inhibitor with the chemical name (*R*)-3-(4-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)-1*H*-pyrazol-1-yl)-3-cyclopentylpropanenitrile phosphate and a molecular weight of 404.36. Ruxolitinib phosphate is a white to off-white to light pink powder and is soluble in aqueous buffers across a pH range of 1 to 8.

8.1.1.2 Product Formulation

Ruxolitinib is an oral medication supplied as 5 mg tablets for this study.

8.1.1.3 Route of Administration

Tablets are for oral administration. Each tablet contains ruxolitinib phosphate equivalent to 5 mg of ruxolitinib free base together with microcrystalline cellulose, lactose monohydrate, magnesium stearate, colloidal silicon dioxide, sodium starch glycolate, povidone and hydroxypropyl cellulose.

8.1.1.4 Agent Ordering

Ruxolitinib is an investigational agent supplied to investigators by Incyte Pharmaceuticals. Ruxolitinib supplied for this protocol is intended for clinical trial use only and is not commercially available. Ruxolitinib is shipped directly from the company to the participating institution (see Appendix H for Drug Order Form). For further details molecule characterization, see the ruxolitinib Investigator's Brochure.

8.1.2 Trastuzumab

8.1.2.1 Product Description

Trastuzumab is a recombinant DNA-derived humanized monoclonal antibody that selectively binds with high affinity in a cell-based assay (Kd=5 nM) to the extracellular domain of the human epidermal growth factor receptor 2 protein, HER2. The antibody is an IgG1 kappa that contains human framework regions with the complementarity-determining regions of a murine antibody (4D5) that binds to HER2.
The humanized antibody against HER2 is produced by a mammalian cell (Chinese Hamster Ovary [CHO]) suspension culture in a nutrient medium containing the antibiotic gentamicin. Gentamicin is not detectable in the final product.

Trastuzumab is a sterile, white to pale yellow, preservative-free lyophilized powder for intravenous (IV) administration. The nominal content of each trastuzumab vial is 440 mg

trastuzumab, 400 mg α,α-trehalose dihydrate, 9.9 mg L-histidine HCl, 6.4 mg L-histidine, and 1.8 mg polysorbate 20, USP. Reconstitution with 20 mL of the supplied Bacteriostatic Water for Injection (BWFI), USP, containing 1.1% benzyl alcohol as a preservative, yields a multi-dose solution containing 21 mg/mL trastuzumab, at a pH of approximately 6.

8.1.2.2 Solution Preparation
Vials of trastuzumab are stable at 2°–8°C (36°–46°F) prior to reconstitution. Do not use beyond the expiration date stamped on the vial. A vial of trastuzumab reconstituted with BWFI, as supplied, is stable for 28 days after reconstitution when stored refrigerated at 2°–8°C (36°–46°F), and the solution is preserved for multiple use. Discard any remaining multi-dose reconstituted solution after 28 days. If unpreserved SWFI (not supplied) is used, the reconstituted trastuzumab solution should be used immediately and any unused portion must be discarded. ***Do not freeze trastuzumab that has been reconstituted.*** The solution of trastuzumab for infusion diluted in polyvinylchloride or polyethylene bags containing 0.9% Sodium Chloride Injection, USP, may be stored at 2°–8°C (36°–46°F) for up to 24 hours prior to use. Diluted trastuzumab has been shown to be stable for up to 24 hours at room temperature 2°–25°C. However, because diluted trastuzumab contains no effective preservative, the reconstituted and diluted solution should be stored refrigerated 2°–8°C.

8.1.2.3 Route of Administration
Trastuzumab is administered intravenously. When administering trastuzumab to a patient with a known hypersensitivity to benzyl alcohol, trastuzumab must be reconstituted with SWFI, and only one dose per trastuzumab vial should be used. ***Trastuzumab, which has been reconstituted, with SWFI must be used immediately and any unused portion must be discarded. Use of other reconstitution diluents should be avoided.***
Shaking the reconstituted trastuzumab or causing excessive foaming during the addition of diluent may result in problems with dissolution and the amount of trastuzumab that can be withdrawn from the vial. Use appropriate aseptic technique when performing the following reconstitution steps:
− Using a sterile syringe, slowly inject the 20 mL of diluent into the vial containing the lyophilized cake of trastuzumab. The stream of diluent should be directed into the lyophilized cake.
− Swirl the vial gently to aid reconstitution. Trastuzumab may be sensitive to shear-induced stress, e.g., agitation or rapid expulsion from a syringe. ***DO NOT SHAKE.***

8.1.2.4 Agent Ordering
Trastuzumab is commercially available.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

Given that this is a proof-of-concept phase I/II study there will be no assessment of integral biomarkers (essential for conducting the study) or integrated biomarkers (testing a hypothesis based on preexisting data). Instead, all correlative studies for this trial will be exploratory and used for either hypothesis generation and/or assay development and testing. Hypotheses generated from these studies will then be tested as integrated biomarkers in follow-up clinical studies.

All analyses will be performed in batched fashion at the end of the study. Plasma and serum samples will be prepared (**Appendix E**) on the same day they are drawn and stored locally. Biopsies will be obtained, processed, and stored as a clinical specimen by the participating

sites clinical pathology labs (**Appendix D**). At the end of the study freshly cut slides will be requested from each site for all the appropriate biopsy specimens. Slides will be cut for all specimens at the same time at the end of the study. Pathology reports for all samples assessed for the study will be entered into the clinical research form. For the blood based biomarkers samples will be shipped directly to the lab performing the analysis at the end of study accrual and sample collection [see appendices for shipping and labeling instructions].

For patients with accessible tumor, 2 punch biopsies (for skin) or 3 core biopsies are recommended. The following will be requested: 10 immunoblanks [each having 4 micron sections on charged slides], one intervening H&E stained slide, and 12 regular slides each with 10 microns sections.

	DNA	Blood Biomarkers	PK	IHC	RNAseq	RPPA
Primary tumor biopsy (archived) *				x	x	x
Biopsy upon recurrence (archived)*				x	x	x
Pre-treatment biopsy (Optional)				x	x	x
On-treatment biopsy (Optional)**				x	x	x
Biopsy upon progression (Optional)				x	x	x
Pre-treatment whole blood	x					
Pre-treatment plasma and serum		x				
On-treatment plasma and serum**		x				
Plasma and serum upon progression		x				
On-treatment plasma**			x			

*If available
**On-treatment assessments will be performed on C2D1. For PK analysis plasma will be drawn before the C2D1 dose and again two hours after.

Transcriptional profiling
RNA-Sequencing will be performed by the Columbia University Genome Center using current best technologies with a goal of at least 40 million reads per sample. RNAseq has been validated from FFPE breast cancer samples. In addition, the quality of sequencing reads from FFPE has been verified by the Columbia University Genome Center. Data analysis will be performed by the Columbia University bioinformatics core under the direction of Andrea Califano. Using the master regulator methodologies pioneered by Dr. Califano and used as the basis for the preclinical studies, they will perform interactome modeling for JAK/SAT pathway dependence. In addition, intrinsic molecular subtype of each sample will be identified, as well as mutation assessment and pathway analysis.

RPPA
We will explore the baseline proteomic profile of patients entered on this study, the profile in the setting of trastuzumab resistance, and the effect on the proteomic profile with treatment with combined trastuzumab and ruxolitinib. We will use is the current technology being used for proteomic profiling in the cancer genome atlas (TCGA) project. Samples will be sent to and analyzed by the MDA Anderson RPPA Functional Proteomics Core Facility.

Protein extracts from FFPE tumor blocks (3-8 untreated 10 micron slides) will be prepared from appropriately isolated regions (as determined by an experienced pathologist). Following deparaffinization, at least 30-40 micrograms of protein per sample will be extracted using an extraction buffer of 20 mM Tris HCl buffer at pH 9, with 2%(w/v)SDS, as previously described. RPPA will be conducted using a total of 161 antibodies (www.mdanderson.org/education-and-research/resources-for-professionals/scientific-resources/core-facilities-and-services/functional-proteomics-rppa-core/index.html), including markers of proliferation (PCNA) and apoptosis (e.g., cleaved caspase-3), as well as antibodies against STAT3, EGFR, HER2, c-MET, IGF-1R, PI3K, PTEN, AKT, mTOR, and their phosphorylated isoforms. These antibodies have been fully validated by direct correlation between RPPA and Western blotting results. Specifically, tumor lysates will be diluted in five 2-fold serial dilutions and arrayed on nitrocellulose-coated slides (Whatman, Schleicher & Schuell BioScience, Inc., Keene, NH). Samples will be probed with validated primary antibodies and biotin-conjugated secondary antibodies and visualized by DAB colorimetric reaction. Slides will be scanned and quantified for raw signal intensities by using MicroVigene automated RPPA software (VigeneTech, Inc., North Billerica, MA). Dilution curves will be fitted to the logistic model "Supercurve Fitting" (http://bioinformatics.mdanderson.org/OOMPA). Relative protein levels for each sample were derived from the supercurve for each lysate by curve-fitting, will be normalized for protein loading, and the log2-scaled protein concentrations will be normalized by global sample median normalization.

IHC

Ten freshly cut slides from each biopsy and archived tissue will be analyzed by the Columbia University Molecular Pathology Lab for immunhistochemical (IHC) staining. IHC for phosphorylated STAT3, Ki67, and cleaved caspase-3, as well as other potential markers identified through transcriptional and/or proteomic profiling, will be performed based on the standard protocol published for each antibody. Standard protocols, institutional and from the antibody vender, will be assessed and modified as needed. Phospho-Stat3 (Tyr705) (D3A7) XP® Rabbit mAb [CellSignaling #9145]. Phospho-Stat3 staining will be measured with an H score calculated from the percent and intensity of staining.

Peripheral blood samples

Peripheral blood samples will be obtained from each patients prior to starting study drug, prior to the observed C2D1 dose and at the time of progression. Plasma and serum biomarkers will be assessed by Biomarkers Core of the Irving Institute for Clinical and Translational Research at Columbia University Medical Center. At minimum, IL-6, IL-8, and C-reactive protein levels will be assessed.

Pharmacokinetics

Ruxolitinib exhibits near complete oral absorption, achieving maximal plasma concentration at approximately 1-2 h post-dose with linear PK over a dose range of 5-200 mg. Full PK analyses will not be performed given the low likelihood of interactions between the trastuzumab and ruxolitinib. However, steady state trough and peak levels [2 hours post dose] of ruxolitinb will be assessed on C2D1 for the first 10 patients on study. Once the samples from the first 10 patients have been obtained they will be sent to Incyte for analysis. If the results are not within expected ranges then the protocol will be amended to include full PK sampling on the remaining patients. The time the trough level is drawn, the time the C2D1 dose is taken, and the time the peak level is drawn must be entered into the clinical research form.

10. STUDY CALENDAR

Baseline evaluations, except for scans and x-rays, are to be conducted within 14 days prior to start of protocol therapy. Scans, including CT scans and MUGA/ECHO, and x-rays must be done ≤ 28 days prior to the start of therapy.

		Cycle 1[c]			Cycle 2[c]	Cycle 3[c]	Cycle 4+[c]	Off Study
Visit Day	**Pre-Study**	**Day 1**	**Day 8**	**Day 15**	**Day 1**	**Day 1**	**Day 1**	**Within 28 days of the last dose**
Visit Window (Days)			**± 2[d]**	**± 2[d]**	**± 4[d]**	**± 4**	**± 4**	
Ruxolitinib[a]		X	X	X	X	X	X	
Trastuzumab		X			X	X	X	
Informed consent[e]	X							
Inclusion/ Exclusion Criteria								
Demographics	X							
Medical history	X							
Concurrent meds[f]		X	X	X	X	X	X[g]	X
History and Physical exam	X	X	X	X	X	X	X[g]	X
Vital signs	X	X	X	X	X	X	X[g]	X
Height	X							
Weight	X	X	X	X	X	X	X[g]	X
Performance status	X	X	X	X	X	X	X[g]	X
Adverse event evaluation	X	X	X	X	X	X	X[g]	X
CBC w/diff, plts	X	X	X	X	X	X	X[g]	X
Serum chemistry, liver function tests[h]	X	X	X	X	X	X	X[g]	X
Lipid panel	X[i]						X[i]	X
B-HCG	X[j]							
Pharmacokinetics					X[k]			
ECG	X		X[l]		X[l]		X[m]	X
EF Assessment (Echo or MUGA)	X				X[n]		X[o]	X
CT C/A/P or PET/CT[p]	X						X[m]	X
Bone Scan[q]	X[q]						X[q]	X[q]
Review Pill Diary			X	X	X	X	X[g]	X
Blood Biomarker	X				X			X

		Cycle 1[c]			Cycle 2[c]	Cycle 3[c]	Cycle 4+[c]	Off Study
Visit Day	**Pre-Study**	**Day 1**	**Day 8**	**Day 15**	**Day 1**	**Day 1**	**Day 1**	**Within 28 days of the last dose**
Visit Window (Days)			**± 2[d]**	**± 2[d]**	**± 4[d]**	**± 4**	**± 4**	
(Section 9)								
Whole blood for DNA	X							
Tumor Analysis: Predictive Biomarker (Section 9)	X[r]				X[s]			X[s]

a. Ruxolitinib: Dose as assigned (phase I dose level 0: 20 mg b.i.d.; phase II dose will be based upon the phase I Maximum Tolerated Dose). **Patients will be instructed not take ruxolitinib until seen by study team on days of study visits. Ideally, the first dose of ruxolitinib will be in the morning so that the first day allow for b.i.d.** On D1 of every cycle prior to drug dispensation and dose administration, subject's disease status/non-progression will be confirmed by the treating investigator, including clinical and radiographic assessment. RECIST restaging will be confirmed for the most recent scans received, including documentation of investigator sign off on calculation of percentage change from baseline and nadir and overall response, prior to treatment. If the new scan information becomes available mid cycle, the study team will inform the subject if treatment discontinuation is required per protocol in real time, and document date of treatment discontinuation.

b. Trastuzumab: Fixed dosing. If no trastuzumab > 28 days from initiating trastuzumab: load 8 mg/kg IV. Otherwise, 6 mg/kg IV. If loaded, patient should remain on 6 mg/kg IV every 21 days (+/- 3 days).

c: Cycle = 21 days (+/-4 days from Day 1 of the prior cycle)

d. During cycle 1, patients will be seen on day 1, 8 (+/- 2 days), 15 (+/- 2 days). Cycle 2 day 1 should be within 21 days (+/- 4 days) from Cycle 1 day 1.

e. Radiographic assessments and EF (ejection fraction) testing may be done before consenting, if performed as part of the subject's routine care/procedures, and if done within the screening window per the protocol-defined method time period.

f. Patients on strong CYP3A4 inhibitors are not eligible. Must be off for at least 1 week prior to being deemed eligible.

g. Every 21 days (+/- 4 days) during phase I and phase II

h. Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.

i. Lipids should be conducted at baseline, prior to cycle 4, and then every 3 cycles (~9 weeks+/- 1 week). Hyperlipidemia is not an exclusion criterion. Lipids do not need to be fasting. If abnormalities are detected, fasting blood lipid levels (at least 8 hours fasting) should be considered and patients should be treated per standard of care.

j. Serum pregnancy test (women of childbearing potential).

k. Trough (prior) and peak (2 hours post) levels should be drawn at Cycle 2 Day 1 (+/- 2 days) during phase I only.

l. ECG prior to dose at Cycle 1 Day 8 (+/- 2 days) and prior to Cycle 2 Day 1 during phase I only.

m. Every 3 cycles (~9 weeks of treatment +/- 4 days) during phase I and phase II. Scans to be conducted every 9 weeks (+/- 4 days) from start of study treatment.

n. During phase I only, EF assessment should occur prior to Cycle 2 Day 1.

o. EF assessment should be performed prior to Cycle 4 Day 1. EF assessment should be repeated 3 cycles (~9 weeks+/- 4 days) prior to treatment with trastuzumab.

p. If patients have known brain metastases and are eligible for this trial, a head CT or brain MRI MUST be performed along with systemic imaging. If a patient does not have known brain metastases and is asymptomatic, no baseline brain imaging is required. . Scans to be conducted every 9 weeks (+/- 4 days) from start of study treatment. Corresponding RECIST restaging will be conducted in real time following scans and signed (electronically or wet ink) by the investigator, including confirmation of overall disease status per RECIST v.1.1

q. Baseline bone scan required in all patients.

NOTE: If bone scan done ≤ 6 weeks prior to registration showed no evidence suggesting bone metastases and no

		Cycle 1[c]			Cycle 2[c]	Cycle 3[c]	Cycle 4+[c]	Off Study
Visit Day	**Pre-Study**	**Day 1**	**Day 8**	**Day 15**	**Day 1**	**Day 1**	**Day 1**	**Within 28 days of the last dose**
Visit Window (Days)			**± 2[d]**	**± 2[d]**	**± 4[d]**	**± 4**	**± 4**	

clinical indication of skeletal pain or other evidence suggesting bone metastases, a repeat baseline bone scan is not required. If there is evidence of disease on the bone scan, bone scans should be continued every 3 cycles (~9 weeks of treatment +/- 4 days), as clinically indicated.

r. From archived primary and/or metastatic biopsy if patient is amenable and has accessible disease repeat biopsy (optional)

s. If patient is amenable and has accessible tumor, repeat biopsy (optional). These biopsies should be considered in patients with accessible disease.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

For the purposes of the phase I/II study, patients should be re-evaluated for response every 3 cycles (~9 weeks of treatment +/- 4 days). In addition to a baseline scan, confirmatory scans should also be obtained 9 (not less than 4) weeks (+/- 4 days) following initial documentation of an objective response.

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

11.1.1 Definitions

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment with ruxolitinib and trastuzumab.

Evaluable for objective response: Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response: Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

11.1.2 Disease Parameters

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

Note: Tumor lesions that are situated in a previously irradiated area may be considered measurable, as long as they meet the criteria above.

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

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

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

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

Target lesions: All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

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

11.1.3 Methods for Evaluation of Measurable Disease

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

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

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

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

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

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

PET-CT: At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site believes 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.

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

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

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

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

11.1.4 Response Criteria

11.1.4.1 Evaluation of Target Lesions

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

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

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is

the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

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

11.1.4.2 Evaluation of Non-Target Lesions

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

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

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

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

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

11.1.4.3 Evaluation of Best Overall Response

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

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

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

Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

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

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

11.1.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

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

Percent progression-free at 24 weeks is defined as the proportion of patients who remain progression-free at 24 weeks from study entry day #1 (binary proportion).

11.1.7 Response Review
All responses are reviewed by an expert(s) independent of the study at the study's completion. Simultaneous review of the patients' files and radiological images is the best approach.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements). The Data Safety Monitoring Plan is described in Section 12.2.

12.1 Data Collection

The Herbert Irving Comprehensive Cancer Center has an electronic clinical trials and data management system that will be used for data collection. CRFs for the study will be built into Velos for data entry. The system has full auditing capabilities which is web-based and housed on a server in a fully HIPAA compliant server room with restricted access and video camera monitoring. All users must login with their own application username and password. Users off campus must first access the Virtual Private Network with their assigned campus username and password and then use their application credentials. Users are only able to view study information if they are indicated as study personnel in our electronic IRB system, or an affiliate IRB system. Users are limited to access based on the role assigned in their corresponding protocol. Subject data is entered directly into the system, which (in the case of Columbia subjects) confirms the correct identity of patients via an interface with the electronic medical patient index. Staff with the appropriate IRB defined roles can run reports within the system for reporting purposes.

12.2 Data Reporting

Responsibility for Data Submission

Case Report Forms will be completed for each subject enrolled into the clinical study through Velos eResearch. It is the investigator's responsibility for ensuring that all clinical and laboratory data entered on the corresponding CRFs are complete, accurate and authentic.

Refer to the table below regarding the form completion and source document submission guidelines.

Phase I:

Redacted Source Documents (if applicable)	Case Report Form	Pre-Study (**Required for Central Registration**)	Baseline	Cycle 1 Day 1	Cycle 1 Day 8	Cycle 1 Day 15	Cycle 2	Cycle 3	Cycle 4	Off Study	SAEs
Required Submission Schedules											
Within 2 business days of visit		At time of Central Registration submission	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 24 hours and ongoing as available and applicable
x	Registration and Demographics		x								
x	Eligibility (Inclusion and Exclusion)		x								
x	Medical and Surgical History		x								
x	Breast Biopsy and Histology		x								
x	Metastatic Breast Cancer Prior to Study		x								
x	HER2 Status Prior to Study		x								
x	Previous Therapy		x								
x	Baseline Physical Exam		x								
x	Baseline Cardiac Assessments		x								
x	Baseline Laboratory & Biomarker Collection (Heme and chemistry)		x								
x	Baseline Imaging		x								
x	Baseline Disease Assessment		x								

Redacted Source Documents (if applicable)	Case Report Form	Pre-Study (**Required for Central Registration**)	Baseline	Cycle 1 Day 1	Cycle 1 Day 8	Cycle 1 Day 15	Cycle 2	Cycle 3	Cycle 4	Off Study	SAEs
Required Submission Schedules											
Within 2 business days of visit		At time of Central Registration submission	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 24 hours and ongoing as available and applicable
x	On Study Physical Exam			x	x	x	x	x	x		
x	On Study Cardiac Assessment				x		x		x[1]		
x	On Study Hematology			x	x	x	x	x	x		
x	On Study Biochemistry			x	x	x	x	x	x		
x	Cycle Form			x	x	x	x	x	x		
x	Agent Interruption Form			x	x	x	x	x	x		
x	Phase I Observation Period						x[2]				
x	On Study Lipid Panel								x[3]	x	
x	Pharmacokinetics						x				
x	Follow Up On Study Tumor Assessment							x			
x	On Study Biomarker Collection						x				
x	Research Related Biopsies			x			x			x	
x	Adverse Events		x	x	x	x	x	x	x	x	x
x	Concomitant Medications, Supplements, Et. Al.		x	x	x	x	x	x	x	x	
x	Off Study Medical History									x	
x	Off Study Physical Exam									x	

Redacted Source Documents (if applicable)	Case Report Form	Pre-Study (**Required for Central Registration**)	Baseline	Cycle 1 Day 1	Cycle 1 Day 8	Cycle 1 Day 15	Cycle 2	Cycle 3	Cycle 4	Off Study	SAEs
Required Submission Schedules											
Within 2 business days of visit		At time of Central Registration submission	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 24 hours and ongoing as available and applicable
x	Off Study Laboratory Data (Hematology)									x	
x	Off Study Laboratory Data (Biochemistry)									x	
x	Off Study Cardiac Assessment									x	
x	Off Study Biomarkers									x	
x	Off Study									x	
x	Death Report[4]										
	Comments*										
	Verification										
x	Off Schedule Imaging*										
x	Follow Up On Study Tumor* Assessment										

[1] On study cardiac assessments to be completed prior to C4D1 and every 3 cycles thereafter; CRF to be completed with C4D1 assessments, C7D1 assessments, C10D1 assessments, etc.
[2] If subject does not complete entire 3 week phase I observation period, a Phase I Observation CRF should be inserted with last day of study assessments to note time point of DLT.
[3] On study lipid panel to be completed prior to C4D1 and every 3 cycles thereafter: CRF to be completed with C4D1 assessments, C7D1 assessments, C10D1 assessments, etc.
[4] Complete at Death Report CRF only if subject dies while on study or within 30 days of last study intervention.
[*] Certain CRF pages can be inserted into any Velos eResearch calendar time point such as Comments or Off Schedule Imaging. Please provide applicable redacted source in these instances.

Phase II:

Redacted Source Documents (If applicable)	Case Report Form	Pre-Study (**Required for Central Registration**)	Baseline	Cycle 1 Day 1	Cycle 1 Day 8	Cycle 1 Day 15	Cycle 2	Cycle 3	Cycle 4	Off Study	SAEs
Required Submission Schedules											
Within 2 business days of visit		At time of Central Registration submission	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 24 hours and ongoing as available and applicable
x	Registration and Demographics		x								
x	Eligibility (Inclusion and Exclusion)		x								
x	Medical and Surgical History		x								
x	Breast Biopsy and Histology		x								
x	Metastatic Breast Cancer Prior to Study		x								
x	HER2 Status Prior to Study		x								
x	Previous Therapy		x								
x	Baseline Physical Exam		x								
x	Baseline Cardiac Assessments		x								
x	Baseline Laboratory & Biomarker Collection (Heme and chemistry)		x								
x	Baseline Imaging		x								
x	Baseline Disease Assessment		x								
x	On Study Physical Exam			x	x	x	x	x	x		

Redacted Source Documents (If applicable)	Case Report Form	Pre-Study (**Required for Central Registration**)	Baseline	Cycle 1 Day 1	Cycle 1 Day 8	Cycle 1 Day 15	Cycle 2	Cycle 3	Cycle 4	Off Study	SAEs
Required Submission Schedules											
Within 2 business days of visit		At time of Central Registration submission	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 24 hours and ongoing as available and applicable
x	On Study Cardiac Assessment								x[1]		
x	On Study Hematology			x	x	x	x	x	x		
x	On Study Biochemistry			x	x	x	x	x	x		
x	Cycle Form			x	x	x	x	x	x		
x	Agent Interruption Form			x	x	x	x	x	x		
x	On Study Lipid Panel								x[2]	x	
x	Follow Up On Study Tumor Assessment							x			
x	On Study Biomarker Collection						x				
x	Research Related Biopsies			x			x			x	
x	Adverse Events		x	x	x	x	x	x	x	x	x
x	Concomitant Medications, Supplements, Et. Al.		x	x	x	x	x	x	x	x	
x	Off Study Medical History									x	
x	Off Study Physical Exam									x	
x	Off Study Laboratory Data (Hematology)									x	
x	Off Study Laboratory Data (Biochemistry)									x	

Redacted Source Documents (If applicable)	Case Report Form	Pre-Study (**Required for Central Registration**)	Baseline	Cycle 1 Day 1	Cycle 1 Day 8	Cycle 1 Day 15	Cycle 2	Cycle 3	Cycle 4	Off Study	SAEs
Required Submission Schedules											
Within 2 business days of visit		At time of Central Registration submission	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 2 business days of visit	Within 24 hours and ongoing as available and applicable
x	Off Study Cardiac Assessment									x	
x	Off Study Biomarkers									x	
x	Off Study									x	
x	Death Report[3]										
	Comments*										
	Verification										
x	Off Schedule Imaging*										
x	Follow Up On Study Tumor* Assessment										

[1] On study cardiac assessments to be completed prior to C4D1 and every 3 cycles thereafter; CRF to be completed with C4D1 assessments, C7D1 assessments, C10D1 assessments, etc.

[2] On study lipid panel to be completed prior to C4D1 and every 3 cycles thereafter: CRF to be completed with C4D1 assessments, C7D1 assessments, C10D1 assessments, etc.

[3] Complete at Death Report CRF only if subject dies while on study or within 30 days of last study intervention.

[*] Certain CRF pages can be inserted into any Velos eResearch calendar time point such as Comments or Off Schedule Imaging. Please provide applicable redacted source in these instances.

12.3 Data and Safety Monitoring Committee

The NCI-approved Data Safety and Monitoring Committee (DSMC) of the Herbert Irving Comprehensive Cancer Center (HICCC) will monitor every subject who receives treatment on this protocol for toxicity. This protocol will adhere to the policies of the currently approved HICCC Data and Safety Monitoring Plan (DSMP), which is in accordance with NCI and CUMC-IRB policy and guidelines. The committee is led by Dr. J. Gregory Mears and consists of HICCC faculty and staff with expertise in oncology, research pharmacy, research nursing, and data management. The DSMC convenes twice a month to review patient safety and the conduct of the trial. The PI will submit data and safety monitoring reports to the DSMC at a frequency to be determined by the DSMC based on risk to the subjects. (**Appendix F**)

At the time of renewal, the study team will submit the most recent DSMC approval letter for safety review to the CUMC IRB. Any modifications that are required by the DSMC to ensure patient safety will be submitted to the IRB. All protocol deviations, violations, and eligibility waivers will be submitted to and approved by the DSMC prior to being reported to the IRB. All study data reviewed and discussed during these meetings will be kept confidential.

The Coordinating Site will assure that there is a mechanism in place to distribute the report to all participating investigators for submission to their local IRB. The report will document that a review of data and outcomes across all centers took place on a given date. It will summarize the DSMC's review of the cumulative toxicities reported from all participating sites without specific disclosure by treatment arm. It will also inform site investigators of the study the DSMC's conclusion with respect to progress or need for modification of the protocol.

12.4 Quality Control and Quality Assurance

Independent monitoring of the clinical study for protocol and GCP compliance will be conducted periodically by the CPDM Compliance Core on behalf of the HICCC DSMC. Additionally, the Compliance Oversight Committee of the IRB at Columbia University Medical Center may audit the study at any time, per institutional policies and procedures.

The investigator-sponsor and Columbia University Medical Center will permit direct access of the study monitors and appropriate regulatory authorities to the study data and to the corresponding source data and documents to verify the accuracy of this data.

A risk-based approach will be used by the Compliance Core to determine the frequency, number of subject charts, and data elements to be monitored. The Compliance Coordinator will review the study status and summarize enrollment, toxicities, SAEs/UPs, dose escalation, statistical endpoints (e.g., stopping rules), etc. for the full DSMC membership at the regularly scheduled meetings.

Internal On-site Monitoring:

1. Initial, recurrent, and close-out on-site monitoring visits will also be conducted at remote clinical sites, as appropriate/feasible. Other sites will have monitoring performed remotely (see below for further details).

a. The study Monitoring Visit Log will be completed and signed by the monitor and the PI/CRNP/CRN and/or CRC and will be filed in the regulatory binder.

2. The Compliance Coordinator will communicate with the site coordinator/Site Principle Investigator to schedule the monitoring visit and arrange for access to study materials and documentation.

3. The assigned Compliance Coordinator will monitor IIT trials within 1 month after the first subject is enrolled and throughout the life of the study to ensure that the study is being conducted in accordance with the protocol, GCP, applicable federal and local regulations, and per all applicable SOPs. The Compliance Coordinator is responsible to notify the PI and CRNP/CRN/CRC of upcoming monitor visits and convey what information and documentation will be required for the visit(s). The Compliance Coordinator is responsible for verifying that informed consent is properly obtained, eligibility is met (via the central registration process), and all study procedures are conducted according to the study protocol. The Compliance Coordinator will also verify that the data reported in the CRF's accurately reflect source documents, that all toxicities have been reported to date, and that all SAE's/UPs/deviations/violations have been reported according to local IRB and HICCC DSMC requirements. The Compliance Coordinator will issue queries and ensure resolution in a timely and efficient manner. The Compliance Coordinator will also monitor for applicable regulatory compliance and research pharmacy compliance (if applicable) and communicate any deficiencies as appropriate.

12.5 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

1. What protected health information (PHI) will be collected from subjects in this study
2. Who will have access to that information and why
3. Who will use or disclose that information
4. The rights of a research subject to revoke their authorization for use of their PHI

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects who have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

12.6 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy

dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

12.7 Reporting to Incyte Corporation

The Overall Principal Investigator will send complete Data transfers every six months during the course of the study to Incyte Corporation. Incyte Corporation may request additional data transfers over the course of the study. The data will be provided in a readable format for all study participants. The data will not include any protected health information as defined under HIPAA.

Additionally, Incyte Corporation will be provide monthly study status updates including a summary of:

- Recruitment (screened, enrolled, discontinued and on-going with dates and study drug dosing regimens,
- SAEs and safety reports to regulatory authorities
- Regulatory correspondence include IRB approval letters, IRB approved protocol, informed consent documents, and documents related to Inycte.
- Investigational product Inventory
- Payment milestones.

12.8 Records Retention

Records relating to a specific research activity, including research records collected by investigators, must be maintained for at least three years after completion of the research (45 CFR 46.115(b); 21 CFR 56.115(b); 21 CFR 312.62). This minimum retention period applies whether or not any subjects were enrolled in the study.
If the research is FDA regulated, records should be retained for at least two years after approval of the investigational agent by FDA; if it is not approved, records should be retained at least two years after the study is terminated and FDA is notified (note the additional requirement below for clinical research studies);
Clinical records, including consent forms that document clinical intervention or clinical diagnostic procedure research-related procedures, must be retained in medical records by the institution for at least seven years, per CUMC and NYP policy, which is based on state law.

Guidelines for Processing IND Safety Reports

The U.S. Food and Drug Administration (FDA) regulations require sponsors of clinical studies to notify the FDA and all participating investigators of any serious and unexpected adverse experiences that are possibly related to the investigational agent. The CUMC Principal Investigator will review all applicable IND Safety Reports and has the responsible for forwarding the IND Safety Reports to the Affiliate Institutions. The Affiliate Institution investigators are to review, send a copy to their IRB according to their local IRB's policies and procedures, and file a copy with their regulatory documents. All Affiliate site INDSR submissions, along with IRB acknowledgment (per local policies and procedures) are to be forwarded to CUMC for placement within the trial master file.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Primary Endpoint (Sample Size)

Phase I (n=10 patients):
The objective of the phase I portion of this study is to determine an acceptable dose of ruxolitinib in combination with trastuzumab. The dose of trastuzumab will be fixed at 6 mg/kg every 3 weeks IV for all patients. If patients have not received trastuzumab for > 28 days prior to the initiation of study drugs, a loading of 8 mg/kg IV should be used. After this dose, patients should remain on 6 mg/kg every 21 days (+/- 4 days). We evaluate four doses of ruxolitinib PO during the phase I. The ordering of these doses is as follows:

Dose Level	Dose
1	25 mg bid
0 (starting dose level)	20 mg bid
-1	15 mg bid
-2	10 mg bid

The maximum tolerated dose (MTD) combination is defined as the dose combination associated with a target probability of dose limiting toxicity of 0.25. A dose-limiting toxicity is defined as the MTD with DLTs defined as any grade 3 non-hematologic toxicities despite maximal supportive care or any grade 4 hematologic toxicity directly related to study drug. In order for a patient to be evaluable for DLT assessments, the patient must incur a DLT during the first 21 days of actively taking ruxolitinib plus trastuzumab. If, for instance, the patient temporarily stops ruxolitinib due to uncomplicated hematologic toxicities, the patient can resume ruxolitinib at the same dose and the DLT period will remain defined as the first 21 days of actively taking the study medication. In order to be considered eligible, the DLT period (i.e. 21 days of actively taking ruxolitinib plus trastuzumab) must be completed within 30 days of the first dose of ruxolitinib.

The MTD will be estimated using the time to event continual reassessment method (TITE-CRM). The TITE-CRM will use an empirical dose-toxicity model, with a sample size of 10. The dose-toxicity model is calibrated such that the method will eventually select a dose that yields between 16% and 34% DLT (Lee and Cheung 2009, Cheung and Chappell 2002). The advantage of the TITE-CRM is that it uses the patient's partial information before a complete follow-up is achieved. As a result, we can conduct the trial in a continuous fashion without having patients being turned away due to waiting time. However, since the MTD will be estimated from partial data, final data may show that patients will have been treated with a dose exceeding the MTD. Given the toxicities reported on previous studies using this drug, we anticipate very low rates of DLTs at 10 and 15 mg. Most likely the MTD will be 25mg or 20mg. In addition, another advantage is an increased likelihood of getting the true MTD with this methodology, as opposed to utilizing a 3+3 dose-finding design. We will start the trial with a dose of 20mg based on the Incyte Investigator's Brochure and assess the performance of the TITE-CRM under two most likely scenarios.

To evaluate the performance of the method, 2000 simulations under the two mostly likely scenarios of toxicity profiles were done. The operating characteristics of our design under these scenarios are displayed in the **Table 8**. With 10 patients, the design

selects the correct MTD with probabilities over 50%. These simulations assume that the patients arrive at a rate of 2 patients every 3 weeks, especially at the initiation of the clinical trial, and that a DLT occurs randomly and uniformly within the observation window.

Table 8: Selection Probabilities of the TITE-CRM

Doses of Ruxolitinib	10mg	15mg	20mg	25mg
Scenario 1				
DLT rate	1%	5%	5%	25%
P(Selection)	0%	2%	20%	78%
Scenario 2				
DLT rate	1%	5%	25%	40%
P(Selection)	1%	21%	51%	27%

Table 9 displays a simulation of a sample design using the method where the MTD is at dose level 1 (i.e. 25 mg po b.i.d.) Given the low likelihood of overlapping toxicities with ruxolitinib plus trastuzumab and favorable side effect profile of ruxolitinib, we anticipate that the table below is the most likely scenario.

Table 9: Sample simulated design with n=10

Patient	Arrival Time	Dose Level Assigned	Toxicity (Y/N)	Time of Toxicity
1	1.5 weeks	0	N	-
2	3 weeks	0	N	-
3	4.5 weeks	1	N	-
4	6 weeks	1	N	-
5	7.5 weeks	1	N	-
6	9 weeks	1	N	-
7	10.5 weeks	1	Y	1.11 weeks
8	12 weeks	1	N	-
9	13.5 weeks	1	N	-
10	15 weeks	1	N	-

Phase II (n=30 patients – evaluable patients from phase I at the recommended phase II trial may be included):
The primary endpoint of the phase II portion of this study is to define the progression free survival (PFS) of the combination of ruxolitinib plus trastuzumab in patients with HER2+ metastatic breast cancer who have previously received HER2 targeted therapy. PFS will be defined as the time from patient registration until objective or disease progression or death from any cause. Assuming a historical PFS of 8 weeks with single-agent agent HER2-targeted therapy in metastatic HER2+ breast cancer after progressing on trastuzumab-based therapy (Blackwell JCO 2010), we predict that patients receiving the combination of ruxolitinib at the MTD dose] plus trastuzumab will have a PFS of at least 13 weeks. With a 2-sided alpha of 0.05, we have 80% power to detect a difference with 30 pts.

The PFS distribution of the treatment arm will be estimated by Kaplan-Meier survival analysis. Ninety-five percent confidence intervals for the Kaplan-Meier PFS estimates will be calculated using Greenwood's formulae. Patients will be analyzed regardless of therapy initiation or compliance for this analysis. A secondary analysis will be performed comparing "evaluable" patients. We will define "evaluable" patients as patients who met eligibility requirements, have initiated therapy, and were not removed from the study for non-compliance or patient withdrawal. Evaluation of disease will be made according to RECIST criteria (version 1.1) in patients with measurable disease. Patients with hormone receptor positive metastatic breast cancer may not have easily measurable disease as defined by RECIST criteria, in particular bone and soft tissue metastases. In these patients CR or PR are not easily assessed. Thus, we have chosen Progression Free Survival as our primary endpoint. The Clinical Benefit Rate (CBR), defined as complete response, partial response or stable disease for > 24 weeks, is our secondary endpoint. In addition, overall response rate, CR and PR will be analyzed in evaluable patients.

13.2 Accrual Rate
Four centers will accrue patients to the phase I/II portions of this trial. These centers are: New York Presbyterian Hospital – Columbia University, Albert Einstein College of Medicine, New York Presbyterian Hospital - Cornell University, and Mount Sinai Medical Center. We anticipate that each site will be able to accrue 5 patients per year and believe that the accrual period will be 2 years. If the accrual is lower than expected within the first 6 months, we will consider expanding the study.

13.3 Stratification Factors
Patients will be stratified by hormone receptor status (binary: positive or negative). Positivity will be defined as estrogen receptor and/or progesterone receptor status $\geq$ 1% by immunohistochemistry.

13.4 Analysis of Secondary Endpoints

13.4.1 Clinical Benefit Rate, Percent Progression-Free at 24 weeks, Objective Response Rate:
Secondary endpoints include clinical benefit rate (CR + PR + SD > 24 weeks) (estimated via binomial proportions) and percent progression-free at 24 weeks. Ninety-five percent confidence intervals will be calculated for the clinical benefit response proportion and the percent progression-free at 24 weeks via binomial proportions. The analysis of objective response rate will be conducted on the

Measurable Disease at baseline analysis set. Exact binomial 2-sided 95% confidence intervals will be generated for the objective response rate.

13.4.2 **Toxicity Evaluation**
The frequency of subjects experiencing toxicities will be tabulated.
The CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. The CTEP Version 4.0 of the CTCAE is identified and located on the CTEP website at (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).
All appropriate treatment areas should have access to a copy of the CTEP Active Version of CTCAE. All patients will be evaluable for toxicity from the time of their first treatment with the study drugs.

13.4.3 **Correlative Studies**
Transcriptional profiling will be assessed by our bioinformatics core under the direction of Dr. Califano using standard analyses, as well as Interactome modeling. Comparisons between pre-treatment to on-treatment will be assessed for significance and trend using the paired t-tests.

13.5 Reporting and Exclusions

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

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

14. PROTECTION OF HUMAN SUBJECTS
This study is to be conducted in accordance with applicable government regulations and Institutional research policies and procedures. AN IND annual report will be submitted to the FDA in accordance with 21.CFR 312.33.

This protocol and any amendments will be submitted to a properly constituted Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using

the IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

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APPENDIX A: PERFORMANCE STATUS CRITERIA

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

APPENDIX B: SUBJECT'S MEDICATION DIARY

SUBJECT'S MEDICATION DIARY

Today's date _____ **Agent: Ruxolitinib _____ mg**

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

Cycle	Day	Date	Time of dose (AM)	Number of Pills (AM)	Time of dose (PM)	Number of Pills (PM)	Comments Side effects
	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						

SUBJECT SIGNATURE **DATE**

APPENDIX C: STRONG KNOWN CYP 3A4 INHIBITORS AND INDUCERS.

Inhibitors
boceprevir
clarithromycin
chloramphenicol
conivaptan
indinavir
itraconazole
ketoconazole
lopinavir/ritonavir
mibefradil
nefazodone
nelfinavir
posaconazole
ritonavir
saquinavir
telaprevir
telithromycin
voriconazole
Patients should limit grapefruit juice intake

Source: Jakafi prescribing information, 2013.

APPENDIX D: ARCHIVED TISSUE AND BIOPSY COLLECTION AND SHIPMENT

All formalin-fixed paraffin embedded (FFPE) from archived tumor and study-related biopsies will be stored onsite until requested by the coordinating center. Ideally, all slides will be cut at the same time, within 6 weeks of time of request, and sent to the coordinating center to distribute for analysis at the Columbia University Genome Center, Columbia University Molecular Pathology Core, and MD Anderson Functional Proteomics Core Facility.
Ship to:
Kevin Kalinsky, MD, MS
Attn: Stephanie Aguilar
Columbia University Medical Center
161 Fort Washington Ave – Mezzanine
New York, NY 10032
Phone: 212-342-4591
Complete these forms separately for each subject; enclose with shipment along with accompanying pathology report.

I. Archived Diagnostic Tissue

Subject ID: _____

Archival tissue, diagnostic biopsy ☐ Not available		
Number of slides	Slide preparation	Slide IDs
	☐ 10 immunoblanks [each having 4 micron sections on charged slides] ☐ 1 intervening H&E stained ☐ 12 regular slides each with 10 microns sections	

Enclose redacted accompanying pathology report.

Archival tissue, biopsy at recurrence available/applicable		☐ Not
Number of slides	Slide preparation	Slide IDs
	☐ 10 immunoblanks [each having 4 micron sections on charged slides] ☐ 1 intervening H&E stained ☐ 12 regular slides each with 10 microns sections	

Enclose redacted accompanying pathology report.

Date	Name of person responsible for this shipment

II. Optional study-related biopsies

For patients with accessible tumor, 2 punch biopsies (for skin) or 3 core biopsies are recommended. The following will be requested: 10 immunoblanks [each having 4 micron sections on charged slides], one intervening H&E stained slide, and 12 regular slides each with 10 microns sections.
Subject ID: _____

FFPE tissue, Pre-treatment biopsy ☐ Core biopsies (3 preferred) ☐ Punch biopsies (2 preferred)			
Number of slides	Slide preparation	Date Collected	Slide IDs
	☐ 10 immunoblanks [each having 4 micron sections on charged slides] ☐ 1 intervening H&E stained ☐ 12 regular slides each with 10 microns sections		

Enclose redacted accompanying pathology report (if available).

FFPE tissue, On-treatment biopsy from C2D1 (prior to treatment) ☐ Core biopsies (3 preferred) ☐ Punch biopsies (2 preferred)			
Number of slides	Slide preparation	Date Collected	Slide IDs
	☐ 10 immunoblanks [each having 4 micron sections on charged slides] ☐ 1 intervening H&E stained ☐ 12 regular slides each with 10 microns sections		

Enclose redacted accompanying pathology report (if available).

Date	Name of person responsible for this shipment

III. Optional biopsy upon progression

For agreeable patients with accessible tumor upon progression, 2 punch biopsies (for skin) or 3 core biopsies are recommended. The following will be requested: 10 immunoblanks [each having 4 micron sections on charged slides], one intervening H&E stained slide, and 12 regular slides each with 10 microns sections.
Subject ID: _____

FFPE tissue, biopsy upon progression ☐ Core biopsies (3 preferred) ☐ Punch biopsies (2 preferred)			
Number of slides	Slide preparation	Date Collected	Slide IDs
	☐ 10 immunoblanks [each having 4 micron sections on charged slides] ☐ 1 intervening H&E stained ☐ 12 regular slides each with 10 microns sections		

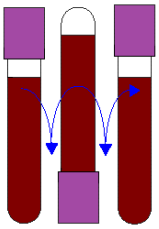
Date	Name of person responsible for this shipment

APPENDIX E: BLOOD BIOMARKER COLLECTION

I. Plasma and Serum Biospecimen Collection

Samples should be obtained at protocol specified time points, using supplies available onsite. All specimens will be stored onsite and shipped out when requested (usually at inception of analysis). Samples are collected according to the calendar in section 9.

I. Whole Blood

Collect	Invert	Store
		
At least 3 ml whole blood in EDTA tube (lavender top)	Invert 8 to 10 times.	Freeze in -80 freezer until ready to ship; alternatively, freeze in -20 freezer up to 4 weeks prior to transferring to -80 freezer for long-term storage.

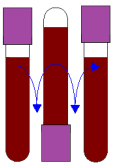
Whole blood is collected pre-study only for all subjects.

- Be sure to label tube with subject ID, date and time point
- When requested, ship to:

  Regina Santella Laboratory
  Attn: Irina Gurvich
  Columbia University Medical Center
  630 West 168th St Room 16-410
  New York, NY 10032
  Phone: 212-305-8158
- *Ship on dry ice for overnight delivery; FedEx airbill can be supplied by study team.*
- Email m1906@columbia.edu and ig112@columbia.edu to inform study team and Irina of incoming samples

II. Plasma PK – C2D1 only

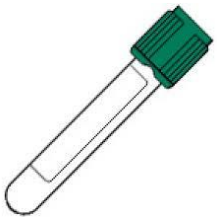
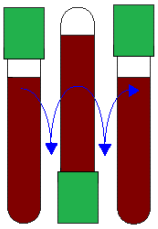
Plasma PK is collected C2D1 prior to treatment only for all phase I subjects.
Trough (prior) and peak (2 hours post) levels should be drawn at Cycle 2 Day 1.

Collect	Invert	Ice/Water Bath	Centrifuge	Aliquot & label	Store
					
4 ml whole blood in K_2EDTA tube (lavender top)	Invert ≥ 5 times.	Place tube on ice or in water bath.	Within 45 min. of collection, centrifuge 2000 x g for 15 minutes at ~5ºC.	With pipette, aliquot equal amounts plasma into 2 cryotubes.	Ship Plasma aliquot A same day as collection or store both in -20 to -80 freezer until ready for shipment.

- Label the cryotubes with protocol number, patient ID, time of collection and Plasma aliquot A or Plasma aliquot B. Place transparent tape over the cryotube labels.
- When prepared, ship Plasma aliquot A samples to:

  Incyte Corporation
  Attn: Tom Emm
  Route 141 & Henry Clay Rd
  Building 400-3431
  Wilmington, DE 19880
  Phone: 302-498-6775
- *Ship on plenty of dry ice for overnight delivery; FedEx airbill can be supplied by study team.*
- Plasma aliquot B samples will remain in frozen storage at the site until specified by Incyte or the study PI to ship
- Email temm@incyte.com and smalhotra@incyte.com with tracking information to inform them of incoming samples

III. Plasma (C-reactive protein, et. al)

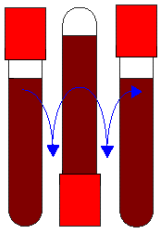
Collect	Invert	Centrifuge	Aliquot	Store
				
At least 4 ml whole blood in lithium heparin tube (dark green top); alternatively, sample can be collected in pink top K_2EDTA tube.	Invert 8 to 10 times (do not shake). Keep at room temperature or on regular ice after inverted.	Within 1 hour of collection, centrifuge 2000 x g for 15 minutes at 4°C.	With pipette, aliquot ~1.0 ml of plasma into 3 to 5 cryovials. (max. volume of cryovials should be 2 ml)	Freeze in -80 freezer until ready to ship.

Plasma is collected pre-study, C2D1 prior to treatment, and at progression for all subjects.

- Be sure to label cryovials with subject ID, date, specimen type and time point
- When requested, ship to:

  Regina Santella Laboratory
  Attn: Irina Gurvich
  Columbia University Medical Center
  630 West 168th St Room 16-410
  New York, NY 10032
  Phone: 212-305-8158
- *Ship on dry ice for overnight delivery; FedEx airbill can be supplied by study team.*
- Email m1906@columbia.edu and ig112@columbia.edu to inform study team and Irina of incoming samples

IV. Serum (IL6, IL8 cytokines & additional serum biomarkers)

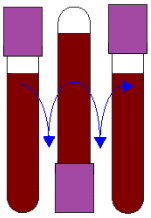
Collect	Invert	Centrifuge	Aliquot	Store
				
Up to 10 ml whole blood in serum tube (red top, no gel separator); alternatively, sample can be collected in multiple serum tubes less than 10 ml. to have adequate aliquots.	Invert 8 to 10 times. Keep at room tempature until adequately clotted (at least 30 minutes).	Within 1 hour of collection, centrifuge 2000 x g for 15 minutes at 4°C.	With pipette, aliquot ~1.0 ml of serum into 4 to 6 cryovials. (max. volume of cryovials should be 2 ml)	Freeze in -80 freezer until ready to ship.

Serum Plasma is collected pre-study, C2D1 prior to treatment, and at progression for all subjects.

- Be sure to label cryovials with subject ID, date, specimen type and time point
- When requested, ship to:

  Regina Santella Laboratory
  Attn: Irina Gurvich
  Columbia University Medical Center
  630 West 168th St Room 16-410
  New York, NY 10032
  Phone: 212-305-8158
- *Ship on dry ice for overnight delivery; FedEx airbill can be supplied by study team.*
- Email m1906@columbia.edu and ig112@columbia.edu to inform study team and Irina of incoming samples

V. Plasma (Additional plasma biomarkers)

Collect	Invert	Centrifuge	Aliquot	Store
				
At least 4 ml whole blood in EDTA tube (lavender top)	Invert 8 to 10 times.	Within 1 hour of collection, centrifuge 2000 x g for 15 minutes at 4°C.	With pipette, aliquot ~1.0 ml of plasma into 3 to 5 cryovials. (max. volume of cryovials should be 2 ml)	Freeze in -80 freezer until ready to ship.

Plasma for biomakers is collected pre-study and C2D1 prior to treatment for all subjects.

- Be sure to label cryovials with subject ID, date, specimen type and time point
- When requested, ship to:

  Regina Santella Laboratory
  Attn: Irina Gurvich
  Columbia University Medical Center
  630 West 168th St Room 16-410
  New York, NY 10032
  Phone: 212-305-8158
- *Ship on dry ice for overnight delivery; FedEx airbill can be supplied by study team.*
- Email m1906@columbia.edu and ig112@columbia.edu to inform study team and Irina of incoming samples

VI. Shipping Document for Biospecimens

Store all biospecimens onsite until they are requested from the coordinating site. Enclose this document with all biospecmen shipments. You may ship multiple specimen types and subjects together, but keep time points and subjects grouped together (i.e. all pre-treatment samples grouped together for one subject; all C2D1 samples for one subject grouped together).
All biospecimens will be shipped to:

Regina Santella Laboratory
Attn: Irina Gurvich
Columbia University Medical Center
630 West 168th St Room 16-410
New York, NY 10032
Phone: 212-305-8158

Keep a copy or multiples copies of this document on site with study subject records. Send shipments on dry ice for overnight delivery.

Subject ID	Whole Blood	Plasma CRP	Serum	Plasma	Total # of cryovials	Comments
	☐ Pre-treatment	☐ Pre-treatment ☐ C2D1	☐ Pre-treatment ☐ C2D1	☐ Pre-treatment ☐ C2D1		
	☐ Pre-treatment	☐ Pre-treatment ☐ C2D1	☐ Pre-treatment ☐ C2D1	☐ Pre-treatment ☐ C2D1		
	☐ Pre-treatment	☐ Pre-treatment ☐ C2D1	☐ Pre-treatment ☐ C2D1	☐ Pre-treatment ☐ C2D1		
	☐ Pre-treatment	☐ Pre-treatment ☐ C2D1	☐ Pre-treatment ☐ C2D1	☐ Pre-treatment ☐ C2D1		
	☐ Pre-treatment	☐ Pre-treatment ☐ C2D1	☐ Pre-treatment ☐ C2D1	☐ Pre-treatment ☐ C2D1		

Use additional shipping documents if needed.

Date	Name of person responsible for this shipment

VII. Shipping Document for PK's

Ship PK's directly to Incyte for processing.
Ship same day if possible on dry ice or store in -80 freezer until ready for shipment.
All PK's will be shipped to:

Thomas Emm
Sr. Director, Drug Metabolism and Biopharmaceutics
Incyte Corporation
1801 Augustine Cut Off
Wilmington, DE 19803

Email temm@incyte.com and smalhotra@incyte.com with tracking information to inform them of incoming samples

Keep a copy of this document on site with study subject records.
Sites should use their own courier services for this activity.
Send shipments on dry ice for overnight delivery; shipments may be completed Monday to Thursday only as Saturday delivery is not available.

Subject ID	Plasma PK	Number of tubes being sent	Comments
	☐ C2D1 only prior to treatment		
	☐ C2D1 only prior to treatment		
	☐ C2D1 only prior to treatment		

Use additional shipping documents if needed.

Date	Name of person responsible for this shipment

COLUMBIA UNIVERSITY MEDICAL CENTER
Discover. Educate. Care. Lead.

HERBERT IRVING COMPREHENSIVE CANCER CENTER
Data and Safety Monitoring Committee

SERIOUS ADVERSE EVENT REPORTING FORM

1 – Trial Information	
SPONSOR	
OVERALL PRINCIPAL INVESTIGATOR	
LOCAL PRINCIPAL INVESTIGATOR	
IRB NUMBER	
STUDY TITLE	
Study team member reporting event	

2 – Type of event				
Are you reporting a UP? (Unanticipated Problem)	Yes	☐	No	☐

3 – SAE report type				
Report type	Initial notification	☐	Follow up	☐

4 – Timeline of the event					
Date Investigator informed		Date of report			
Date of Onset		Date Resolved			

5 – Participant information								
SUBJECT ID NUMBER		**Age**		**Gender**	Male	☐	Female	☐

6 – Description of the Event(s)					
	Seriousness		**Causality**		**Expectedness**
Death	☐	Not related	☐	Expected	☐
Life threatening	☐	Unlikely	☐	Not expected	☐
Hospitalization or prolongation of hospital stay	☐	Possibly	☐		
Persistent or significant disability or incapacity	☐	Probably	☐		
Congenital abnormality or birth defect	☐	Definitely	☐		
Otherwise considered serious	☐				

Primary Diagnosis			
CTCAE term for Primary SAE		Grade	Attribution
Start date of Primary SAE		End date of Primary AE	

Secondary SAEs during hospitalization/prolongation of hospitalization (if applicable)					
CTCAE term	Grade	Start date of AE	End date of AE	Attribution	Comments

Brief description and treatment of events							
Outcome							
Fatal	☐	On-going	☐	Recovered with sequelae	☐	Recovered	☐
If *recovered* (or *recovered with sequelae*) selected, enter date of recovery							
If *fatal* enter date of death							
Cause of death:							

7 – Medication details

Information about the CHEMOTHERAPY/ STUDY DRUG

Drug (please specify action taken next to the agent) Legend is in the last column	Dose	Route	Start date	Stop date	Action Taken
					1- Dose stopped 2- Dose reduced 3- Dose unchanged 4- Dose increased

Relevant Concomitant Medication

Drug (please specify action taken)	Indication	Dose	Route	Start date	Stop date	Action Taken
						1- Dose stopped 2- Dose reduced 3- Dose unchanged 4- Dose increased 5- N/A

Other clinical information, including relevant tests (laboratory, CT, ECG etc.) **Please include relevant redacted source documentation.

CONTACT DETAILS	
Clinical Research Coordinator or Research Nurse	
Signature and date	
Email	
Fax	
Telephone	

CONTACT DETAILS	
CUMC Principal Investigator or Co-Investigator providing coverage	
Signature and date	
Email	
Fax	
Telephone	

CONTACT DETAILS	
Sub-Site Principal Investigator	
Signature and date	
Email	
Fax	
Telephone	

APPENDIX G: GUIDELEINES FOR AFFILIATE INSTITUTIONS IN MULTICENTER STUDIES

1. Multi-site Communication:

The CPDM office at CUMC provides administration, data management, and organizational support for the affiliate sites in the conduct of a multicenter clinical trial. The CPDM office will coordinate regularly scheduled conference calls with affiliate sites.
The following issues will be discussed as appropriate:

- Enrollment information
- Cohort updates (i.e. DLTs)
- Adverse events (i.e. new adverse events and updates on unresolved adverse events and new safety information)
- Protocol violations
- Other issues affecting the conduct of the study

2. New Protocol Distribution, IRB Submission, Modifications and Annual Renewals

- Protocol specific documents are distributed to affiliate sites once CUMC IRB approval has been obtained.
- The affiliate site must submit a draft of site specific revisions to protocol and/or consent form documents for review and approval by the sponsor-investigator prior to submission to the local IRB. Draft documents should be sent to the study specific email address. The site will be provided confirmation that they are approved to submit to their local IRB.
- Protocol amendments must be approved by the affiliate site's local IRB within 90 days of distribution to the site by the sponsor-investigator.

3. Regulatory Documents:

3.1 Prior to Site Initiation:
Sponsor-Investigator will ensure that proper requests are made of sites and that the following documentation is collected prior to the initiation of an affiliate site.

- CV of PI, Sub-I's and other research staff listed on FDA 1572 (signed and dated copy within 2 years)
- Medical Licenses of PI and Sub-I's (current copy)
- Human subjects training certificates for PI and Sub-I's
- CLIA/Laboratory Certifications for Local Laboratories listed on FDA 1572
- Local Laboratory Director's CV and License
- Local Laboratory Reference Ranges
- IRB roster or statement of compliance
- FDA Form 1572, if applicable (wet ink originals required)
- Financial Disclosure forms for all members listed on FDA 1572 (wet ink originals required)

3.2 Ongoing Regulatory Documentation: Sponsor-Investigator will ensure that proper requests are made of sites and that the following documentation is collected throughout the course of the study.

- IRB approval letters for all protocol modifications and all renewals
- IRB-approved consent forms
- Current IRB roster, if statement of compliance is not provided as part of site initiation
- FDA Form 1572, if applicable as updates are required
- Updated investigator and site information where relevant (e.g., CV, medical licensure and Financial Disclosure for new sub-investigator, local laboratory information)

Regulatory documents may be sent to M1906@columbia.edu or to the following address if wet ink originals are required:

Clinical Protocol & Data Management Office
161 Fort Washington Ave.
Herbert Irving Pavilion
Mezzanine Level, M-203
New York, NY 10032

4. Protocol Deviation/Subject Waiver request for Affiliate Sites:

The Affiliate site MUST submit a prospective deviation request to the CUMC lead PI for review and submission to the HICCC DSMC and CUMC IRB. Approvals must be obtained from all entities prior to implementation at the Affiliate site. If a prospective protocol deviation request is submitted for review (from an Affiliate site), the PI/site memo(s), HICCC DSMC approval(s) and correspondence and CUMC IRB eligibility deviation approval letter(s) should be forwarded to the Affiliate site for documentation. The Affiliate site is also required to obtain prospective local IRB approval as per institutional policies/procedures prior to implementing the proposed deviation and registering/enrolling the subject via CUMC Central Registration. All documents and determinations must be clearly documented in the study subject's medical record, research chart and regulatory binder, as described.

5. Guidelines for Affiliate Site Monitoring

6.1 On-Site MCT Monitoring:

1. Initial, recurrent, and close-out on-site monitoring visits will also be conducted at Affiliate sites, as appropriate/feasible. Other sites will have monitoring performed remotely (see below for further details).
    - The study Monitoring Visit Log will be completed and signed by the monitor and the PI/CRNP/CRN and/or CRC and will be filed in the regulatory binder.

2. The Compliance Coordinator will communicate with the Affiliate site coordinator/Site Principle Investigator to schedule the monitoring visit and arrange for access to study materials and documentation.

3. The Compliance Coordinator will monitor IIT trials within 1 month after the first subject is enrolled at the Affiliate site and throughout the life of the study to ensure that the study is being conducted in accordance with the protocol, GCP, applicable federal and local regulations, and per all applicable SOPs. The Compliance Coordinator is responsible to notify the participating site PI and CRNP/CRN/CRC of upcoming monitor visits and convey what information and documentation will be required for the visit(s). The Compliance Coordinator is responsible for verifying that informed consent is properly obtained, eligibility is met (via the central registration process), and all study procedures are conducted according to the study protocol. The Compliance Coordinator will also verify that the data reported in the CRF's accurately reflect source documents, that all toxicities have been reported to date, and that all SAE's/UPs/deviations/violations have been reported according to Coordinating Center, local IRB and HICCC DSMC requirements. The Compliance Coordinator will issue queries and ensure resolution in a timely and efficient manner. The Compliance Coordinator will also monitor for applicable regulatory compliance and research pharmacy compliance (if applicable) and communicate any deficiencies as appropriate.

4. An SIV (or) teleconference will be scheduled and conducted prior to study drug being made available (if applicable) and before any subjects are enrolled on a study at the Affiliate site.

6.2 MCT Remote Monitoring:

1. When necessary (due to logistical constraints), Affiliate sites will be monitored remotely by a designated Compliance Coordinator. Sites will be informed of this remote monitoring process on a site by site basis.

2. Affiliate sites will be monitored by the Compliance Coordinator on both a regulatory level, as well as a clinical data/source documentation review level.

3. Redacted source documents (applicable to supporting the protocol specific CRF data requirements) will be sent to the designated Compliance Coordinator via fax or secure email for all subjects enrolled at Affiliate sites. Timelines for submission procedures will be defined on a case-by-case basis.

4. The Compliance Coordinator will review all submitted redacted source documents against the data entered on the protocol specific CRFs. The Compliance Coordinator will issue queries when/if necessary.

5. The Affiliate site research staff will respond to queries within 30 days. If queries remain outstanding, the Compliance Coordinator will send a delinquent query reminder for the outstanding items.

3. The remote monitoring procedures will include review of applicable redacted source documentation and supporting applicable documents to determine compliance regarding:
    - Informed consent procedures

- Eligibility criteria
- Protocol specific treatment compliance
- Protocol specific toxicity/outcome documentation/compliance
- Protocol specific schedule of events (e.g., baseline visits, pre-treatment, on study, follow-up)
- Participating site IRB documents (e.g., IRB amendment approvals, annual renewals, SAE/UP submissions, violation/deviation submissions, INDSR submissions, etc).
- Required specimen submissions (e.g., tissue specimens, research blood specimens, etc.)
- Pharmacy accountability records
- Adherence to the CRF submission timeframes to CUMC (within the protocol specified timeframes)

4. Affiliate site remote monitoring reports will be sent to the lead PI, HICCC DSMC, and Affiliate sites after each remote monitoring review. Reports will include information regarding data submission timeliness/accuracy, protocol adherence items, query resolution status, regulatory status, and overall Affiliate site performance. These reports will be generated by the Compliance Coordinator and reviewed with the Compliance Core Manager prior to dissemination.

6. Dose Level Determinations:

The sponsor-investigator will review enrollment for each dose level cohort during the regularly scheduled conference call with the affiliate sites.

The dose level for newly enrolled subjects will be determined by the study statistician upon notification that a subject has signed informed consent to participate in the study. The assigned dose level for any subject to begin study treatment will be communicated to the affiliate site along with the determination by Central Registration that the subject is eligible for enrollment in the study.

If a Dose Limiting Toxicity (DLT) is identified in a subject, the affiliate site must notify the sponsor-investigator via email at the study specific email address within 1 business day of identification. The lead site will communicate that a DLT has been experienced within 1 business day.

7. Confidentiality

Each affiliate site will be assigned a site number. Each subject that signs consent should be assigned a unique code number consisting of site number followed by a number with each new subject being assigned the next sequential number (e.g. 04-10). All sites will be required to enter their data in the Velos eResearch, the Clinical Trial Management System used for all Cancer-related clinical research at CUMC. All users must login with their own application username and password. Users off campus must first access the Virtual Private Network with their assigned campus username and password and then use their application credentials.

Subject confidentiality must be maintained according to HIPAA regulations and GCP recommendations.

Except when required by law, study information shared with persons and organizations outside of Columbia University Medical Center must not identify the patient by name, social security number, address, telephone number, or any other direct personal identifier.
If the results of this research project are published or presented at a scientific or medical meeting, the patient not be identified. Otherwise, all results will be kept confidential and will not be divulged (except as required by law) without permission.

Data Reporting Plan
Columbia University Medical Center (CUMC) is deeply committed to research integrity and strong credibility when it comes to the discovery of new treatment concepts, implementation of new clinical research techniques, and acceptance of its researcher's findings by the medical establishment. In accord with these ethics, CUMC encourages and supports its investigators in the sharing of final research data and/or details of newly developed clinical treatments.

CUMC's policies that pertain to patient data sharing conform to CUMC IRB rules, local and state laws, and HIPAA privacy regulations. The primary reason for this is to protect the privacy of patients who participate in clinical trials. The data can be made available for continuing review by federal agencies upon request and for ongoing study safety reviews by the Principal Investigator, Statistician, Data Safety and Monitoring Board (DSMC), and, in other instances, the CUMC IRB.

Data collected during the course of this clinical trial will primarily be shared with other investigators and University staff, the IRB, FDA, and other reporting agencies, and/or transferred to other collaborators. Prior to transfer, the data collected must comply with, and must be limited by, the CUMC's guidelines for Protecting the Rights and Privacy of Human Subjects.

Data Acquisition and Submission
Informed consent, including HIPPA authorization, must be obtained on all subjects prior to their participation. Always keep the original signed and dated consent form, with the redacted source documents and eligibility checklist. Velos eResearch will be used as the electronic clinical trials and data management system. Affiliate sites will enter data directly into Velos eResearch via customized case report forms for the study. The research staff will generate reports from Velos eResearch to ensure timely submission of data by affiliate sites. This resource allows for the timely analysis of particular data sets for safety analysis.

APPENDIX H: DRUG ORDER FORM

Completed Form should be e-mailed to: gmb-pci-clinicalrequests@catalent.com and cc: Evyonne.Shands@catalent.com or fax to (215)613-3360.

INVESTIGATIONAL SUPPLIES SHIPMENT AND RECEIPT VERIFICATION FORM

Incyte: Protocol: I-RUX-13-02 (Site Protocol # AAAM1906)

Section I: (PRINT/TYPE)

Authorized Requestor: Sales Order #: 9015870 Today's Date:

Date needed on site: Domestic Carrier: Catalent Preferred Carrier

Site No.: 1 **Investigator Information:**

Full Ship To Address: (No P.O. Boxes) **Name:**

Attention to:

Telephone:

Authorized Requestor: **Date:**

Description	Catalent Part #	Quantity Requested to Ship
I-RUX-13-02 5MG 60CT BT LABELED	CLR-FG-9015870-001	

Section II: (to be completed by Pharmacy, Site Coordinator or Investigator)

Date Shipment Received: **Inventoried By:**

Discrepancies:

Authorized Site Signature: **Date:**

Upon Receipt, please conduct an inventory of the above shipment and file a copy of this form with the drug accountability records.

1.0 Attachment 3 Shipment Checklist

Incyte Corporation	Catalent	**I-RUX-13-02**
Pack Slip#:	**SO#:** 9015870	**Consign#**
Shipment Condition: Ambient	**Carrier: FedEx**	

Documents Included w/shipment:

- ☐ Packing Slip
- ☐ Shipment Request Form

International Requirements: N/A

Shipping Timelines:

- 48 hour turnaround unless expedited services requested by client.

Special Instructions:

Study Material is CL1

Packout: Standard Corrugated Shipper