TBCRC Protocol #: TBCRC 039

Local Protocol #: 16-151

TITLE: Phase II study of combination ruxolitinib (INCB018424) with preoperative chemotherapy for triple negative inflammatory breast cancer

Coordinating Center: Dana-Farber Cancer Institute

Study Liaison:
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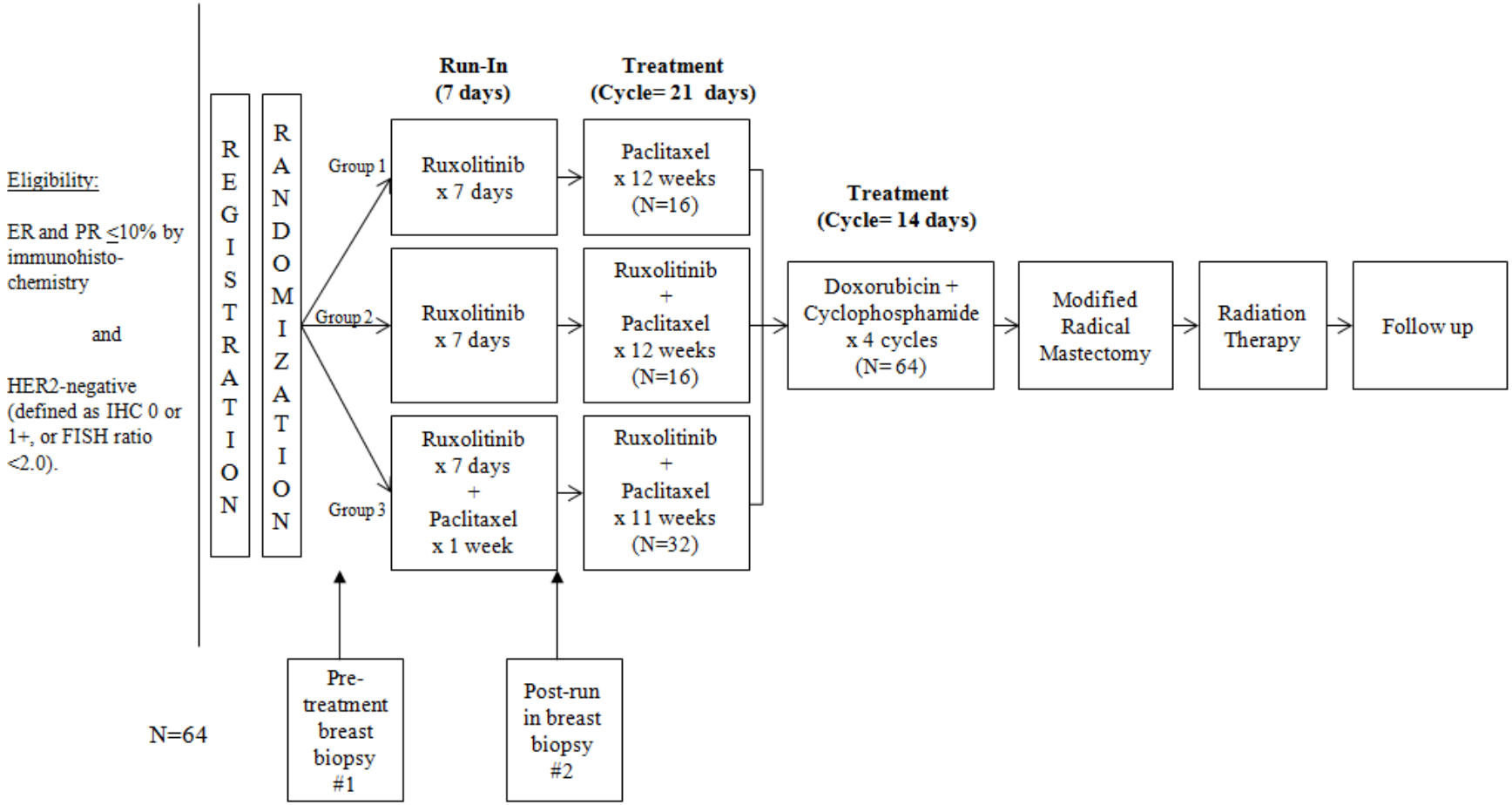
Supporters: [redacted]

Agents: Ruxolitinib [redacted], paclitaxel [redacted]), cyclophosphamide ([redacted], doxorubicin ([redacted])

Study Exempt from IND Requirements per 21 CFR 312.2(b).

Protocol Type / Version # / Version Date: Version #6/May 6, 2021

SCHEMA


Eligibility:
ER and PR ≤10% by immunohisto-chemistry
and
HER2-negative (defined as IHC 0 or 1+, or FISH ratio <2.0).
N=64
REGISTRATION
RANDOMIZATION
Run-In (7 days)
Treatment (Cycle= 21 days)
Group 1
Ruxolitinib x 7 days
Paclitaxel x 12 weeks (N=16)
Group 2
Ruxolitinib x 7 days
Ruxolitinib + Paclitaxel x 12 weeks (N=16)
Group 3
Ruxolitinib x 7 days + Paclitaxel x 1 week
Ruxolitinib + Paclitaxel x 11 weeks (N=32)
Treatment (Cycle= 14 days)
Doxorubicin + Cyclophosphamide x 4 cycles (N= 64)
Modified Radical Mastectomy
Radiation Therapy
Follow up
Pre-treatment breast biopsy #1
Post-run in breast biopsy #2

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1 OBJECTIVES

1.1 Study Design

- Patients with triple negative inflammatory breast cancer without evidence of visceral or bone metastasis will be randomized 1:1 to receive either single agent ruxolitinib daily for 7 days or ruxolitinib daily for 7 days administered with one dose of weekly paclitaxel.

- Those patients randomized to combined ruxolitinib and paclitaxel will continue to receive a total dose of 12 weeks of weekly paclitaxel administered with daily ruxolitinib, followed by standard doxorubicin and cyclophosphamide (AC) given every 2 weeks for 4 cycles preoperatively.

- Those patients randomized to single agent ruxolitinib for 7 days will have been randomized 1:1 to either receive 12 weeks of weekly paclitaxel followed by standard doxorubicin and cyclophosphamide (AC) given every 2 weeks for 4 cycles preoperatively; or 12 weeks of weekly paclitaxel administered with daily ruxolitinib, followed by standard doxorubicin and cyclophosphamide (AC) given every 2 weeks for 4 cycles preoperatively.

- Three to five weeks following the completion of preoperative study therapy, patients deemed surgically operable proceed to total mastectomy and axillary lymph node dissection, where residual cancer is obtained for correlative studies.

    - Patients whose disease is not surgically resectable following the completion of preoperative study therapy, may proceed to definitive radiation given to the affected breast and regional lymph nodes beginning approximately 3 to 6 weeks after completion of preoperative therapy.

    - Patients whose disease is rendered surgically resectable following radiation therapy may proceed to total mastectomy and axillary lymph node dissection approximately 4 to 8 weeks following the completion of radiation therapy. Residual cancer will be obtained for correlative studies.

- Definitive radiation is given to the post-surgical chest wall and regional lymph nodes beginning approximately 3 to 6 weeks after surgery.

- For participants that complete protocol therapy, participants will be followed every 3 months for 1 year, then every 6 months for 4 years, then annually until death.

- For participants that are removed from study for unacceptable adverse events, they will be followed closely until resolution or stabilization of the adverse event. They will then be contacted either directly or their current treating physician will be contacted every 6 months to determine disease status until death.

- For participants that are no longer being followed at the treating institution, they will be contacted either directly or their current treating physician will be contacted every 6 months to determine disease status until death.

1.2 Primary Objectives

- Proof of Principle: To assess the effect of JAK inhibition with ruxolitinib on pSTAT3 level and STAT3 related gene expression by comparing molecular and genomic markers in pretreatment biopsy specimens to post-ruxolitinib run-in biopsy specimens.

- Changes in expression will be assessed on breast specimens exposed to single agent ruxolitinib and ruxolitinib combined with one dose of paclitaxel.

- Assess the difference in pSTAT3 level and STAT3 related gene expression following exposure to ruxolitinib versus ruxolitinib with paclitaxel.

1.3 Secondary Objectives

- To determine pathologic complete response (pCR) rate after preoperative therapy.
- To correlate effects on pSTAT3+ and STAT3 related gene expression with pCR: Assess pSTAT3 level and STAT3 related gene expression on pre-treatment tumor biopsy specimens and to correlate expression with pCR.
- To assess changes in pSTAT3 level and STAT3 related gene expression following either ruxolitinib or ruxolitinib with paclitaxel determined in tumor biopsy specimens and to correlate changes in expression with pCR.
- Assess difference in pCR rate following preoperative treatment with combination ruxolitinib with paclitaxel compared with paclitaxel alone.
- To determine the efficacy of therapy defined as disease-free survival (DFS), time to treatment failure (TTF), and overall survival (OS).
- To assess the residual cancer burden (RCB) after preoperative therapy with combination ruxolitinib with paclitaxel or paclitaxel alone followed by doxorubicin / cyclophosphamide (AC) in triple negative inflammatory breast cancer.
- To assess any difference in RCB comparing preoperative combination ruxolitinib with paclitaxel compared with paclitaxel alone.
- To describe changes in IL-6 and CRP plasma levels during treatment and to correlate pre-treatment IL-6 and CRP plasma levels with pCR and exposure to ruxolitinib, either alone or in combination with paclitaxel.
- To describe the distribution of CD44+/CD24- stem cell population in tumor pre- and post-exposure to ruxolitinib.

2 BACKGROUND

2.1 Ruxolitinib

Ruxolitinib (INCB18424) is a potent, orally bioavailable inhibitor of JAK (Janus kinase) 1 and 2 activation. As a result of this inhibitory activity, ruxolitinib can down modulate the biologic activity of a number of cytokines and growth factors which are involved in hematopoiesis and immune function. JAK signaling involves recruitment of signal transducers and activators of transcription Interference of JAK signaling results in decreased activity of STATs (signal transducers and activators of transcription) which function to modulate gene expression. Dysregulation of the JAK/STAT pathway is seen in patients with Philadelphia chromosome negative myeloproliferative diseases, and can result in aberrancies involving cellular differentiation, angiogenesis, proliferation, metastasis and apoptosis. [1], [2] JAK/STAT activation has also been found to be important in non-hematologic tumor formation and disease progression.

2.1.1 Clinical Pharmacology

The pharmacology of ruxolitinib has been studied in both healthy volunteers and patients with hematologic and solid tumor malignancies. Ruxolitinib is more than 95% absorbed orally (regardless of food ingestion) with peak plasma concentrations occurring 1-2 hours after dosing. (Investigator's Brochure, Edition 14) The pharmacokinetics are linear and there is 97% plasma protein binding with limited penetrance across the bloodbrain barrier. The majority of drug is excreted in the urine (75%) with less than 1% excreted as unchanged parent drug. The mean terminal half-life is 3-5 hours, and it is metabolized through the cytochrome P450 isozyme CYP3A4 resulting in oxygenated and conjugative metabolites. Twice daily dosing does not result in significant accumulation of the parent compound or its metabolites. The total daily dose should be reduced by 50% when ruxolitinib is administered with strong CYP3A4 inhibitors, but not dose-adjusted when given with CYP3A4 inducers.

2.1.2 Clinical Safety Data

The most common toxicity associated with ruxolitinib administration is thrombocytopenia and anemia; the majority of events are grade 1-2 and could be managed by dose reduction or interruption. The need to discontinue drug is rare. Among the 146 patients with myelofibrosis receiving ruxolitinib on the phase III COMFORT-II trial, the mean hemoglobin (Hbg) levels reached their nadir at 12 weeks of therapy, then recovered to steady state by 24 weeks (baseline Hbg = 109.3 g/l; nadir 94.1 g/l; steady-state 101.8 g/l). [3] There were increased rates of packed red blood cell (PRBC) transfusions among patients receiving ruxolitinib (51% received at least one PRBC), however platelet transfusions were rare. The most frequent nonhematologic toxicity was diarrhea (23% all grades; 1% grade 3-4), and the most frequent grade 3-4 toxicity was abdominal pain (3%).

Toxicity has also been assessed in 198 healthy volunteers with renal or hepatic impairment, and in 59 patients with rheumatoid arthritis. (Investigator's Brochure, Edition 11) The adverse events were mild and did not require intervention. Ruxolitinib has no effect on QT/QTc prolongation.

2.1.3 Combination Ruxolitinib and Chemotherapy

The FDA approved single agent dose is 15 to 20 mg twice daily (bid) based upon platelet level. The dose can then be escalated to 25mg bid which is associated with 50-60% inhibition of circulating STAT activity. Several phase I studies are investigating the recommended phase 2 dose (RP2D) of ruxolitinib in combination with chemotherapy. (Personal Communication) The starting dose level of ruxolitinib combined with full-dose capecitabine is 15 mg bid administered in a study for pancreatic cancer. The Phase I component of DF/HCC 13-494 utilized a 10 mg bid starting dose of ruxolitinib in combination with weekly paclitaxel and dose escalated in a standard 3+3 design. The RP2D was determined to be 15mg BID (personal communication).

2.2 Inflammatory Breast Cancer

Inflammatory breast cancer (IBC) accounts for 2-5% of all invasive breast cancer. IBC is classified as a "cliniopathologic" diagnosis, whereby documentation of invasive breast carcinoma is established in the setting of unique clinical characteristics including a rapid onset of breast enlargement, pain, diffuse erythema and edema (peau d'orange) usually occurring within 3-6 months. The breast cancer often presents without a palpable mass, and dermal lymphatic involvement with cancer is demonstrated in approximately 75% of the cases.[4] It is the effect of dermal lymphatic involvement, not infiltration of inflammatory cells that result in the clinical changes observed in IBC. The median age at presentation is less than that seen in non-IBC, and there is a greater incidence of IBC among African American women.[5], [6]

The intrinsic biology of IBC is such that advanced disease is present at the time of diagnosis. Approximately 55-85% of patients present with metastasis to the axillary and / or supraclavicular lymph nodes, and distant metastatic disease is present at diagnosis in approximately 20-40% of women. [7] Even in the absence of metastatic disease at presentation, the likelihood of developing distant metastasis is extremely high, supporting a role for chemotherapy as the mainstay of treatment. Historically, surgery and radiation therapy alone resulted in a 54% relapse rate within 18 months, translating into a median survival of 1.2 years. [8] The addition of chemotherapy to the initial treatment of IBC, i.e. trimodality therapy with neoadjuvant chemotherapy, followed by mastectomy and radiation therapy, improved the median overall survival to 3.8 years, translating into an approximately 50% 5-year overall survival. [4]

The extent of locoregional disease precludes mastectomy as primary treatment for IBC, therefore preoperative chemotherapy has become the standard of care; however, the optimal chemotherapy regimen has yet to be determined. Anthracycline-containing regimens employed preoperatively have resulted in 40-45% 5-year overall survival, whereas the addition of taxanes to these regimens improved the pathologic complete response rate and overall survival. [9-11] The current

chemotherapy regimens utilized as preoperative treatment for IBC most commonly include anthracyclines and taxanes, yet the poor overall survival rates still necessitate ongoing investigation into improved preoperative regimens. Triple negative inflammatory breast cancer is associated with a particularly grave prognosis, with a median time to treatment failure (TTF) of 19 months, time to distant metastasis (TDM) of 20 months, and a median disease free interval from mastectomy of 15 months (SABCS 2014 abs P6-14-09).12 This translates into a median overall survival of 34 months, which signifies the need to explore novel therapeutic approaches given the failure of conventional chemotherapy to significantly impact the outcome of this disease. The focus on examining novel preoperative systemic therapy for IBC has occurred in the setting of an explosion of basic science investigation into the unique biologic mechanisms that differentiate IBC from non-IBC locally advanced breast cancer.

2.3 Rationale

In general, breast cancer is comprised of at least four distinct molecular subtypes: luminal A, luminal B, Her2-enhanced and basal like. The same cell-of-origin subtypes exist in inflammatory breast cancer (IBC) as in non-IBC tumors, however, IBC has a propensity to segregate into the more proliferative subtypes, i.e., the basal-like or "triple negative" subtype (i.e., negative for estrogen receptor (ER), progesterone receptor (PR) and HER2 expression), and HER2-overexpressing subtype.13, 14 For example, in a large registry dataset from the California Cancer Registry (1999-2007), the receptor status and clinical outcome was assessed among 2,014 IBC patients, 1,268 non-IBC LABC patients, 3,059 patients with metastatic breast cancer (MBC) and 73,758 non-T4 breast cancer patients. 15 Patients with IBC were more likely to have HR negative disease (both ER and PR negative) compared with the other groups of breast cancer patients: 40% (IBC), 31% (non-IBC LABC), 25% (MBC), 18% (non-T4); and IBC patients were more likely to have HER2 positive breast cancer: 40% (IBC), 35% (non-IBC LABC), 35% (MBC), 22% (non-T4).

Within these intrinsic subtypes, there exists significant molecular heterogeneity, including a subpopulation of CD44+CD24- cells with stem cell-like characteristics. This cell population displays a more invasive and angiogenic phenotype and is thought to contribute to metastatic progression and therapeutic resistance to chemotherapy. 16 Of interest, CD44+CD24- cells can be found across breast cancer subtypes, including 69% of luminal A, 70% of luminal B, 52% of HER2, and nearly 100% of basal-like tumors 17.

The frequency of CD44+CD24- cells is extremely high in inflammatory breast cancer, particularly in the lymphovascular emboli. 18 In addition, a human xenograft model of IBC known as MARY-X, recapitulates the IBC phenotype and expresses cellular markers (CD44+/CD24-, CD133, ALDH1) that correspond to those present on cancer stem cells.19 The high prevalence of CD44+/CD24- phenotype among human IBC was confirmed by Polyak's laboratory who examined tumor specimens from all subtypes of IBC and found that >80% expressed the stem-cell phenotype of CD44+/CD24-, regardless of whether they were triple negative or of luminal subtype.20 The IL6/Jak2/Stat3 pathway appears to be preferentially activated and required for the growth of CD44+CD24- stem cell-like breast cancer, such as IBC.25 This pathway may be utilized as a significant therapeutic target for triple negative IBC

which, unlike the estrogen receptor or HER2 positive IBC, has no known therapeutic target to date.

The carcinoma of the human IBC xenograft model, Mary X, is triple negative and overexpresses the membrane bound adhesion molecule E-cadherin and cell-surface MUC-1 glycoprotein up to 10-20 times greater than in non-IBC xenograft models. When further examined, the carcinoma within the lymphovascular spaces are spheroids, exhibiting significant homotypic tumor cell adhesion due to overexpression of E-cadherin along the entire membrane surface of the carcinoma. 21 Although MARY-X overexpressed MUC-1, it is a dysfunctional protein, with decreased cell-surface sialyl-Lewis X/A residues which bind to E-cadherin. This contributes to the absence of tumor cell heterotypic adhesion to the endothelial cells causing visible retraction of the tumor emboli from the endothelial wall lining the lymphovascular structures. The pattern of these effector molecules result in the ability of IBC to form tight adhesive tumor emboli that lack the ability to adhere to the endothelial wall of vasculature, thus enabling a more metastatic phenotype. Alpaugh et al confirmed the overexpression of E-cadherin and MUC-1 in human IBC specimens and also found that the tumor emboli were retracted from the endothelial lining of the vasculature in these human samples. 21,22,23

The E-cadherin adhesion complex remains structurally and functionally intact in IBC, with overexpression of its associated α-catenin and β-catenin membrane-bound proteins as part of its E-cadherin/α,β-catenin functional axis. 23 In MARY-X, when this axis is disrupted, the spheroids disassociate and apoptosis occurs suggesting that the intact E-cadherin/α,β-catenin functional axis is necessary for the survival of IBC cells. These characteristic spheroids are also present and functional in the pulmonary metastasis that develop in the MARY-X xenograft, supporting the hypothesis that this structural integrity is necessary and maintained throughout the progression to metastasis that occurs in IBC. Recent investigation has shown that increased pSTAT3 activation is associated with increased cellular density. 24 Thus cellular aggregation, such as that occurring in IBC, triggers STAT3 activation by several mechanisms. One mechanism appears to be adhesion-dependent activation of interleukin-6 (IL6) via E-cadherin stimulation of IL6 transcription, resulting in the activation of JAK2 through an autocrine mechanism. The unique cellular characteristics present in IBC, namely the development of tight tumor emboli due to E-cadherin overexpression, also supports the importance of the JAK2/STAT3 signaling pathway as a mechanism of cell survival and proliferation in IBC.

Dr. Polyak's laboratory has examined the role of the JAK2/STAT3 pathway in IBC and found nearly 100% of the triple negative IBC specimens overexpressed activated STAT3 (pSTAT3), supporting the therapeutic focus of this pathway in the treatment of triple negative IBC.20 Her laboratory has derived xenografts from pStat3+ IBC (IDC31) and demonstrated that its in vivo growth is abolished by treatment with BSK805 Jak2 inhibitor.25 They subsequently characterized the genetic and gene expression profiles of this IBC using SNP (Single Nucleotide Polymorphism) array and SAGE-seq (Serial Analysis of Gene Expression combined with high-throughput sequencing) and identified a focal amplicon on 9p24 containing the JAK2 gene implicating an important role for it in the pathology of IBC. Evidence for suppression of pSTAT3 with JAK2 inhibition in vitro was replicated in triple negative IBC cell lines.20 Xenograft models of IBC demonstrated synergistic reduction in tumor volume when exposed to

combination ruxolitinib and paclitaxel compared with either agent alone (personal communication, N. Polyak). Based on our preliminary data we hypothesize that the Jak2/Stat3 pathway plays a key role in promoting the growth of IBC and thus, its inhibition represents a new therapeutic option for the treatment of this disease (manuscript in process).

Ruxolitinib has received FDA approval for use in intermediate and high-risk myelofibrosis, supported by 2 phase III studies. Compared with placebo, oral ruxolitinib given twice daily was associated with a reduction in spleen size and disease-related symptom improvement (night sweats, fatigue, early satiety, pruritus, abdominal discomfort). 26 _ENREF_4 Similar endpoints were obtained in the second pivotal study comparing ruxolitinib with the "best available therapy". 3 The toxicity was primarily anemia and thrombocytopenia. These toxicities do not overlap with the common toxicities that are associated with effective chemotherapy for breast cancer. The most common hematologic toxicity associated with paclitaxel is neutropenia, with little effect on anemia and thrombocytopenia when administered in a standard regimen. This allowed further evaluation of ruxolitinib in combination with standard preoperative chemotherapy for triple negative IBC, with the goal to inhibit JAK2 when given concurrent with paclitaxel followed by combination doxorubicin and cyclophosphamide. A completed phase I study involving 20 patients with metastatic breast cancer treated with combination ruxolitinib and weekly paclitaxel, determined the RP2D of ruxolitinib to be 15mg BID when administered with standard dose weekly paclitaxel. The toxicity profile was acceptable (personal communication), and permitted the expansion of this therapeutic approach into the treatment of newly diagnosed triple negative breast cancer. Once efficacy of combination JAK2 inhibition and chemotherapy is demonstrated, this may become the new standard of care for the preoperative treatment of triple negative inflammatory breast cancer.

2.3.1 Rationale for Addition of Interim Analysis

The rarity of inflammatory breast cancer within the United States combined with the aggressive behavior of triple negative IBC has resulted in difficulties completing accrual to this project within an acceptable timeframe. In addition, new therapies explored in non-IBC have demonstrated improved outcomes which may also benefit the IBC population. For this reason, Sponsor Amendment 5 adds an interim analysis of the primary endpoint in order to find a biologic signal from the addition of ruxolitinib to chemotherapy as preoperative treatment for triple negative IBC. If a biologic signal is found, then the protocol may be amended to include newer systemic therapies for triple negative breast cancer added to the treatment with ruxolitinib.

2.4 Correlative Studies Background

2.4.1 pSTAT3 Assessment

We will describe Stat3 status by assessing pStat3 IHC, pSTAT3 immunoflorescence and using a previously described Stat3 activation signature.[25] pStat3 status in baseline, post-ruxolitinib exposure, post-ruxolitinib discontinuation, and residual disease tumor samples will be assessed by central IHC testing, scored based on the percentage and intensity of tumor cells that immunostain for pStat3 (see Appendix D). In addition, pSTAT3

evaluation will be assessed by immunoflorescence at these noted timepoints. We hypothesize that pStat3 status will decline upon exposure to ruxolitinib, which will be assessed by analyzing the pretreatment and post-ruxolitinib run-in tumor biopsies. We will also pursue analysis of STAT3 targets by gene expression profiling by RNA-seq or qRT-PCR and ChIP-PCR or ChIP-seq for pSTAT3 as well as evaluate JAK2 and KDM4C copy number (both genes are part of the 9p23-24 amplicon common in TNBCs). _ENREF_16

Our preliminary data indicate that JAK inhibition (INC424) inhibits IBC cell growth in vitro and in vivo and it also enhanced the tumor suppressive effects of paclitaxel in vivo. Therefore, to identify key downstream targets of pSTAT3 that could be used as biomarkers of response , we defined the gene expression and pSTAT3 chromatin binding profiles of IBC cell lines (SUM149PT, SUM190PT, FC-IBC02, and MDA-MB-IBC3) after the treatment with commonly used chemotherapeutic drugs paclitaxel and doxorubicin. IBC cell lines were treated with either paclitaxel or doxorubicin (2 x IC50 or 10 x IC50) for 24hrs to collect chromatin for pSTAT3 ChIP-seq or 72h to collect mRNA for RNA-seq. We expect to find pSTAT3 targets after paclitaxel and doxorubicin treatments that could be inhibited upon JAK2 inhibition and account for the observed improvement in chemotherapeutic response. In addition we are developing paclitaxel or doxorubicin resistant IBC cell lines. We plan to perform pSTAT3 ChIP-seq and RNA-seq with these cell lines. The aim is to elucidate if STAT3 activation underlies the resistance mechanisms to common chemotherapeutic drugs and if it is possible to overcome resistance by concomitantly inhibiting the JAK2 pathway.

During the interim analysis, we will be assessing pSTAT3 activity by immunohistochemistry, and assess the activation of pSTAT3 targets GLIS1, CYP26B1, GLI2, ZEB1, ZEB2, BNC2, NKAIN2, and LRP2. If there is sufficient tissue available, we will also perform single cell RNA-seq, single cell ATAC-seq, and immuno-FISH on select tissue sections.

2.4.2 IL-6 and CRP

Many, though not all, studies have found an association between serum IL-6 levels and clinical outcomes in patients with breast cancer. [27] Higher IL-6 levels have been identified in patients with metastatic, as compared to early-stage breast cancer, and appear higher still in patients with progressive disease. [28],[29],[30],[31] Furthermore, IL-6 induces hepatic production of C-reactive protein (CRP). Thus, CRP level may serve as a simple, pharmacodynamic readout of inhibition of IL-6/JAK/Stat3 signaling. Elevations in CRP have long been correlated with worse all-cause and cardiovascular mortality.[32] More recently, high CRP levels have been described in association with adverse breast cancer outcomes. In a study of 734 patients with stage I-III breast cancer, patients with elevated CRP experienced inferior disease-free and overall survival, even after censoring for cardiovascular death.[33]

We hypothesize that an elevated baseline IL-6 or CRP level will be associated with a greater tumor dependence on the IL-6/JAK/Stat3 pathway, and therefore be associated with a higher likelihood of objective response to ruxolitinib as manifested by an increased pCR rate. We also hypothesize that these levels will decrease upon exposure to ruxolitinib.

2.4.3 Distribution of CD44/CD24/pStat3 subpopulations:

In preclinical studies, CD44+/CD24- cells were frequently pStat3 positive. However, the proportion of CD44+/CD24- cells varies by tumor subtype and even between tumors from different individuals with the same general tumor subtype. We will assess pretreatment, post-ruxolitinib run-in, post-ruxolitinib exposure, and residual tumor samples for the frequency of CD44+/CD24- cells (versus the frequency of CD44+/CD24+, CD44-/CD24+, and CD44-/CD24- cells) and describe the frequency of pStat3 positivity in each subpopulation using triple immunofluorescence techniques. We will tabulate the frequency of each of four CD44/CD24 cell types in these noted biopsy specimens and describe the frequency of pStat3 positivity in each subpopulation.

During the interim analysis, we will assess the frequency of CD44+/CD24- cells and perform immunofluorescence analysis of pSTAT3, CD44+/CD24- cells.

3 PARTICIPANT SELECTION

3.1 Eligibility Criteria

3.1.1 Participants must have histologically confirmed invasive breast cancer. All histologic subtypes are eligible.

3.1.2 Patients must have known ER, PR, and HER2 status defined as triple-negative breast cancer (TNBC), defined as:

- ER and PR ≤10% by immunohistochemistry, and HER2-negative (as per ASCO/CAP guidelines, defined as IHC 0 or 1+, or FISH ratio <2.0 or HER2 copy number < 6.0).

3.1.3 Patients must have the clinical diagnosis of inflammatory breast cancer involving an intact breast.

3.1.4 Age ≥ 18 years. Because no dosing or adverse event data are currently available on the use of ruxolitinib in participants <18 years of age, children are excluded from this study.

3.1.5 ECOG performance status 0 or 1 (see Appendix A).

3.1.6 Participants must have normal organ and marrow function as defined below:

- Leukocytes ≥ 3,000/mm3

- Absolute neutrophil count ≥ 1,500/mm3
- Platelets ≥ 100,000/mm3
- Bilirubin ≤ 1.5 x institutional upper limit of normal (ULN)
- AST (SGOT)/ALT (SGPT) ≤ 2.5 X institutional upper limit of normal
- Creatinine ≤1.5 x institutional upper limit of normal OR creatinine clearance ≥ 60 mL/min/1.73 m^2 for subjects with creatinine levels above institutional normal

3.1.7 Patients with evidence of extensive nodal involvement are allowed. Extensive nodal involvement is defined as metastatic disease involving any nodal region outside of the involved breast.

3.1.8 Patients with minimal metastatic disease involvement in bone or viscera are allowed. Minimal metastatic disease is defined as: evidence of metastatic involvement as demonstrated by imaging only, not amenable to biopsy confirmation.

3.1.9 Both men and women are allowed.

3.1.10 The effects of ruxolitinib on the developing human fetus are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry until completion of chemotherapy. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

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

3.1.12 LVEF ≥ 50% calculated by echocardiogram (ECHO) or MUGA

3.1.13 Patients may have bilateral breast cancer so long as one breast meets criteria for inflammatory breast cancer, and neither breast cancer has received prior therapy.

3.2 Exclusion Criteria

Participants who exhibit any of the following conditions at screening will not be eligible for admission into the study.

3.2.1 Participants may not be receiving any other investigational agents.

3.2.2 Participants with known brain metastases should be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.

3.2.3 History of allergic reactions attributed to compounds of similar chemical or biologic composition to ruxolitinib or other agents used in this study.

3.2.4 Participants receiving any medications or substances that are potent inhibitors of CYP3A4, including grapefruit juice are ineligible. Participants receiving fluconazole are also ineligible. (Please refer to Appendix B for the full list of potent inhibitors and washout periods).

3.2.5 Chronic corticosteroid use in excess of the equivalent of prednisone 10 mg once daily.

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

3.2.7 Pregnant women are excluded from this study because paclitaxel, doxorubicin, and cyclophosphamide have the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk of adverse events in nursing infants secondary to treatment of the mother with these agents, breastfeeding should be discontinued if the mother is treated on study. These potential risks may also apply to other agents used in this study.

3.2.8 Individuals with a history of a different malignancy are ineligible except for the following circumstances. Individuals with a history of other malignancies are eligible if they have been disease-free for at least 3 years and are deemed by the investigator to be at low risk for recurrence of that malignancy. Individuals with the following cancers are eligible if diagnosed and treated within the past 3 years: cervical cancer *in situ*, and basal cell or squamous cell carcinoma of the skin.

3.2.9 Known HIV-positive individuals on combination antiretroviral therapy are eligible so long as they meet all other criteria. Known HIV-positive individuals who are not on combination antiretroviral therapy are not eligible because these individuals are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in participants receiving combination antiretroviral therapy when indicated.

3.2.10 Clinically significant malabsorption syndrome.

3.2.11 Patients may not have received paclitaxel, doxorubicin, or cyclophosphamide as anti-neoplastic therapy.

3.2.12 Patients with prior radiation to the affected breast.

3.3 Inclusion of Women, Minorities and Other Underrepresented Populations

Both men and women and members of all races and ethnic groups are eligible for this trial. Because of the epidemiology of breast cancer, it is anticipated that the vast majority of participants in this trial will be female.

4 REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC and DF/PCC Institutions

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

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

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

4.2 Registration Process for DF/HCC and DF/PCC Institutions

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

4.3 General Guidelines for Other Investigative Sites

Eligible participants will be entered on study centrally at the Dana-Farber Cancer Institute by the Project Manager. The required forms can be found in Section 4.4. Following registration, participants must begin protocol treatment within 7 calendar days. Issues that would cause treatment delays should be discussed with the Protocol Chair. If a participant does not receive protocol therapy following registration, the participant's registration on the study may be canceled. The Project Manager should be notified of cancellations as soon as possible.

4.4 Registration Process for Other Investigative Sites

To register a participant, the following documents should be completed by the research nurse or data manager and e-mailed to the Study Project Manager at [redacted] or faxed to [redacted]

- Clinic visit note documenting history and physical exam
- Copy of required laboratory tests including: Hematology (CBC with differential), serum chemistries (creatinine and/or creatinine clearance, bilirubin, ALT, and AST, Alkaline phosphatase) pregnancy test for women of childbearing potential only (serum or urine)
- Pathology report and documentation of ER/PR status and HER2 status.
- Breast Imaging: Mammogram, MRI, +/- Ultrasound report & PET/CT, CAP CT reports
- MUGA or ECHO report

- EKG report
- Signed participant consent form
- HIPAA authorization form (if separate from the informed consent document)
- Completed Eligibility checklist

The research nurse or data manager at the participating site will then e-mail [redacted] the Project Manager to verify eligibility. To complete the registration process, the Project Manager will follow DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) and register the participant on the protocol. The Project Manager will fax or e-mail the participant study number, and if applicable the dose treatment level, to the participating site.

Treatment may not begin without confirmation from the Coordinating Center that the participant has been registered.

NOTE: Registration and randomization can only be conducted during the business hours of 8:00 AM and 5:00 PM Eastern Standard Time Monday through Friday. Same day treatment registrations will only be accepted with prior notice and discussion with the DF/HCC Lead Institution.

5 TREATMENT PLAN

Treatment will be administered on an outpatient basis. Expected toxicities and potential risks as well as dose modifications for ruxolitinib, paclitaxel, and doxorubicin/cyclophosphamide are described in Section 6 (Expected Toxicities and Dosing Delays/Dose Modification). No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

5.1 Randomization

Patients are randomized 1:1:2 to one of three regimens in the run-in and treatment phases:

Group	# Patients	Run-in Phase	Treatment Phase
1	16	Ruxolitinib	Paclitaxel
2	16	Ruxolitinib	Ruxolitinib + paclitaxel
3	32	Ruxolitinib + paclitaxel	Ruxolitinib + paclitaxel

5.2 Run-in Phase

According to randomization, patients receive either (see TABLE 1: run-in phase)

- 7 days of ruxolitinib alone (Groups 1 and 2) or
- 7 days of ruxolitinib with one dose of weekly paclitaxel administered on day -7 (Group 3)

5.3 Treatment Phase

5.3.1 Preoperative paclitaxel with or without ruxolitinib

- Group 1: Those patients randomized to receive ruxolitinib alone during the run-in phase and paclitaxel alone during the treatment phase will receive paclitaxel weekly x 12 doses.
- Group 2: Those patients randomized to receive ruxolitinib alone during the run-in phase and ruxolitinib with paclitaxel during the treatment phase will receive 12 doses of weekly paclitaxel with ruxolitinib.
- Group 3: Those patients randomized to receive ruxolitinib with paclitaxel during the run-in phase and during the treatment phases will continue to receive a total of 12 doses of weekly paclitaxel with ruxolitinib (i.e., 11 additional weeks of treatment).
- When ruxolitinib is given with paclitaxel, ruxolitinib will be continued for 8 days beginning on the last dose of paclitaxel (dose 12).

5.3.2 Preoperative doxorubicin and cyclophosphamide

- Two weeks after the last dose of paclitaxel (12 doses total) all patients will receive doxorubicin and cyclophosphamide (AC) every 2 weeks +/- 2 days for 4 cycles. Group 1 may start AC one to two weeks after the last dose of paclitaxel, depending on institutional guidelines. Cycle length of AC is 14 days.
- If AC is delayed due to toxicity and an investigator prefers drug administration every 3 weeks in order to maintain full dose, AC may be given on a 21 day cycle with approval of the Principal Investigator.
- For patients who are not deemed surgical candidates after 4 cycles of AC and are felt to benefit from more systemic treatment, up to 2 additional doses of AC will be allowed, given either every 2 or 3 weeks

TABLE 1: Preoperative ruxolitinib and paclitaxel followed by doxorubicin/cyclophosphamide

Run-in Phase: Preoperative ruxolitinib with or without paclitaxel					
Agent	**Pre-medications; Precautions**	**Dose**	**Route**	**Schedule**	**Cycle Length**
Ruxolitinib	None	Variable[a]	PO	Twice Daily (Days -7 to -1)	7 days

Run-in Phase: Preoperative ruxolitinib with or without paclitaxel					
Paclitaxel **(Group 3 only)**	Pre-medicate with dexamethasone 10 mg po or IV; diphenhydramine 12.5-50 mg po or IV; famotidine 20 mg IV all administered 30-60 min prior to paclitaxel; or per institutional guidelines.	80 mg/m^2	IV over 1 hour or per institutional guidelines	One dose, Day -7	

Treatment Phase: Preoperative paclitaxel with or without ruxolitinib					
Agent	**Pre-medications; Precautions**	**Dose**	**Route**	**Schedule**	**Cycle Length**
Ruxolitinib	None	15 mg BID (twice daily)	PO	Twice Daily	21 days
paclitaxel	Pre-medicate with dexamethasone 10 mg po or IV; diphenhydramine 12.5-50 mg po or IV; famotidine 20 mg IV all administered 30-60 min prior to paclitaxel; or per institutional guidelines.	80 mg/m^2 weekly	IV over 1 hour or per institutional guidelines	4 Cycles* *Group 3 (patients randomized to paclitaxel during the run-in phase) will receive only 11 weeks of paclitaxel	21 days (3 weeks)

Treatment Phase: Preoperative doxorubicin/cyclophosphamide					
Agent	**Pre-medications; Precautions**	**Dose**	**Route**	**Schedule**	**Cycle Length**
doxorubicin	No premedication is necessary.	60 mg/m^2 every 14 days	IVP over 3-5 minutes or per institutional guidelines; given prior to cyclophosphamide	Every 14 days for 4 doses	14 days (2 weeks)

Treatment Phase: Preoperative doxorubicin/cyclophosphamide					
Cyclophos-phamide	No premedication is necessary.	600 mg/m^2 every 14 days	IV infusion in 250-500 ml administered per institutional guidelines; given after doxorubicin	Every 14 days for 4 doses.	

***See Section 6 for Dose Modification Criteria and Instructions**

a. Ruxolitinib will be administered beginning on Day -7. When administered alone, ruxolitinib will be administered at starting doses of either 15 mg twice daily or at 20 mg twice daily depending on the patient's Cycle 1 Day -7 platelet counts (20 mg twice daily for patients with initial platelet counts of 200,000 or greater and 15 mg twice daily for patients with initial platelet counts equal to or greater than 100,000 and less than 200,000). If ruxolitinib is given with paclitaxel, then the dose will be the RP2D of ruxolitinib, which is 15 mg bid.

- No dose reductions of ruxolitinib will be allowed during the run-in phase for either 15mg or 20mg dosed patients.
- Minor schedule changes owing to observed holidays, inclement weather, etc. are permitted.
- Patients may interrupt therapy for protocol-directed reasons (i.e. toxicity) or for personal preferences (holidays, vacations, etc.). Treatment should resume according to protocol guidelines.
- [redacted] will provide study drug (ruxolitinib) to participating institutions for use in the study for dispensing free of cost to participants. Patients and/or their insurance companies will be billed for the cost of paclitaxel, doxorubicin and cyclophosphamide and their administration, as it is considered standard of care for inflammatory breast cancer.
- Drug will be dispensed on day -7 and on day 1 of every cycle of paclitaxel.
- Ruxolitinib will be self-administered at approximately the same times each day at least 10-12 hours apart.
- If it is not feasible for a patient to start with the morning (AM) dose of ruxolitinib on day -7 (due to required 10-12 hours between doses), the patient may start with the evening (PM) dose.
- Ruxolitinib will continue daily for 8 days beginning on the last dose of paclitaxel (dose 12), then is discontinued.
- Preoperative treatment will be paclitaxel x 12 weeks either with or without ruxolitinib, followed by doxorubicin/cyclophosphamide (AC) x 4 cycles. For Groups 2 and 3, AC begins two weeks after receiving the 12th dose of paclitaxel. For Group 1, AC may begin one to two weeks after the 12th dose of paclitaxel, depending on institutional guidelines.

5.4 Pre-treatment Criteria

5.4.1 Prior to all drug dosing, including Run-in treatment and Cycle 1 Day 1, the following criteria must be met:

Paclitaxel with or without ruxolitinib or ruxolitinib alone (see Table 1, footnote a)

- ANC ≥1,000/mm^3
- Platelet count ≥100,000/mm^3
- Bilirubin ≤ 1.5 x institutional upper limit of normal (ULN)
- ALT and AST ≤ 2.5 X ULN

Doxorubicin/cyclophosphamide

- ANC ≥1,000/mm^3
- Platelet count ≥100,000/mm^3
- Bilirubin ≤ 1.5 x institutional upper limit of normal
- ALT and AST ≤ 2.5 X institutional ULN
- LVEF ≥ 50% - only assessed prior to cycle 1.

5.5 Agent Administration

5.5.1 Ruxolitinib

A one-cycle supply of ruxolitinib will be dispensed on day 1 of each cycle. Study participants will self-administer ruxolitinib at approximately the same times each day at least 10-12 hours apart. Patients will self-dose ruxolitinib at home, on all cycle days. Therefore, ruxolitinib will always be taken at home first. Ruxolitinib will be dispensed in such a way to allow for continuous dosing even on day 1. Day 1 labs do not need to be available prior to dosing of ruxolitinib. The AUC of ruxolitinib is not appreciably altered in the presence of food; therefore, ruxolitinib may be taken either with or without food. Ruxolitinib should not be crushed, chewed or dissolved. If a dose is missed (i.e. more than 4 hours have elapsed since the scheduled administration time), it should be skipped, and this should be recorded in the drug diary. If a dose is vomited, it should not be retaken. Instead, it should be skipped, and this should be recorded in the drug diary. See Appendix C for the drug diary.

Patients should be given a 9 day supply of ruxolitinib on Day -7 (Run-In Phase) to ensure that continuous dosing will be available on Cycle 1 Day 1 for those patients who will continue to receive ruxolitinib. Patients will be instructed to take at least 5 days of drug, with at least 7 days preferred. See Appendix C for Run-In phase patient diary.

The research biopsy should preferentially be obtained within 2-4 hours after ruxolitinib administration (see Section 10).

5.5.2 Paclitaxel

- Paclitaxel is administered at a dose of 80 mg/m2 IV weekly
- Paclitaxel is administered weekly +/- 2 days x 12 doses total, preoperatively. Cycle length is 21 days. Premedication should be administered 30-60 min. or per institutional guidelines prior to paclitaxel and include: dexamethasone 10 mg po or IV; diphenhydramine 12.5-50 mg po or IV; famotidine 20 mg IV. Drugs may be modified or eliminated as per institutional standard.
- If the patient does not experience an allergic reaction, the premedication regimen may be altered at the discretion of the treating physician.
- Anaphylaxis precautions should be observed during paclitaxel infusion.
- Paclitaxel reactions will be managed per institutional guidelines.
- The timing of paclitaxel administration is not dependent upon the timing of ruxolitinib dosing.
- Participants with disease progression on paclitaxel may proceed to AC treatment as outlined in the protocol below. For these participants, AC can begin 1 week following discontinuation of paclitaxel. Ruxolitinib should be discontinued prior to AC but does not require a washout period.

Dosing –Should be per institutional guidelines.

5.5.3 Doxorubicin

- Doxorubicin is administered at a dose of 60 mg/m2 IV over 3-5 minutes or per institutional guidelines every 14 days x 4 cycles.
- It is given for a total of 4 cycles preoperatively.
- Doxorubicin is administered prior to cyclophosphamide.

Dosing –Should be per institutional guidelines.

5.5.4 Cyclophosphamide

- Cyclophosphamide is administered at a dose of 600 mg/m2 IV infusion over 30 minutes or per institutional guidelines.
- It is given for a total of 4 cycles preoperatively.
- Cyclophosphamide is administered after doxorubicin.

Dosing –Should be per institutional guidelines.

5.5.5 Surgery

Primary breast surgery should be performed within 3 - 5 weeks after the last dose of doxorubicin/cyclophosphamide. Surgery must be performed at one of the participating institutions. Prior to surgery, patients should be assessed for clinical response to

preoperative treatment by physical exam and imaging of the breasts (see Study Calendar). Patients should undergo surgery with a total mastectomy; removal of the breast and level 1 and 2 axillary lymph node dissection. Pathological specimens will be analyzed for tumor extent and grade, ER and PR status, HER2 expression, and other markers of tumor biology. Given the high risk of local regional disease recurrence with IBC, reconstruction surgery should be delayed for 6 – 12 months following the completion of radiation therapy.

Patients whose disease is not surgically resectable following the completion of preoperative study therapy, may either receive 2 additional cycles of AC or proceed to definitive radiation given to the affected breast and regional lymph nodes beginning approximately 3 to 6 weeks after completion of preoperative therapy. Patients whose disease is rendered surgically resectable following radiation therapy may proceed to total mastectomy and axillary lymph node dissection approximately 4 to 8 weeks following the completion of radiation therapy. Patients may also proceed to definitive radiation after completing 6 cycles of AC if the disease remains unresectable.

5.5.6 Radiation Therapy

Radiation therapy should be initiated within 3-6 weeks following surgery. Radiation should be delivered to the chest wall and regional lymph nodes. Treatment will be administered per institutional guidelines. Radiation can be administered at a local facility.

Patients whose disease is not surgically resectable following the completion of preoperative study therapy, may proceed to definitive radiation given to the affected breast and regional lymph nodes beginning approximately 3 to 6 weeks after completion of preoperative systemic therapy.

5.6 General Concomitant Medication and Supportive Care Guidelines

Because there is a potential for interaction of ruxolitinib with other concomitantly administered drugs through the cytochrome P450 system, the concurrent use of all other drugs, over-the-counter medications, or alternative therapies must be captured. See Appendix B for a list of prohibited medications.

5.6.1 Antiemetics

Patients may be given antiemetics at the discretion of the treating physician. The following antiemetics are recommended if the patient experiences symptoms: Prochlorperazine 10 mg PO Q6 hours PRN, and/or Lorazepam 1 mg PO Q6 hours PRN. Other antiemetics are allowed at the discretion of the treating physician. Aprepitant (a moderate CYP3A4 inhibitor) is allowed, but its use should be reserved to patients in whom other anti-emetics are insufficient or contraindicated.

5.6.2 Anticoagulants

Anticoagulation with heparin, heparin derivatives, and/or warfarin may be given at the discretion of the treating physician. Patients receiving warfarin with therapeutic INR should have PT-INR checks according to standard institutional practice to maintain the target INR as determined by the treating investigator.

5.6.3 Antiinfectives

Because of potential interactions with ruxolitinib, patients may not receive fluconazole while on study. Additional excluded CYP3A inhibitors are listed in Appendix B.

5.6.4 Growth Factors

Patients may not receive erythropoietin while on study. The use of G-CSF should generally be limited to clinical scenarios such as febrile neutropenia or active infection and should be administered on a chronic basis to maintain neutrophil counts as part of ongoing therapy in the administration of doxorubicin/cyclophosphamide. G-CSF should be routinely administered to maintain a 14 day cycle for doxorubicin/cyclophosphamide per institutional guidelines. Ruxolitinib may abrogate G-CSF effectiveness [redacted]. [34, 35] The use of platelet growth factors are specifically prohibited on this protocol. Please consult the Principal Investigator with questions. Investigational growth factors are not permitted on this study.

5.7 Criteria for Taking a Participant Off Protocol Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse events, treatment may continue until completion of protocol assigned therapy or until one of the following criteria applies:

- Disease progression (as evidenced by clinical examination and/or imaging with or without confirmatory biopsy) requiring therapy not specified in the protocol
    - Participants who progress and are deemed safe to continue on to the next protocol-specified therapy (See Section 5.7.1 for details) will remain on treatment.
- Intercurrent illness that prevents further administration of treatment.
- Unacceptable adverse event(s). See additional criteria below:
    - Preoperative treatment: Patients who discontinue chemotherapy due to toxicity should not systematically be withdrawn from all study treatments. The following recommendations are given:
        - Paclitaxel: patients who received at least 2 cycles of paclitaxel with ruxolitinib can proceed to doxorubicin/cyclophosphamide. Patient should then complete the treatment as per protocol.

  - Doxorubicin / cyclophosphamide: in the case of discontinuation of doxorubicin/cyclophosphamide, patients should be assessed for eligibility for mastectomy, and if they are deemed surgical candidates, then they should proceed to mastectomy and continue on treatment as per protocol.
- Participant demonstrates an inability or unwillingness to comply with the medication regimen and/or documentation requirements.
- Participant decides to withdraw from the study.
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the opinion of the treating investigator.

Note: For patients who do not complete systemic therapy for any reason, if:

- They proceed directly to surgery; surgical specimens will be collected as appropriate.
- They go on to receive additional chemotherapy not specified as protocol therapy, patients will proceed directly to follow up (see section 5.8). An attempt at collecting surgical specimens should be made.

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF). In general, completion of protocol assigned therapy (off treatment) is defined as the date of the post-surgical evaluation visit (unless participant meets one of the other criteria for coming off treatment early). Alternative care options will be discussed with the participant.

When a participant is removed from protocol therapy and/or is off of the study, the participant's status must be updated in OnCore in accordance with REGIST-OP-1.

In the event of unusual or life-threatening complications, participating investigators must immediately notify the Protocol Chair, Dr. Filipa Lynce at 617-632-3800 or pager #40394.

5.7.1 Progression:

For participants with disease progression, surgical data/specimen collection, off treatment status, and duration of follow up are dependent on the planned course of treatment after progression. Details are outlined below. Please notify the Overall PI of any participants with disease progression.

Progression and amenable to protocol-specified therapy:
Participant with disease progression that is deemed safe to continue on to the next protocol-specified therapy. The Protocol Chair must provide confirmation that the participant will remain on treatment and can proceed with protocol-specified therapy/requirements.

- Participant remains on treatment and on study

- Participant surgical data, surgical specimen, and follow-up information will be collected as outlined in the protocol.

Progression and subsequent treatment with non-protocol therapy:
Participant with disease progression that will go on to receive therapy that deviates from the study treatment and schedule specified in the protocol (i.e. paclitaxel, AC).

- Participant will come off treatment but remain on study
- Surgical and follow-up information will be collected; An attempt will be made to collect surgical specimens.

Details of protocol-treatment continuation are outlined in Section 5.5.

5.8 Duration of Follow Up

For participants that complete protocol therapy, participants will be followed every 3 months for 1 year, then every 6 months for 4 years, then annually until death as outlined in Section 9 (Study Calendar).

For participants that are removed from treatment for unacceptable adverse events, they will be followed closely until resolution or stabilization of the adverse event. They will then be contacted either directly or their current treating physician will be contacted every 3 months for 1 year, then every 6 months for 4 years, then annually until death as outlined in Section 9 (Study Calendar).

For participants with disease progression, they will be followed every 3 months for 1 year, then every 6 months for 4 years, then annually until death as outlined in Section 9 (Study Calendar).

For participants that are removed from treatment for other reasons, participants will be followed every 3 months for 1 year, then every 6 months for 4 years, then annually until death as outlined in Section 9 (Study Calendar).

For participants that are no longer being followed at the treating institution, they will be contacted either directly or their current treating physician will be contacted every 3 months for 1 year, then every 6 months for 4 years, then annually until death as outlined in Section 9 (Study Calendar).

The follow up time line starts from the date off treatment (i.e. the post-surgical evaluation visit, unless participant comes off treatment early for other reasons, See Section 5.7).

5.9 Criteria for Taking a Participant Off Study

Participants will be removed from study when any of the following criteria apply:

- Lost to follow-up

- Withdrawal of consent for data submission
- Death

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF). In addition, the study team will ensure the participant's status is updated in OnCore in accordance with REGIST-OP-1.

6 DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made as indicated in the following table(s). The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

If possible, symptoms should be managed symptomatically. In the case of toxicity, appropriate medical treatment should be used (including anti-emetics, anti-diarrheals, etc.).

All adverse events experienced by participants will be collected from the time of the first dose of study treatment, through the study and until the final study visit. Participants continuing to experience toxicity at the off study visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

6.1 Toxicity Management

The most commonly reported toxicities with ruxolitinib are hematologic. Please refer to Section 5.6 for guidance regarding the use of growth factors on this protocol. For other toxicities, patients should be managed according to standard institutional practice. Please see Section 5.6 and Appendix B for guidance regarding allowed and prohibited medications.

6.2 Dose Modifications/Delays

Dose adjustments are to be made according to the highest grade toxicity or hematologic toxicity, whichever is more likely to result in a dose reduction. Toxicities will be graded using NCI Common Terminology Criteria for Adverse Events (CTCAE).

6.2.1 Ruxolitinib

Ruxolitinib dose modifications are listed in TABLES 2 and 3. Ruxolitinib is administered at a dose of 20 mg twice daily ONLY during the Run In phase (ruxolitinib alone). Once ruxolitinib is added to paclitaxel, either during the Run In or Treatment Phase, the dose of ruxolitinib starts at the RP2D of 15 mg twice daily. There is no dose reduction for either the ruxolitinib dose of 15mg or 20 mg twice daily administered during the Run In phase.

Ruxolitinib may be held up to a maximum of 2 consecutive weeks. If there is a delay > 2 consecutive weeks, subjects should be taken off treatment unless there is demonstrated evidence of clinical benefit. In that case, the treating investigator must contact the overall study PI to discuss whether it would be appropriate to resume ruxolitinib and receive approval for patient to resume. Up to 2 dose reductions are allowed.

- Patients should be assessed for toxicity prior to each dose of paclitaxel with ruxolitinib; dosing will occur only if the clinical assessment and laboratory test values are acceptable. The dose delays and reduction instructions are to serve as guidelines to allow ongoing treatment for patients experiencing clinical benefit while ensuring patient safety.

- In general, it is recommended that all ruxolitinib-related toxicities have resolved to Grade $\leq$1 or to the baseline grade before the next scheduled dose. Patients in whom other significant ruxolitinib–related toxicities have not recovered to Grade $\leq$1 or baseline grade at the time of their next scheduled dose may have their dose of ruxolitinib delayed for up to 14 days.

- Patients should be re-evaluated weekly during their treatment delay, whenever possible. If retreatment criteria are met, patients may receive their next scheduled dose of ruxolitinib either at the previous dose level or at one dose level lower (see Table 2), according to the guidelines in Table 3. Subjects who have had a ruxolitinib or chemotherapy dose reduction due to toxicity will not have subsequent dose increases.

- The criteria presented in this section for dose modification and/or delay of treatment of ruxolitinib, doxorubicin and cyclophosphamide, and paclitaxel are meant as general guidelines. However, dose modification or delay may occur in the setting of lower grade toxicity if the Principal Investigator believes that it is in the interest of a subject's safety. When an adverse event is judged by the investigator to be related to chemotherapy (e.g. diarrhea, neuropathy) only, the investigator can decide, in consultation with the Principal Investigator, to only dose reduce chemotherapy, not ruxolitinib. Additional dose modifications may be considered following discussion with the Principal Investigator

- In the setting of hematologic toxicity, both drugs (ruxolitinib and paclitaxel) should be modified per toxicity tables (Table 3 and Table 8, respectively).

Criteria for dose reductions and discontinuation of therapy for some specific hematological and non-hematological toxicity are provided in Table 3. The final dose modification criteria according to the following tables should be based upon the worst grade of toxicity experienced.

TABLE 2. Ruxolitinib Dose Reduction Schedule

Dose Level	Ruxolitinib Dose
Starting Dose	RP2D, 15 mg orally twice daily
First dose reduction	10 mg orally twice daily
Second dose reduction	5 mg orally twice daily

TABLE 3. Dose Reduction Criteria and Guidelines for Management of Ruxolitinib-Associated Toxicity

Event	Action to be Taken
Thrombocytopenia	
75,000 to <100,000/ mm^3	Hold ruxolitinib. Check platelet count once weekly until platelets ≥ 100,000/ mm^3, then reduce by one dose level. Recheck platelet count one week after restarting ruxolitinib.
50,000 to < 75,000/ mm^3	Hold ruxolitinib. Check platelet count once weekly until platelets ≥ 100,000/ mm^3, then reduce by one dose level. Recheck platelet count once weekly x 2 after restarting ruxolitinib.
25,000 to < 50,000/ mm^3	Hold ruxolitinib. Check platelet count twice weekly until platelets ≥ 100,000/ mm^3, then reduce by two dose levels. Recheck platelet count twice weekly x 2 after restarting ruxolitinib.
<25,000/ mm^3	Stop ruxolitinib. Patients should be taken off treatment unless there is evidence of clinical benefit, in which case, approval to restart may be considered with written permission from overall study PI. In such a case, hold ruxolitinib until platelets ≥ 100,000/ mm^3, then reduce by two dose levels, and recheck platelet count weekly x 3 after restarting ruxolitinib.
Anemia	
Grade 3	1st occurrence: transfuse PRBC as clinically indicated per institutional guidelines. Ruxolitinib may be resumed at same dose once anemia resolves to Grade 2 or less. 2nd occurrence: transfuse PRBC as clinically indicated per institutional guidelines, and, reduce by one dose level. Ruxolitinib may be resumed once anemia is Grade 2 or less.
Grade 4	1st occurrence: Hold ruxolitinib. Work up for alternative causes of blood loss. Transfuse per institutional guidelines. If, upon discussion with the PI, it is felt that the patient is experiencing clinical benefit from ruxolitinib, it may be

	reduced by two dose levels and resumed once anemia improves to Grade 2 or less. 2nd occurrence: Off treatment
Absolute neutrophil count <1,000/mm3	Hold ruxolitinib until ANC improves to ≥ 1,000/mm3 then, reduce by one dose level. Check ANC daily until it improves to ≥ 1,000/mm3. Recheck ANC one week after restarting ruxolitinib.
Other Treatment-Related Toxicity Grade 2 non-hematological (with the exception of alopecia) Grade 3 non-hematological Grade 4 non-hematological	1st occurrence: ***If*** unacceptable to patient or medically concerning then hold until recovery to baseline or ≤ grade 1, then restart at same dose. 2nd occurrence: ***If*** unacceptable to patient or medically concerning then hold until recovery to baseline or ≤ grade 1, then reduce by one dose level. Hold ruxolitinib until recovery to baseline or ≤ grade 1, then reduce by one dose level. Off treatment
Toxicity leading to delay > 2 weeks	Off treatment unless there is demonstrated evidence of clinical benefit. In that case, the treating investigator must contact the overall study PI to discuss whether it would be appropriate to resume ruxolitinib and receive written permission from overall PI to resume treatment.

6.2.2 Paclitaxel, and doxorubicin/cyclophosphamide (AC)

Dose Modifications and Delays for Paclitaxel:

- The principle is to attempt to administer full doses of therapy on schedule.
- No more than 1 dose-reduction of paclitaxel chemotherapy should be made for toxicity. If more than 1 dose-reduction would be required, paclitaxel should be discontinued.
- If Paclitaxel is held for > 2 weeks after anticipated scheduled dose for toxicity, paclitaxel should be discontinued. In certain instances, if paclitaxel is held or delayed for > 2 weeks and subject has demonstrated clinical benefit, treating investigator may request approval from the Protocol Chair, Dr. Filipa Lynce, to resume protocol therapy.

- If paclitaxel is discontinued, the patient should proceed to doxorubicin/cyclophosphamide and proceed as outlined in the protocol.

- All dose modifications for paclitaxel are based on the dose level changes outlined below (TABLE 4).

TABLE 4: Dose Levels for Paclitaxel

	Dose level 0 Starting dose	**Dose Level -1**	**Dose Level -2**
Paclitaxel (mg/m2)	80	65	Discontinue

- If a Grade 3 or 4 non-hematological toxicity is experienced, paclitaxel may be reduced from 80 mg/m^2 to 65 mg/m^2.

- If paclitaxel-related hypersensitivity reaction occurs despite pre-medication, treatment as medically indicated will be instituted.

    - For hypersensitivity reaction less than or equal to CTCAE Grade 3, continuation of paclitaxel is at the Investigator's discretion.

    - If Grade 4 hypersensitivity is experienced, paclitaxel must be permanently discontinued.

- See TABLE 5 for the management of taxane-related neurosensory toxicity and TABLE 6 for taxane-related musculoskeletal pain. Instructions for management of all other toxicities related to paclitaxel are listed in TABLE 8.

TABLE 5: Dose Modifications for Paclitaxel-Related Neurosensory Toxicity

Paresthesias/Dysesthesias	**1 – 7 Days Duration**	**Persistent for > 7 Days *or* Caused the Next Cycle to be delayed**
Grade 1 Paresthesias/dysesthesias that do not interfere with function	Maintain paclitaxel dose	Maintain paclitaxel dose
Grade 2 Paresthesias/dysesthesias interfering with function, but not activities of daily living	Maintain paclitaxel dose [a]	Decrease paclitaxel one dose level [b]
Grade 3 Paresthesias/dysesthesias with pain or with function impairment interfering with activities of daily living [C]	**First episode:** Decrease paclitaxel one dose level[a] **Second episode:** Discontinue paclitaxel	Discontinue paclitaxel

[a] Must be resolved to ≤ Grade 1 on Day 1 of the next cycle.
[b] Hold paclitaxel for *persistent* Grade 2 neurotoxicity. When ≤ grade 1, resume treatment with dose modification. If grade 2 toxicity persists after 2 weeks of delay, discontinue paclitaxel.
[c] For persistent paresthesias/ dysesthesias that are disabling or life-threatening, paclitaxel should be discontinued.

TABLE 6: Dose Modifications for Paclitaxel Musculoskeletal Pain *Not Controlled by Analgesia*[a]

Musculoskeletal Pain	1 – 7 Days Duration	Persistent for > 7 Days *or* Caused the Next Cycle to be Delayed
Grade 1	Maintain paclitaxel dose	Maintain paclitaxel dose
Grade 2	Maintain paclitaxel dose	Decrease paclitaxel one dose level [a]
Grade 3	**First episode:** Decrease paclitaxel one dose level **Second episode:** Discontinue paclitaxel	**First episode:** Decrease paclitaxel one dose level[a] OR Discontinue paclitaxel **Second episode**: Discontinue paclitaxel

[a] Use of narcotics and NSAIDs is encouraged to maintain dose of paclitaxel if possible. Hold paclitaxel for persistent Grade 2 or 3 musculoskeletal pain. When ≤ grade 1, resume treatment with dose modification for paclitaxel. If Grade 2 or Grade 3 toxicity persists after 2 weeks of delay, discontinue paclitaxel.

Doxorubicin/cyclophosphamide (AC)

- The principle is to attempt to administer full doses of therapy on schedule.
- No more than 1 dose-reduction of AC chemotherapy should be made for toxicity. If more than 1 dose-reduction would be required, AC should be discontinued, and the patient should proceed to surgery as outlined in the protocol.
- If AC is held for > 2 weeks for toxicity, AC should be discontinued. Patients may proceed to surgery as outlined in the protocol. In certain instances, if AC is held or delayed for > 2 weeks and subject has demonstrated clinical benefit, treating investigator may request approval from the Protocol Chair, Dr. Filipa Lynce, to resume protocol therapy.
- All dose modifications for AC are based on the dose level changes outlined below (TABLE 7).

- Instructions for management of all other toxicities related to AC are listed in TABLE 8.

TABLE 7: Dose Levels for doxorubicin/cyclophosphamide (AC)

	Dose level 0 Starting dose	**Dose Level -1**	**Dose Level -2**
doxorubicin (mg/m^2)	60	45	discontinue
Cyclophosphamide (mg/m^2)	600	450	discontinue

If a Grade 3 or 4 non-hematological toxicity is experienced, doxorubicin may be reduced from 60 mg/m^2 to 45 mg/m^2, and cyclophosphamide may be reduced from 600 mg/m^2 to 450 mg/m^2**.**

TABLE 8: Dose Modifications and Delays for Paclitaxel and doxorubicin/cyclophosphamide (AC)

NCI CTCAE v 4.0 [Category] Grade	**Modifications for AEs that occur during a cycle but RESOLVE PRIOR TO THE NEXT TREATMENT CYCLE [a]**	**Modifications for AEs that REQUIRE A DELAY IN ADMINISTRATION OF THE TREATMENT CYCLE[b]**
HEMATOLOGICAL:		
Neutrophils count decreased		
Grades 2, 3, 4	Maintain dose	*Hold until ≥ 1000/mm^3 for paclitaxel and until ≥ 1000/mm^{3}for AC.* *If recovery takes:* • 1 wk: maintain dose • 2 wks: ↓ one dose level
Febrile neutropenia	↓ one dose level or discontinue.	
Platelet count decreased		
Grades 2, 3 Grade 4	Maintain dose ↓ one dose level	*Hold until ≥ 100,000/mm3.* *If recovery takes:* • 1 wk: maintain dose • 2 wks: ↓ one dose level ↓ one dose level
GASTROINTESTINAL DISORDERS *(if related to chemotherapy)*:		
Diarrhea		
Grade 2 Grade 3 Grade 4	Maintain dose ↓ one dose level discontinue	↓ one dose level ↓ one dose level discontinue
Mucositis oral (stomatitis)		

Grade 2 Grade 3 Grade 4	Maintain dose ↓ one dose level discontinue	↓ one dose level ↓ one dose level discontinue
Vomiting *(despite antiemetics)*		
Grade 2 Grades 3, 4	↓ one dose level (optional) ↓ one dose level or discontinue	↓ one dose level discontinue
HEPATIC FUNCTION:		
Bilirubin or AST or ALP increased		
Grade 2 Grade 3 Grade 4	↓ one dose level ↓ one dose level Discontinue	*Hold until bilirubin returns to the baseline grade, and AST and alkaline phosphatase have returned to≤ grade 1.* Then ↓ one dose level ↓ one dose level Discontinue
OTHER CLINICALLY SIGNIFICANT AEs[c]:		
Grade 3 Grade 4	↓ one dose level discontinue	↓ one dose level Discontinue

- Dose modifications must be based on AEs that occur during the cycle (column 2) *and* AEs present on the scheduled cycle Day 1 (column 3).
- Dose modifications must be based on the AE requiring the greatest modification.
- The same modality of cardiac imaging used at baseline should be used throughout the study.

[a] Resolved means that all requiring dose modification are ≤ grade 1 (except ANC/AGC [which must be ≥ 1000/mm3] and bilirubin [which must be ≤ the baseline grade]) on each week of paclitaxel or (except ANC/AGC [which must be ≥ 1000/mm3] and bilirubin [which must be ≤ the baseline grade]) on Day 1 of the next scheduled cycle of AC (i.e., treatment can be given without delay).
[b] Hold and check weekly. With exception of ANC/AGC and bilirubin, resume treatment when toxicity is ≤ grade 1. If toxicity has not resolved after 2 weeks of delay, discontinue paclitaxel or doxorubicin/cyclophosphamide.
[c] Determination of "clinically significant" AEs is at the discretion of the investigator.

7 DRUG FORMULATION AND ADMINISTRATION

7.1 Ruxolitinib

7.1.1 Description

Ruxolitinib (INCB018424; INC424) is an inhibitor of the Janus kinase (JAK) family of protein tyrosine kinases. It represents a novel, potent, and selective inhibitor of JAK1 (IC50 = 3.3 + 1.2 nM) and JAK2 (IC50 = 2.8 + 1.2 nM) with modest to marked selectivity against TYK2 (IC50 = 19 + 3.2 nM) and JAK3 (IC50 =428 + 243 nM), respectively. Ruxolitinib is inactive (i.e. less than 30% inhibition) against 28 additional kinases when tested at 200 nM.

The chemical name of ruxolitinib phosphate is (*R*)-3-(4-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)-1H-pyrazol-1-yl)-3-cyclopentylpropanetrile phosphate. Ruxolitinib has a molecular formula of C17H21N6O4P and a molecular weight of 404.36.

Ruxolitinib has high solubility and permeability, exhibits moderate-to-high clearance, volume of distribution, and oral bioavailability in preclinical species. Ruxolitinib exhibits high plasma protein binding in humans with an unbound fraction of 3.3%.

PK information is available from single and repeat dose studies in healthy volunteers and studies in patients with myelofibrosis and rheumatoid arthritis. Oral absorption is rapid and nearly complete, with >/= 95% absorption consistent with a Biopharmaceutical Classification System (BCS) Class I compound. Mean peak plasma concentrations were reached within 1-2 hours of administration. Administration of 25 mg tablets with food increased the Tmax from 1.3 to 2.7 hours and lowered the man Cmax by 25% but did not significantly affect the AUC compared to fasted administration. Thus, the drug may be administered either with or without food. Dose proportional exposure is observed between 5 to 200 mg dose range with linear PK. Plasma protein binding is approximately 97% in vitro. PK parameters in rheumatoid arthritis and myelofibrosis patients were similar to those in healthy volunteers.

Ruxolitinib's primary clearance pathway is oxidative metabolism. The apparent elimination half-life is short (<5 hours) in all species tested. There is no long-term retention of drug-related material in preclinical species and limited drug penetration into the central nervous system or across the bloodbrain barrier. The metabolism by human liver microsomes is catalyzed predominantly by CYP3A4. However, the potential for ruxolitinib to cause clinical drug interactions via CYP inhibition or induction is low. Tissue distribution studies in rats indicate rapid and complete elimination of radioactivity in most tissues. Oxidative metabolites retain pharmacological activity albeit with one half to one fifth of the activity of the parent compound.

Ruxolitinib was well tolerated in single dose drug-drug interaction studies with inhibitors and an inducer of the CYP P450 family of metabolizing enzymes, when co-administered with methotrexate, in subjects with renal insufficiency, and in subjects with hepatic dysfunction. Co-administration of erythromycin or methotrexate does not alter the PK parameters of ruxolitinib. Therefore, no dose adjustments are required with ruxolitinib is administered with moderate CYP3A4 inhibitors such as erythromycin, grapefruit juice, or methotrexate. Ketoconazole significantly increases the exposure to ruxolitinib when coadministered. Co-administration with rifampin significantly decreased the exposure to

ruxolitinib; however, the enhanced formation of active metabolites resulted in nearly identical levels of overall JAK inhibitory activity as assessed by measurement of the inhibition of IL-6-induced STAT phosphorylation. Therefore, there is no required dose adjustment when ruxolitinib is coadministered with rifampin or other CYP3A4 inducers, but these agents should be used with caution in combination with ruxolitinib and alternative therapy used if available. The dose of ruxolitinib should be reduced by approximately 50% if given with strong CYP3A4 inhibitors; however potent CYP3A inhibitors (see Appendix B for full list) and fluconazole are excluded from use while on study. No dose adjustment is necessary when co-administering ruxolitinib with CYP3A4 inducers.

In lactating rats, the milk:plasma ratio of radioactivity was 13.4, indicating that ruxolitinib-derived radioactivity preferentially partitions into milk. Milk concentrations were <1% of Cmax by 24 hours. As stated in Section 3.2.8, because there is an unknown but potential risk of adverse events in nursing infants secondary to treatment of the mother with ruxolitinib, breastfeeding should be discontinued if the mother is treated with ruxolitinib.

In subjects with mild, moderate, or severe renal impairment, ruxolitinib pharmacokinetics and pharmacodynamics (as measured by IL-6 induced STAT3 phosphorylation) was similar compared to subjects with normal renal function. In patients with end stage renal disease on dialysis, ruxolitinib PK parameters were also similar to healthy subjects, whether dosed prior to or following a dialysis session, but ruxolitinib pharmacodynamics were prolonged in subjects dosed following a dialysis session. In patients with severe renal impairment (CrCl < 30 mL/min) the starting dose should be reduced by 50% with subsequent dose titration based on individual safety and efficacy. Although ruxolitinib can be dosed in hemodialysis patients, patients with severe renal impairment or requiring hemodialysis are not eligible for this trial.

In a hepatic impairment study, no significant differences in pharmacodynamics were observed among subjects with mild or moderate hepatic dysfunction compared to healthy subjects. In subjects categorized as having severe hepatic impairment, the inhibition of IL-6 induced pStat3 levels appeared to be prolonged by a single dose of ruxolitinib.

7.1.2 Form

Ruxolitinib [redacted] is commercially available in the US in 5, 10, 15, 20, and 25 mg strength tablets. The tablet contains the active ingredient and may include the following commonly used excipients: microcrystalline cellulose, lactose, stearic acid, magnesium stearate, colloidal silicone dioxide, sodium starch glycolate, Povidone, and hydroxyl propyl cellulose. All excipients are of US and EuPh compendial grade.

The 5 mg (free base equivalent) and 25 mg (free base equivalent) tablets are packaged in HDPE bottles. All bottles of [redacted] investigational product contain the following

language: "Caution: New Drug-Limited by Federal Law to Investigational Use." The clinical supply for investigational use is the 5 mg tablet.

7.1.3 Storage and Stability

Ruxolitinib has been shown to be stable for up to 6 months at 40 degrees C and up to 24 months when stored at 25 degrees C.

7.1.4 Compatibility

Ruxolitinib may be taken either with food or without food.

7.1.5 Handling

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

7.1.6 Availability

Ruxolitinib is an investigational agent and will be supplied free-of-charge from [redacted] in 5 mg tablets.

7.1.7 Administration

Ruxolitinib will be taken orally and self-administered by study participants at approximately the same times each day at least 10-12 hours apart. Participants will be instructed to complete a drug diary, which will be collected on day 1 of each cycle.

7.1.8 Ordering

Shipment request for ruxolitinib generated by each individual site will be submitted via email or fax directly to [redacted] vendor for shipment to the site for the study. No supplies will be shipped on Friday unless directed in writing by [redacted].

7.1.9 Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the agent (investigational or free of charge) using the NCI Drug Accountability Record or another comparable drug accountability form. (See the CTEP website at http://ctep.cancer.gov/protocolDevelopment for the "Policy and Guidelines for Accountability and Storage of Investigational Agents" or to obtain a copy of the drug accountability form.)

Responsibility for drug accountability at the study site rests with the Investigator; however, the Investigator may assign some of the drug accountability duties to an appropriate pharmacist or designee. Inventory and accountability records must be maintained and readily available for inspection by the study monitor and are open to inspection at any time by any applicable regulatory authorities. All used or partially used drugs may be destroyed on site upon return to the clinic.

The Investigator or designee must maintain records that document:

- investigational product delivery to the study site
- the inventory at the site
- use by each subject including pill/unit counts from each supply dispensed
- return to the Investigator or designee.

These records should include dates, quantities, batch/serial numbers (if available), and the unique code numbers (if available) assigned to the investigational product and study subjects. The investigational product must be used only in accordance with the protocol.

The Investigator will also maintain records adequately documenting that the subjects were provided the correct study drug specified.

Completed accountability records will be archived by the site.

7.1.10 Destruction and Return

Unused supplies of Ruxolitinib should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

7.2 Paclitaxel

7.2.1 Formulation

Paclitaxel is commercially available in 30 mg/5ml, 100 mg/16.7 ml, and 300 mg/50 ml multidose vials containing a clear colorless to slightly yellow viscous solution. Each ml of sterile nonpyrogenic solution contains 6 mg of paclitaxel, 527 mg of purified Cremophor EL (polyoxyethylated castor oil), and 49.7% (v/v) dehydrated alcohol, USP. Please refer to the FDA-approved package insert for complete product information.

7.2.2 Preparation

Paclitaxel must be diluted before administration with 0.9% sodium chloride for injection, USP; 5% dextrose for injection, USP for a final concentration of 0.3 to 1.2 mg/ml. Paclitaxel should be prepared and stored in glass, polypropylene, or polyolefin containers because of the leaching of DEHP [di(2ethylhexyl)phthalate] plasticizer from polyvinyl

chloride (PVC) containers. Non-PVC-containing tubing and connectors should be used, such as the IV administration sets (polyethylene or polyolefin) used to infuse parenteral nitroglycerin. In-line filtration should be accomplished by incorporating a hydrophilic, microporous filter of pore size not greater than 0.22 micron (e.g. IVEX-2) into the IV fluid pathway distal to the infusion pump. The Chemo Dispensing Pin device and similar devices with spikes should not be used with vials of paclitaxel since they can cause the stopper to collapse, resulting in loss of sterile integrity of the paclitaxel solution. Drug may be prepared per institutional standard of practice.

7.2.3 Administration

Paclitaxel will be administered as an IV infusion with the use of an in-line 0.22-micron filter. Please see Section 5.5.2 for premedication regimen. Administration time is per institutional standards.

7.3 Doxorubicin

7.3.1 Formulation

Commercially available as lyophilized powder for reconstitution in 10-, 20-, 50-, 100-and 150-mg vials. Also available as solution (2 mg/ml) in 10-, 20-, 50- and 200-mg vials for injection. Please refer to FDA-approved package insert for complete product information.

7.3.2 Preparation

Reconstitute the vials with 5, 10, 25, 50, or 75 ml, respectively, of sodium chloride for injection, USP. Drug may be prepared per institutional standard of practice.

7.3.3 Storage and Stability

Intact vials of doxorubicin should be stored in the refrigerator. Intact vials of powder for reconstitution should be stored at room temperature. Reconstituted solutions are stable for 7 days at room temperature and 15 days under refrigeration when protected from light. Commercially available solutions labeled as such are intended to be multi-dose vials.

7.3.4 Administration

Administer doxorubicin intravenously, either peripherally as a bolus injection or through a central venous line. Avoid extravasation, since severe local tissue necrosis may result. Administration time is per institutional standards.

7.4 Cyclophosphamide

7.4.1 Formulation

Commercially available as powder for injection in 100-mg, 200-mg, 500-mg, 1-g, and 2-g vials. Please refer to the FDA-approved package insert for complete product information.

7.4.2 Preparation

Reconstitute 100-mg, 200-mg, 500-mg, 1-g, and 2-g vials with 5, 10, 25, 50, or 100 ml of sterile water for injection respectively, for a final concentration of 20 mg/ml. Vigorous shaking and/or gentle warming may be necessary. Bacteriostatic water (paraben-preserved only) may also be used for reconstitution. 0.9% sodium chloride or 5% dextrose may also be used for reconstitution. Drug may be prepared per institutional standard of practice.

7.4.3 Storage and Stability

Store intact vials of powder at room temperature (15° to 30°C). Reconstitute lyophilized cyclophosphamide is chemically and physically stable for 24 hours at room temperature or for 6 days in the refrigerator (2° to 8°C). It does not contain any antimicrobial preservative, and care must therefore be taken to ensure the sterility of prepared solution.

7.4.4 Administration

Intravenous injection. Administration time is per institutional standards.

8 CORRELATIVE/SPECIAL STUDIES

8.1 STAT3 Activation

- Stat3 phosphorylation status will be assessed centrally by immunohistochemistry (IHC) on formalin fixed paraffin embedded tissue. We hypothesize that pStat3 status will decrease upon exposure to ruxolitinib.

- All pStat3 testing for the purpose of endpoint determination will be performed in the Immunohistochemistry Laboratory of Brigham & Women's Hospital (Jason L. Hornick, M.D., Ph.D., Director). Dr. Jane Brock, a breast pathology and co-investigator on this study, will supervise the conduct and scoring of pStat3 immunohistochemistry on the trial.

- We will use the Cell Signaling phospho-Stat3 (Tyr705) polyclonal antibody. pStat3 status will be determined by evaluating the percent positive staining cells and the strength of staining (weak vs moderate/strong) in relation to a positive (xenograft from SUM159 cell

line) and negative control (xenograft from MCF7 cell line) which will be run simultaneously each time the tumor samples are processed. Please refer to Appendix D for Standard Operating Procedures regarding pStat3 testing.

- Patients will have pStat3 analysis performed on breast cancer biopsies obtained prior to treatment, after 7 days of run-in treatment (cycle 1, day 1), and on any residual tumor at time of surgical resection.

- Triple immunofluorescence for CD44, CD24, and pStat3 will be performed on the breast core biopsies and residual disease in mastectomy specimens (as outlined above) in the laboratory of Dr. Polyak.

- Other markers of interest may also be examined by immunohistochemistry using excess biopsy sample or residual disease from mastectomy.

8.2 Stat3 Expression Signature

- We will characterize baseline biopsy, post-ruxolitinib exposure biopsy, and residual disease tumor samples using a previously characterized Stat3 activation signature.[25]

- We may also pursue analysis of STAT3 targets by gene expression profiling by RNAseq or qRT-PCR and ChIP-PCR or CHIPseq as well as evaluate JAK2 and 9p21 amplicons and assess their association with KDM4C.

8.3 Sample Acquisition

Table 9. Specimen Collection

Specimen Type	Time Point			Shipping Condition	Ship to
	Screening	C1D1	Surgery		
Lavender Top Blood Sample	X[a]			Ambient	DF/HCC Core Blood and Tissue Bank
4-6 Cores total (2-3 FFPE, 2-3 OCT)	X	X	X[b]	FFPE: ambient OCT: frozen (dry ice)	DF/HCC Core Blood and Tissue Bank
1 block/10-15 USS			X[c]	Ambient	DF/HCC Core Blood and Tissue Bank

a. At screening or on Cycle 1, Day -7 prior to start of run-in therapy
b. If adequate residual disease present; per pathology discretion (See Lab Manual)
c. If gross disease is not found at the time of surgery, residual microscopic specimens may be requested (See Lab manual).

8.3.1 Blood Collection

- Lavender Top Blood Sample

  One 10mL lavender top (EDTA Fisher #366643) tube of whole blood will be collected at baseline or on Cycle 1, Day -7 prior to start of run-in therapy.

Blood at non-DFCI sites is to be shipped to DFCI within 24 hours of collection. Blood will be processed and banked in the DF/HCC Core Blood and Tissue Bank for future research purposes or until sent to the Broad Institute for sequencing.

8.3.2 Guidelines for Tissue Biopsies

- A core needle tumor biopsy will be acquired from the patients enrolled into this clinical trial prior to starting protocol therapy (baseline), and prior to cycle 1 day 1 of preoperative treatment.
- When the patient is randomized to a treatment arm where ruxolitinib is included, a dose of ruxolitinib should preferentially be taken within two to four hours of any biopsy.
- Tissue specimens will be collected from tumor lesions using standard institutional procedures. Refer to Lab Manual for research collection guidelines.
- NOTE: Tissue from any routine diagnostic procedures (e.g., diagnostic biopsy) may be accessed for research analyses, if needed.

8.3.3 Residual Tumor collection on mastectomy specimen

If adequate residual tumor is present (per pathology discretion) in the mastectomy specimen, research samples will also be obtained. Refer to the Lab Manual for specimen guidelines.

8.3.4 Specimen Shipping

Refer to the Lab Manual for specimen shipping guidelines.

8.4 Pharmacodynamic Studies

8.4.1 Laboratory Correlative Studies

C-reactive protein

Dana-Farber patients will use the BWH chemistry laboratories for CRP measurements. Other investigative sites should process per institutional guidelines.

- CRP will be measured at baseline, cycle 1 day 1, cycle 5 day 1, and before surgery.

CRP level will be recorded as a continuous variable in the case report forms. We will use the American Heart Association and U.S. CDC defined cutoffs for hs-CRP based on cardiovascular risk. Elevated CRP is defined as level > 3.0 mg/L. Average CRP is defined as level between 1.0 to 3.0 mg/L. "Low risk" CRP is defined as level less than 1.0 mg/L.

IL-6

Dana-Farber patients will use the BWH chemistry laboratories for IL-6 measurements. Other investigative sites should process per institutional guidelines.

- IL-6 level will be measured at baseline, cycle 1 day 1, cycle 5 day 1, and before surgery.

IL-6 level will be recorded as a continuous variable in the case report forms.

8.5 Specimen Banking

Any leftover study blood and tissue samples may be stored for future research studies. The subjects will consent to the future use of samples in the consent form for the study. Any samples will only be released for use in future studies after approval by the Protocol Chair and other regulatory bodies, as appropriate.

The Protocol Chair and collaborators have approval by the TBCRC to use all research bio-specimens collected during the conduct of this trial to address the research questions described in the protocol document. All future use of residual or repository specimens collected in this trial for purposes not prospectively defined will require review and approval by the TBCRC according to its established policies, whether the specimens are stored in a central site or at a local institution in a virtual repository.

Secondary use of bio-specimens for new endpoints must be submitted to the TBCRC Central Office for possible review by the TBCRC Correlative Science Review Committee.

9 STUDY CALENDAR

Baseline evaluations are to be conducted within 28 days prior to start of protocol therapy (except pregnancy test within 14 days). Breast imaging and scans must be done ≤4 weeks prior to the start of therapy. All assessments must be performed prior to administration of any study medication. All on-treatment assessments should be administered within +/- 3 days of the protocol-specified date, unless otherwise noted. For cycle 1 day 1 ONLY, the treatment assessments must be within +1/-2 days.

Study Flowchart

	Screening (within 28 days prior to run-in therapy)	Run-In Therapy Cycle 1, Day -7	Preoperative Therapy Cycle 1-4 paclitaxel +/- ruxolitinib[o] (Day 1 of each cycle)	Preoperative Therapy Cycle 5-8 AC (Day 1 of each cycle)	Pre-Surgical Evaluation (1-2 weeks after ending chemotherapy)	Post-Surgical Evaluation [k] (1-3weeks post surgery)	Follow-up Visits [l]
Complete medical history	x						
Complete Physical Exam	x	x [i]	x	x	x	x	
Clinical tumor assessment	x	x	x	x	x		
Weight, vital signs, height (height at baseline only)	x	x	x	x	x	x	
Performance status (ECOG)	x						
Toxicity evaluation		x	x	x	x	x	
EKG (12 lead)	x						
Mammogram +/- ultrasound [a]	x				x [j]		x [m]
Clinical breast MRI [a]	x				x [j]		x [m]
Research Breast Biopsy [b]	x		x				
PET/CT [a]	x						
CT chest/abdomen/pelvis [a]	x [g]						
MUGA scan or Echocardiogram [c]	x						
Pregnancy test [d]	x						
Hematology (CBC with differential)	x	x	x[n]	x	x		
Serum chemistry [e]	x	x	x[n]	x	x		
IL-6, CRP [f]	x		x	x	x		
Research blood sample	x[h]						

[a] Breast imaging and scans must be done ≤ 28 days prior to the start of therapy (C1D-7 Run-In Therapy).
[b] See section 8.3. Baseline biopsy can be done any time prior to initiating therapy (C1D-7 Run-In Therapy). Cycle 1 day 1 biopsy can be done within 2 days prior to cycle 1 day 1 therapy provided the patient has had at least 5 days of ruxolitinib. For tissue collection guidelines, please refer to the Lab Manual.
[c] Cardiac evaluations should consistently use one modality.

[d] Women of childbearing potential. Serum or urine pregnancy test.
[e] Sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, total protein, albumin, total bilirubin, SGOT(AST), SGPT(ALT), alkaline phosphatase.
[f] At least 2mL should be collected into a yellow SST tube for CRP measurements. At least 2 mL serum should be collected in a separate yellow SST tube for IL-6 measurements. IL-6 and CRP should be drawn baseline prior to run-in, prior to chemotherapy on cycle 1 day 1 and cycle 5 day 1, and before surgery.
[g] If PET-CT is of CT-approved diagnostic quality, CT chest/abdomen/pelvis not required. NOTE: If questions and/or if patient has a contrast allergy, this is under the direction of institutional radiology guidelines.
[h] One 10mL lavender top tube. At screening or on Cycle 1, Day -7 prior to start of run-in therapy. See section 8.3.
[i] If the screening physical exam occurs ≤ 3 days of Day -7 Run in therapy, the physical exam does not need to be repeated on Day -7
[j] Breast imaging must be done 1-2 weeks after completing preoperative treatment, prior to surgery. Bilateral breast MRI. Mammogram+/- ultrasound performed on affected breast.
[k] Documentation of initiation of radiation therapy (See Section 5.5.6).
[l] Every 3 months (+/- 1 month) x 1 year, then every 6 months (+/- 1 month) x 4 years, then annually (+/- 1 month). Patients can be evaluated at any time as deemed clinically necessary by the investigator. See Section 5.8
[m] Breast imaging per standard practice recommendations.
[n] Laboratory tests should be checked weekly with the administration of paclitaxel (Section 5.4)
[o] For cycle 1 day 1 ONLY, the treatment assessments must be within +1/-2 days.

10 MEASUREMENT OF EFFECT

10.1 Antitumor Effect

For the purposes of this study, participants should be reevaluated every 1 cycle by physical examination. Photographs of the affected breast may be obtained. If extensive nodal involvement is present, patients should be evaluated with scans prior to cycle 5 day 1 (beginning AC) and prior to surgery.

Tumor response will be evaluated based upon resolution of edema, erythema and any density palpable within the affected breast. Breast imaging and clinical exam will determine final criteria for surgical respectability.

10.1.1 Definitions

Evaluable for toxicity. All participants who receive at least one dose of study treatment will be evaluable for toxicity from the time of their first treatment.

Evaluable for efficacy objectives. All participants randomized who initiate treatment will be evaluable for clinical efficacy endpoints, as specified in the definition of each endpoint.

Evaluable for biologic objectives. All participants randomized whose pre-treatment breast biopsy is scored "pStat3-positive" and have an assessable post-run-in breast biopsy will be evaluable for the primary biologic endpoint.

10.1.2 Disease-Free Survival, Time to Treatment Failure (TTF), and Overall Survival (OS)

DFS will be defined among patients who undergo surgery, as time from surgery until occurrence of one of the events below. Time to treatment failure will be defined among all patients from time of treatment initiation until occurrence of one of the events below or occurrence of progressive disease during preoperative therapy or treatment of disease that is not surgically resectable.

- Ipsilateral local-regional invasive breast cancer recurrence (i.e., an invasive breast cancer in the axilla, regional lymph nodes, chest wall and / or skin of the ipsilateral breast, or skin of the contralateral breast)
- Distant recurrence (i.e., evidence of breast cancer in any anatomic site other than local-regional disease described above) that has either been histologically confirmed or clinically diagnosed as recurrent invasive breast cancer.
- Contralateral invasive breast cancer.
- Death attributable to any cause including breast cancer, non-breast cancer, or unknown cause (but cause of death should be specified if at all possible).
- Second primary cancers other than breast.

For DFS and TTF, patients without an event will be censored at the date of last disease assessment.

OS will be defined two ways: among patients who undergo surgery, as time from surgery until death from any cause; and among all patients, as the time from treatment initiation until death from any cause. Censoring will use the date last known alive.

10.2 Other Response Parameters

10.2.1 Pathologic Complete Response pCR

Complete pathologic disease response (pCR) is defined as absence of invasive carcinoma within the breast and axillary lymph nodes following preoperative therapy. Participants whose disease is not surgically resectable following preoperative treatment are considered as not having pCR.

10.2.2. Residual cancer burden (RCB) after preoperative therapy will be determined, as defined by Symmans et al.[36]

11 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of reported and/or potential AEs (Section 11.1) and the characteristics of an observed AE (Section 11.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

11.1 Expected Toxicities

11.1.1 Adverse Event Lists(s) for ruxolitinib

The following summarizes the known toxicity profile of ruxolitinib. Please refer to the Investigator's Brochure for additional details.

Ten Phase I, five Phase II, and two Phase III clinical studies were conducted to explore the clinical pharmacology of ruxolitinib in healthy volunteers and in patients with myelofibrosis, essential thrombocythemia, polycythemia vera, subjects with renal or hepatic impairment, prostate cancer, multiple myeloma, or rheumatoid arthritis (see Investigator's Brochure, version 10).

Ruxolitinib has been administered to 198 healthy volunteers in single dose and repeat dosing studies of up to 10 days' duration. In healthy volunteer studies, a transient, reversible decrease in neutrophil count has been frequently seen, which reverses after 12 to 24 hours off drug, consistent with a margination effect. This was seen in 33% of subjects receiving 25 mg BID and 13% of subjects receiving 15 mg BID and at these doses were all either grade 1 or grade 2. Grade 4 neutropenia was observed in one subject

treated at 50 mg BID and led to study drug discontinuation. The maximum tolerated dose in healthy volunteers was determined to be 25 mg BID or 100 mg QD.

A phase II study of ruxolitinib was conducted in patients with rheumatoid arthritis. Efficacy greater than placebo was demonstrated in the top three doses in this study (i.e. 15 mg BID, 25 mg BID, and 50 mg QD) according to ACR20 response criteria. Ruxolitinib was well tolerated at these doses and AEs were similar to those seen in patients receiving placebo. There were no clinically significant changes in mean Hgb or platelet count and mean neutrophil counts remained in the normal range. One patient dosed with 25 mg BID exhibited grade 3 neutropenia which improved despite continued dosing and one patient who had a prior history of 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 QD) in a patient taking methotrexate with coronary artery disease.

INCB 18424-138 was a thorough QT study. The study was conducted to assess the heart rate corrected QT intervals in healthy subjects dosed with single doses of 25 mg and 200 mg ruxolitinib compared with placebo and moxifloxacin. The study was conducted double-blind with regard to ruxolitinib and placebo and open label for moxifloxacin. The study enrolled 50 and was completed by 47 normal healthy volunteer subjects (24 men and 23 women). The primary endpoint of this study was change in corrected QTcF. Based on the ICH E14 this study met the requirements for a negative QT study. Assay sensitivity was established with moxifloxacin. For ruxolitinib, at no time did the upper bound of the 2-sided 90% confidence interval exceed 10 msec. After subtracting placebo and baseline, there were no systematic changes in heart rate following the 25 mg dose. There were no systematic placebo and baseline adjusted changes in the PR interval or QRS duration.

In a study of patients with advanced prostate cancer, SAEs were generally related to expected progression of disease. One patient was reported to have sudden death while on study, felt by the investigator to be possibly related to ruxolitinib. Of note, this occurred in the context of a pre-existing history of congestive heart failure, hypertension, hyperlipidemia, and progressive prostate carcinoma.

In a study of patients with advanced multiple myeloma, observed toxicities included thrombocytopenia and anemia. Reported SAEs included pneumonia, urinary tract infection, anemia, pyrexia, and other cardiac and pulmonary complications. However, overall safety results were similar with those observed in other studies of ruxolitinib.

In a study of patients with advanced polycythemia vera or essential thrombocytopenia, the most commonly observed AEs were anemia in ET subjects and anemia and thrombocytopenia in PV subjects, and these were reversible with dose modifications. SAEs deemed at least possibly related included a case of atrial flutter which resolved, acute renal failure which resolved, and a renal tumor which was surgically resected.

In a phase I-II study among 153 patients with myelofibrosis, the nonhematologic toxicity profile (adverse events though to be at least possibly related to the study medication) was reported as below[37])

Event	All Grades (%)	Grade 3 or 4 (%)
Diarrhea	5.9	0
Fatigue	4.3	1.3
Headache	3.3	0
Peripheral edema	2.6	0
Pain in extremities	2.6	0
Urinary tract infection	2.6	0
Dizziness	2.6	0
Dyspnea	2.6	0
Asthenia	2.6	2.6
Fever	2.0	0.7
Cardiac murmur	2.0	0
Musculoskeletal pain	2.0	0
Peripheral neuropathy	2.0	0
Edema	2.0	0
Anxiety	2.0	1.3
Insomnia	2.0	1.3
Epistaxis	2.0	0
Flatulence	2.0	0
Nausea	2.0	0

With respect to hematologic toxicity, among 47 patients who received the 25 mg BID dose, grade 3 thrombocytopenia was reported in 23% of patients and grade 4 thrombocytopenia was reported in 6% of patients. Among 30 patients who were transfusion-independent at baseline, new onset grade 3 or 4 anemia was reported in 27% of patients. In general, the incidence of thrombocytopenia was dose dependent as was rapidly reversible and manageable with dose interruption and/or dose reduction.

Two phase III trials have recently been reported at the 2011 ASCO meeting. COMFORT-1 was a randomized, double-blind phase III trial of ruxolitinib (n=155) versus placebo (n=154) in patients with myelofibrosis (Verstovek et al, ASCO 2011). The most common AEs of any grade were thrombocytopenia (34% vs. 9%), fatigue (25% vs 34%), anemia (31% vs. 14%), diarrhea (23% vs 21%), abdominal pain (10% vs. 41%), and peripheral edema (19% vs. 22%). Anemia and thrombocytopenia were manageable and rarely (0.6%) led to withdrawal from the study.

COMFORT-2 was a randomized, double-blind phase III trial of ruxolitinib (n=146) versus best available therapy (BAT) (n=73) in primary myelofibrosis, post-polycythemia vera myelofibrosis, or post-essential thrombocythemia myelofibrosis (Harrison et al, ASCO 2011). The rate of grade 3 or 4 anemia was 42% on ruxolitinib and 31% on BAT; the rate of grade 3 or 4 thrombocytopenia was 8% on ruxolitinib and 7% on BAT. Grade 3 or 4 nonhematologic toxicities were uncommon, with none reported at a frequency of

above 3%. The most common non-hematologic toxicities of any grade included diarrhea (23% vs 11%), peripheral edema (22% vs 26%), asthenia (16% vs 10%), dyspnea (16% vs 18%), nasopharyngitis (15% vs 14%), pyrexia (14% vs 10%), nausea (13% vs 7%), arthralgia (12% vs 7%), cough (13% vs 15%), fatigue (12% vs 8%), and pain in extremity (12% vs 4%). The Investigator Brochure lists the most frequent adverse events (at least 5%) with suspected study drug in the Phase III patients (See Table 10 below).

Table 10.

	Study INCB 18424-351		Study CINC424A2352		Total
	Ruxolitinib	Placebo	Ruxolitinib	BAT	Ruxolitinib
Preferred term **Maximum Grade**	**N=155** **n (%)**	**N=151** **n (%)**	**N=146** **n (%)**	**N=73** **n (%)**	**N=301** **n (%)**
Any preferred term	115 (74.2)	84 (55.6)	117 (80.1)	14 (19.2)	232 (77.1)
Grade 3	31 (20.0)	25 (16.6)	31 (21.2)	1 (1.4)	62 (20.6)
Grade 4	12 (7.7)	0	2 (1.4)	0	14 (4.7)
Thrombocytopenia	47 (30.3)	8 (5.3)	62 (42.5)	1 (1.4)	109 (36.2)
Grade 3	10 (6.5)	1 (0.7)	9 (6.2)	0	19 (6.3)
Grade 4	1 (0.6)	0	1 (0.7)	0	2 (0.7)
Anemia	38 (24.5)	9 (6.0)	44 (30.1)	3 (4.1)	82 (27.2)
Grade 3	10 (6.5)	5 (3.3)	13 (8.9)	0	23 (7.6)
Grade 4	6 (3.9)	0	0	0	6 (2.0)
Diarrhea	17 (11.0)	9 (6.0)	12 (8.2)	1 (1.4)	29 (9.6)
Grade 3	1 (0.6)	0	0	0	1 (0.3)
Fatigue	19 (12.3)	20 (13.2)	5 (3.4)	1 (1.4)	24 (8.0)

Grade 3	3 (1.9)	1 (0.7)	0	0	3 (1.0)
Platelet count decreased	14 (9.0)	2 (1.3)	10 (6.8)	1 (1.4)	24 (8.0)
Grade 3	2 (1.3)	0	1 (0.7)	0	3 (1.0)
Edema peripheral	9 (5.8)	10 (6.6)	9 (6.2)	0	18 (6.0)
Hemoglobin decreased	13 (8.4)	2 (1.3)	4 (2.7)	1 (1.4)	17 (5.6)
Grade 3	8 (5.2)	2 (1.3)	0	0	8 (2.7)
Grade 4	2 (1.3)	0	1 (0.7)	0	3 (1.0)
Nausea	10 (6.5)	10 (6.6)	6 (4.1)	0	16 (5.3)
Grade 3	0	1 (0.7)	0	0	0
Weight increased	5 (3.2)	0	10 (6.8)	0	15 (5.0)
Grade 3	0	0	2 (1.4)	0	2 (0.7)
Headache	8 (5.2)	2 (1.3)	6 (4.1)	0	14 (4.7)
Grade 3	0	0	1 (0.7)	0	1 (0.3)
Dizziness	8 (5.2)	3 (2.0)	2 (1.4)	0	10 (3.3)
Asthenia	1 (0.6)	3 (2.0)	8 (5.5)	1 (1.4)	9 (3.0)
Grade 3	0	1 (0.7)	0	0	0
Abdominal pain	3 (1.9)	13 (8.6)	5 (3.4)	1 (1.4)	8 (2.7)
Grade 3	1 (0.6)	3 (2.0)	1 (0.7)	0	2 (0.7)

Single cases of Grade 5 AEs were reported, these are included in category "Grade 4"
The adverse events reported in the table were considered at least possibly related by individual investigator assessment.
Source: [Summary of Clinical Safety - Appendix 1–Table 2.1-1.7].

11.1.2 Adverse Event Lists(s) for Paclitaxel

The following summarizes the known toxicity profile of paclitaxel. Please refer to the package insert for additional details.

- Fatigue
- Alopecia
- Nausea and/or vomiting
- Leukopenia
- Anemia
- Neuropathy
- Arthralgias, Myalgias
- Mucositis
- Thrombocytopenia
- Diarrhea
- Headaches
- Skin rash, redness
- Skin or nail darkening
- Infusion reactions
- Dehydration
- Changes in vision

- Seizure
- Stevens-Johnson syndrome
- Deep vein thrombosis
- Pneumonia
- Heart failure, heart attack, or irregular heart rhythm
- Skin irritation and swelling

11.1.3 Adverse Event Lists(s) for Cyclophosphamide

The following summarizes the known toxicity profile of cyclophosphamide. Please refer to the package insert for additional details.

- Alopecia
- Fertility complications
- Nausea/vomiting
- Loss of appetite
- Diarrhea
- Mucositis
- Bladder inflammation and bleeding
- Thrombocytopenia,
- Anemia
- Febrile neutropenia
- Facial flushing
- Headache
- Skin rash
- Abnormal chemistries leading to fluid imbalance
- Kidney Failure
- Nasal congestion
- Sneezing
- Heart failure
- Heart inflammation
- Interstitial pneumonitis
- Anaphylaxis
- Bleeding of the colon
- Bleeding of the urethra
- Abnormal liver function tests
- Hyperuricemia
- Hypokalemia
- Malaise
- Radiation recall
- Bladder cancer
- Stevens-Johnsons syndrome

- Toxic epidermal necrolysis
- Muscle weakness of the whole body

11.1.4 Adverse Event Lists(s) for Doxorubicin

The following summarizes the known toxicity profile of doxorubicin. Please refer to the package insert for additional details.

- Neutropenia
- Nausea/vomiting
- Mucositis
- Diarrhea
- Dehydration
- Skin rash
- Low energy
- Photosensitivity
- Radiation recall reaction
- Amenorrhea
- Irregular heart beats
- Heart failure
- Hyperpigmentation
- Abdominal pain
- Fever, chills,
- Myelosuppression
- Ulceration of the colon
- Hearing loss
- Conjunctivitis
- Cornea scarring
- Epiphora

11.2 Adverse Event Characteristics

- CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- For expedited reporting purposes only
  - AEs for the agent(s) that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.

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

- **Expectedness** of the AE:
  - Expected adverse event

    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.

  - Unexpected adverse event

    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.

11.3 Expedited Adverse Event Reporting

11.3.1 Investigators **must** report to the Protocol Chair any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last protocol therapy (surgery) on a MedWatch Form 3500A or local institutional SAE form.

11.3.2 For multi-institution studies where a DF/HCC investigator is serving as the Overall Principal Investigator, each participating institution **must** abide by the reporting requirements set by the DF/HCC. This applies to any medical event equivalent to an unexpected grade 2 or 3 with a possible, probable or definite attribution, unexpected grade 4 toxicities, and grade 5 (death) regardless of study phase or attribution.

11.3.3 DF/HCC Expedited Reporting Guidelines

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

Other investigative sites will report SAEs to their respective IRB according to the local IRB's policies and procedures in reporting adverse events. A copy of the submitted institutional AE form should be forwarded to the Protocol Chair within the timeframes detailed in the table below.

Attribution	DF/HCC Reportable AEs				
	Gr. 2 & 3 AE Expected	**Gr. 2 & 3 AE Unexpected**	**Gr. 4 AE Expected**	**Gr. 4 AE Unexpected**	**Gr. 5 AE Expected or Unexpected**
Unrelated Unlikely	Not required	Not required	5 calendar days#	5 calendar days	24 hours*
Possible Probable Definite	Not required	5 calendar days	5 calendar days#	5 calendar days	24 hours*
# If listed in protocol as expected and not requiring expedited reporting, event does not need to be reported.					
* For participants enrolled and actively participating in the study ***or*** for AEs occurring within 30 days of the last intervention, the AE should be reported within 1 business day of learning of the event.					

The Overall PI will submit SAE reports from outside institutions to the DFCI OHRS according to DFCI IRB policies and procedures in reporting adverse events.

11.3.4 Serious Adverse Event Reporting to [redacted]

All serious adverse events that occur after the initial dose of study treatment, during treatment, or within 30 days of the last protocol therapy (surgery) must be reported to [redacted] on a MedWatch Form 3500A or DF/HCC local institution SAE form. This includes events meeting the criteria outlined below:

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

Grade 2 (moderate) and Grade 3 (severe) Events – Only events that are unexpected and possibly, probably or definitely related/associated with the intervention.

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 DF/HCC Overall Principal Investigator and to [redacted] 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:

Filipa Lynce MD

[redacted]

And to:

[redacted] for e-mail transmission of individual SAE reports; Within the following 24-48 hours, the participating investigator must provide follow-up information on the serious adverse event.

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.

11.3.5 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 [redacted] within 24 hours of learning of the occurrence
- Study drug must be discontinued immediately
- Subject must be withdrawn from the study
- Perform the required End-of-treatment Visit evaluations
- Investigator must complete and submit the Pregnancy Initial and Follow-up report forms to the Principal Investigator and to [redacted]
- 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 [redacted] 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 [redacted].

11.4 Expedited Reporting to Hospital Risk Management

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

11.5 Routine Adverse Event Reporting

All Grade 2 or higher Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, etc.) must <u>also</u> be reported in routine study data submissions.**

12 DATA AND SAFETY MONITORING

12.1 Data Reporting

12.1.1 Method

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

12.1.2 Responsibility for Data Submission

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

12.2 Data Safety Meetings

The DF/HCC Data and Safety Monitoring Board (DSMB) will review and monitor study progress, toxicity, safety and other data from this study. The board is chaired by a medical oncologist from outside of DF/HCC and has external and internal representation. Information that raises any questions about participant safety or protocol performance will be addressed by

the Overall PI, statistician and study team. Should any major concerns arise, the DSMB will offer recommendations regarding whether or not to suspend the study.

The DSMB will meet twice a year to review accrual, toxicity, response and reporting information. Information to be provided to the DSMB may include: participant accrual; treatment regimen information; adverse events and serious adverse events reported by category; summary of any deaths on study; audit results; and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

We also plan on reporting the success with obtaining research biopsies and the status of completion of surgery (mastectomy) and pCR rate (without regard to treatment assignment). Currently, pSTAT+ status of biopsy specimens are being assessed in batches, but when that data is available, it will be provided to the DSMB.

As part of the TBCRC, the progress of the study will be discussed at twice-monthly teleconferences and semi-annual TBCRC meetings. These discussions will provide information on the safety and scientific progress of the study and will be reported to the DSMB.

12.3 Multicenter Guidelines

This protocol will adhere to the policies and requirements of the DF/HCC Multi-Center Data and Safety Monitoring Plan. The specific responsibilities of the Overall PI, Coordinating Center, and Participating Institutions and the procedures for auditing are presented in Appendix E.

- The Overall PI/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports to all participating institutions for submission to their individual IRBs for action as required.

- Mechanisms will be in place to ensure quality assurance, protocol compliance, and adverse event reporting at each site.

- Except in very unusual circumstances, each participating institution will order the study agent(s) directly from supplier. A participating site may order the agent(s) only after the initial IRB approval for the site has been forwarded to the Coordinating Center.

12.4 Collaborative Research and Future Use of Data and Samples

Tissue, blood, bodily fluids, and other materials derived from these will be collected in this study to analyze genes, DNA, RNA, proteins and cells for the study's correlative endpoints and potential future research, utilizing new types of biomarker testing as it becomes available.

These samples and any data generated as a part of these clinical trials may be used for future research studies and may be provided to collaborating investigators both within and outside of the Translational Breast Cancer Research Consortium (TBCRC) for either correlative endpoints or

secondary use. Samples and data may be shared with outside non-profit academic investigators, as well as with for-profit pharmaceutical investigators or commercial entities, with whom we collaborate. When samples or data are sent to collaborators and when any research is performed on them, all information will be identified with a code, and will not contain any PHI, such as name, birthday, or MRNs.

In order to allow the greatest amount of research to be performed on the specimens and information generated as a part of this trial, researchers in this study may share results of genetic sequencing with other scientists. De-identified specimen or genetic data may be placed into one of more publicly-accessible scientific databases, such as the National Institutes of Health's Database for Genotypes and Phenotypes (dbGaP). The results from the correlative research on this study will be shared with these public databases. Through such databases, researchers from around the world will have access to de-identified samples or data for future research. More detailed information, beyond the public database, may only be accessed by scientists at other research centers who have received special permission to review de-identified data.

13 STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

The primary objective of the study is proof of principle, to assess the effect of JAK inhibition with ruxolitinib on pStat3+ expression by comparing expression in pretreatment biopsy specimens to post-ruxolitinib (after 7 days of ruxolitinib administered alone or with one dose of weekly paclitaxel; 32 patients per group) biopsy specimens. Patients will subsequently receive neoadjuvant treatment with weekly paclitaxel x 12 doses within or without ruxolitinib (32 patients with and 16 patients without ruxolitinib), followed by AC x 4 cycles. Treatment allocation is achieved by randomization to one of 3 treatment regimens defining treatment during the 7 day run-in phase and during the neoadjuvant treatment phase. Pathologic complete response (pCR) and residual cancer burden (RCB) will be assessed in patients undergoing surgical resection and residual tumor sample will be collected at surgery. Patients will be followed after surgery and radiation regularly for 5 years and yearly thereafter for survival. Serum will be collected at 4 timepoints prior to surgery to measure IL-6 and CRP.

As the primary endpoint, "biologic response," is whether the tumor demonstrated a reduction in pStat3 activity and is defined as a change in pStat3 scoring from moderate/high positive ("pStat3-positive") prior to treatment to negative or weakly positive/equivocal ("pStat3-negative") in post-ruxolitinib biopsy sample (defined in **Appendix D**). About 80% of patients tumors are expected to be pStat3-positive, based on data from the Polyak laboratory.[25]

13.2 Sample Size/Accrual Rate

Up to 64 patients will be enrolled to include 50 patients with a pre-treatment biopsy assessed as pStat3-positive and an assessable post-ruxolitinib biopsy, assuming 80% of pre-treatment tumors

will have positive expression and allowing for ~10% non-assessability.[25] Accrual is expected to be 21-22 patients per year for 3 years.

About 80% of patients' tumors pre-treatment are expected to be pStat3-positive. If at least 33% of these tumors have biologic response (change to pStat3-negative) after 7 days of single-agent ruxolitinib, the treatment will be considered as promising in demonstrating reduction in pStat3 activity; a rate of 10% or less would be considered as not promising. This will be compared with a 66% biologic response (change to pSTAT3 negative) after 7 days of combination ruxolitinib and paclitaxel, based upon presumed synergy between ruxolitinib and paclitaxel. Assuming 25 patients per treatment group, if at least 5 of 25 patients' tumors treated with single-agent ruxolitinib have biologic response then the hypothesis that biologic response is ≤10% is rejected with an error rate of 0. 098 (target error 0.10). If 4 or fewer of 25 patients' tumors have biologic response then the hypothesis that biologic response is ≥33% is rejected with an error rate of 0.05 (target error 0.10). For the comparison between treatment groups, 23 patients per group provides 74% power to detect a difference in biologic response of 33% vs. 66% using Fisher's exact test (1-sided α=0.10). In secondary analyses of continuous expression levels related to Stat3 status, 25 patients per group provides 90% power to detect an 0.876 SD between-group difference (one-sided α =0.05).

Sample size also considers the secondary endpoint of pCR, which will be assessed in all patients who initiate treatment. In a retrospective analysis of 2 trials including 60 IBC patients treated with FAC followed by paclitaxel, of which 65% had ER-negative disease, a pCR of 25% was characterized and is considered as an historical pCR rate, below which the experimental regimen would not be considered as promising. [11] A pCR rate of about 50% with ruxolitinib+paclitaxel for 12 weeks would be considered as promising. For the comparison between treatment groups, 48 and 16 patients in the two groups provides 60% power to detect a difference in pCR of 50% vs. 25% using Fisher's exact test (1-sided α=0.10); and 51% power within the subgroup that are pSTAT3+ pre-treatment (estimated 38 and 14 patients).

13.3 Analysis of Primary Objectives

The number, percent and two-sided 80% exact binomial CI for biologic response (change from pStat3-positive to pStat3-negative) among the subgroup with pStat3-positive tumors prior to treatment will be summarized. Summaries will be overall and according to treatment assignment (run-in with ruxolitinib alone vs ruxolitinib + paclitaxel). Biologic response will be compared between treatment groups using Fisher's exact test. Pre- vs. post-ruxolitinib pStat3 scoring will also be summarized descriptively in a cross-tabulation, among all treated patients.

13.3.1 Interim Analysis

Sponsor Amendment 5 adds an interim analysis for futility. This analysis will investigate whether there is adequate preliminary evidence of feasibility and/or biological activity to proceed with enrollment. The analysis will include the first 20-25 patients enrolled, depending on enrollment at time the plan is approved. Accrual may continue as the interim analysis is underway. There are two facets to the interim analysis.

13.3.1.1 The primary objective population includes those with pStat3-positive pre-treatment biopsies. The study assumes 80% pStat3+. If the observed positivity is too much lower than assumed, then enrollment will take longer to achieve the n=50 pairs, which is not feasible. With 20-25 patients, the study could reasonably distinguish between 80% vs 50% pStat-positive (n.b, where not positive are those pStat3-negative or not assessable). The analysis will use the principle of a single-stage design with the available denominator as guidance for declaring worthy to proceed (one-sided α=0.05, β=0.10) and calculate 2-sided exact binomial 90% CI. For example, if <16/23 are pStat3-positive, then the preliminary data would question feasibility to proceed (15/23=0.652 pStat3-positive with 90% CI [0.459 to 0.814] as the upper CL of 90% CI barely reaches the assumed 80% positivity).

13.3.1.2 The primary objective aims for ≥33% of those pStat3-positive biopsies to have biologic response (vs ≤10%) after single-agent ruxolitinib, needing ≥5/25 to declare worthy of further study. For a secondary objective, the study hypothesis is ≥66% of those pStat3-positive will have biologic response after combination ruxolitinib + paclitaxel. For the interim analysis, if evidence is that biologic response among all pStat3-positive samples (without regard to treatment) is unlikely to reach 33%, then the preliminary data would question whether to proceed. Similarly, the assessment will use principle of a single-stage design with observed pStat3-positive denominator to set a threshold for declaring worthy to proceed (one-sided α=0.05, β=0.10) and calculate 2-sided 90% CI. For example, if <3/18 pStat3-positive have biologic response, then the preliminary data would question proceeding (2/18=0.111, with 90% CI [0.020 to 0.310] and thus upper CL of 90% CI is well below a 33% biologic response rate. The underlying idea is that if 0 of 9-10 treated with ruxolitinib and 1 of 9-10 treated with ruxolitinib + paclitaxel have biologic response, then upper CLs of 90% CIs would be below 33% and well below 66%, respectively. Although with 3/18 biologic responses the upper 90% CL is 0.377, either 1/9 ruxolitinib or (0/9 + 3/9) in the two treatment groups, respectively, would be interesting to pursue.

13.4 Analysis of Secondary Objectives

The association of pre-treatment, post-treatment and change in pStat3 expression with pCR will be assessed. Number, percent and two-sided 80% CI for pCR will be summarized for pStat3 subgroups, but formal hypothesis testing is not planned. Summaries will also be according to treatment assignment. Further analysis will explore continuous measures of pStat3 expression in relation to pCR, descriptively using boxplots for example.

We will assess early measures of efficacy, pCR and RCB; pCR is strongly associated with longer-term outcomes in TNBC. The within-group pCR rates with confidence intervals will be considered relative to a historical control of 25%; the protocol acknowledges that the sample size provides limited power for the two group comparison of pCR but the hypothesis test will be conducted. pCR will be summarized among all treated patients according to treatment assignment (12 weeks paclitaxel alone vs ruxolitinib + paclitaxel) as number, percent and 2-sided 80% CI; patients who are not surgically resectable after treatment are counted in the denominator and considered as not having pCR. Summaries will be overall and according to treatment assignment, and groups will be compared between using Fisher's exact test.

Patients will be followed long term, and longer-term efficacy outcomes, including DFS and OS, will be summarized. DFS and OS after surgery will be summarized using Kaplan-Meier method, among all patients who have surgery, according to treatment group. TTF and OS from enrollment will be summarized among all treated patients, according to treatment group.

RCB will be summarized as number, percent and two-sided 80% CI within each category, according to treatment group.

IL-6 and CRP levels pre-treatment, after 7 days ruxolitinib (with or without paclitaxel - cycle 1 day 1) and at completion of systemic treatment (prior to surgery) will be summarized descriptively, according to treatment. The association of pre-treatment IL-6 and CRP levels with pCR will be investigated by graphically summarizing levels according to pCR status using boxplots, according to treatment group.

In addition to correlative studies, the distribution of CD44/CD24/pStat3 subpopulation expression among pre-treatment, post-treatment and post-discontinuation of ruxolitinib will be summarized descriptively, but formal hypothesis testing is not planned. Additional measures of pSTAT3 expression, STAT3 target gene expression and JAK2 and KDM4C copy number, will also be summarized descriptively.

13.5 Reporting and Exclusions

Analysis populations are described throughout Section 13.4.

14 PUBLICATION PLAN

The data will be collected and analyzed by Dr. Filipa Lynce, [redacted] from the Department of Biostatistics and Computational Biology, and key co-investigators. The results will be shared with [redacted] and the TBCRC in advance of publication. It is anticipated that results will be made public within 12 months of the end of data collection. Initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. The primary endpoint of the study will not be reported until the study is closed to accrual and there is adequate follow-up time to estimate the response rate. A report will also be published in a peer-reviewed journal. A full report of the outcomes will be made public no later than three years after the end of data collection.

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16 APPENDICES

APPENDIX A: Performance Status Criteria
APPENDIX B: Prohibited Medications List
APPENDIX C: Drug Diary
APPENDIX D: Immunohistochemistry for pSTAT3 on Paraffin Embedded Tissue
APPENDIX E: Multicenter Guidelines

16.1 APPENDIX A: Performance Status Criteria

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Description	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.

16.2 APPENDIX B: Prohibited Medications List

Drug Class	Agent	Wash-out
CYP3A4 Inhibitors		
Antibiotics	Clarithromycin, telithromycin, troleandomycin	2 weeks
Antifungals	Itraconazole, ketoconazole, fluconazole (>200 mg daily), voriconazole, posaconazole	2 weeks
Antiretrovirals, protease inhibitors	Tipranavir, nelfinavir, ritonavir, indinavir, saquinavir, lopinivir, telaprevir, danoprevir, elviegravir, boceprevir	2 weeks
Calcium channel blockers	Mibefradil	2 weeks
Antidepressants	Nefazodone	2 weeks
Diuretics	Conivaptan	2 weeks
Food Product	Grapefruit Juice	5 days
Other	Cobicistat	2 weeks
Other	LCL161	2 weeks

Note: Erythromycin, azithromycin, aprepitant, and fluoxetine ARE allowed on protocol, as only potent inhibitors and fluconazole are prohibited. Please contact the Principal Investigator in any cases of uncertainty.

16.3 APPENDIX C: Ruxolitinib Drug Diaries

Ruxolitinib Run-In Phase Drug Diary

Name: _____ Subject#____ Group _____ Dose:_____

Date	(AM) Number of Pills	Time pills Taken	(PM) Number of Pills	Time pills Taken
DAY 1		____:____AM		____:____PM
DAY 2		____:____AM		____:____PM
DAY 3		____:____AM		____:____PM
DAY 4		____:____AM		____:____PM
DAY 5		____:____AM		____:____PM
DAY 6		____:____AM		____:____PM
DAY 7		____:____AM		____:____PM
DAY 8		____:____AM		____:____PM
DAY 9		____:____AM		____:____AM

*Please take your ruxolitinib dose at approximately the same times each day at least 10-12 hours apart. For example if you take your morning dose at 8am you should take your evening dose no earlier than 6pm.

*** You may have left over pills at the time of your second biopsy:**
Group 1: Take your AM dose of ruxolitinib on day of your second biopsy. Stop the ruxolitinib after your second biopsy.
Group 2: Continue to take the ruxolitinib until you are seen in clinic
Group 3: Continue to take the ruxolitinib until you are seen in clinic

Please record the approximate time you will take your doses: _____AM, _____PM

*Ruxolitinib may be taken with or without food twice per day.

*If a dose is missed (i.e. more than 4 hours have elapsed since the regular time to take your AM or PM dose) it should be skipped and this should be recorded in the drug diary.

*If you vomit a dose, it should not be retaken. Instead, it should be skipped and recorded in the drug diary.

Please do not chew, crush or dissolve ruxolitinib.

*Please contact your study doctor or study nurse with any questions.

*Subject Signature_____ Date_____

Please return diary and drug bottles at next visit

Ruxolitinib Treatment Phase Drug Diary

Name: _____ Subject#____ Group _____Cycle#_____ Dose:_____

Date	**(AM) Number of Pills**	**Time pills Taken**	**(PM) Number of Pills**	**Time pills Taken**
DAY 1		____:____AM		____:____PM
DAY 2		____:____AM		____:____PM
DAY 3		____:____AM		____:____PM
DAY 4		____:____AM		____:____PM
DAY 5		____:____AM		____:____PM
DAY 6		____:____AM		____:____PM
DAY 7		____:____AM		____:____PM
DAY 8		____:____AM		____:____PM
DAY 9		____:____AM		____:____PM
DAY 10		____:____AM		____:____PM
DAY 11		____:____AM		____:____PM
DAY 12		____:____AM		____:____PM
DAY 13		____:____AM		____:____PM
DAY 14		____:____AM		____:____PM
DAY 15		____:____AM		____:____PM
DAY 16		____:____AM		____:____PM
DAY 17		____:____AM		____:____PM
DAY 18		____:____AM		____:____PM
DAY 19		____:____AM		____:____PM
DAY 20		____:____AM		____:____PM
DAY 21		____:____AM		____:____PM

*Please take your ruxolitinib dose at approximately the same times each day at least 10-12 hours apart. For example if you take your morning dose at 8am you should take your evening dose no earlier than 6pm.

Please record the approximate time you will take your doses: _____AM, _____PM

*Ruxolitinib may be taken with or without food twice per day.

*If a dose is missed (i.e. more than 4 hours have elapsed since the regular time to take your AM or PM dose) it should be skipped and this should be recorded in the drug diary.

*If you vomit a dose, it should not be retaken. Instead, it should be skipped and recorded in the drug diary.

Please <u>do not</u> chew, crush or dissolve ruxolitinib.

*Please contact your study doctor or study nurse with any questions.

*Subject Signature_____ Date_____

Please return diary and drug bottles at next visit

16.4 APPENDIX D: Immunohistochemistry for pSTAT3 on Paraffin Embedded Tissue

ENVISION+ HORSERADISH PEROXIDASE METHOD

To Demonstrate - Antigenic sites

Method

1. Dewax sections in Hemo-De - 10 minutes
2. Take sections to absolute alcohol – x6 changes
3. Block endogenous peroxidase activity

Paraffin sections

70mls of 3% hydrogen peroxide – 30 minutes
130mls of absolute alcohol Wash
sections in running tap water - 3 minutes

MAKE UP ALL ANTISERA

4. Perform antigen retrieval:
5. Ring around sections using a pap pen
6. Place slides in a 500ml container of TBS containing approximately
10mlsl%BRIJ -1 minute
7. Apply primary antibody and incubate sections - O/N 4oC
8. Rinse sections in TBS to remove excess antibody, then wash in 500ml container of TBS containing
10ml of 1% BRIJ (on a magnetic stirrer) - 10 minutes
9. Incubate sections with secondary antibody - 30 minutes
For polyclonals use Rabbit Envision +^
10. Repeat step "8" - 10 minutes
11. Incubate sections with DAB+ - 5 minutes for cytoplasmic antibodies
- 10 minutes for nuclear antibodies

DAB+ SOLUTION - For every 1ml of buffer solution use 1 drop of DAB.

12. Wash well in running tap water
13. Counterstain using Mayer's Hematoxylin -11/2 minutes
14. Wash in running tap water
15. "Blue" sections using Scott's Tap Water. - 1 minute
16. Wash in running tap water
17. Dehydrate, clear and mount sections

RESULTS

Antigenic Sites - brown
Nuclei - blue
Background – clear

pStat3 SCORING System

% stained cells	**0**	**1-24%**	**25-49%**	**50-74%**	**75-100%**
Score	**0**	**1**	**2**	**3**	**4**
Patient score					

Intensity of stain	**Weak**	**Moderate**	**Strong**
Score	**2**	**3**	**4**
Patient score			

Total Score:

Score interpretation:
Score of ≥ 6 is high positive
Score of 5 is moderate positive
Score of 3-4 is weakly positive/equivocal
Score of 0 is negative

Publication materials and methods version:
Specimen fixation time is at least 6 hours for all cases. Four □m thick sections are baked at 37°C overnight, then deparaffinized and rehydrated (100% xylene x 4 for 3 minutes each, 100% ethanol x 4 for 3 minutes each, and running water for 5 minutes). Endogenous peroxidase activity is blocked with 3% hydrogen peroxide in methanol for 10 minutes and washed under running water for 5 minutes. Heat induced epitope retrieval is performed in EDTA buffer (pH 8.0) with a pressure cooker (Biocare Medical) at 122°C, to between 14 -17 PSI with the cycle lasting on average 45 minutes and cool down period approximately 20 minutes. IHC is performed on an automated instrument (Dako Autostainer Plus). A range of titers is tested for antibody and titer is calibrated using positive control staining, and negative staining of known (mouse) control tissue. Primary antibody (1:200) is incubated overnight at 4oC, followed by detection with the Envision plus system (Dako) for 30 minutes in a humid chamber at room temperature. Sections are developed using 3,3'-diaminobenzidine (DAB) (Sigma, St. Louis, MO) as substrate and counterstained with Mayer's hematoxylin.

Reagents
Phospho-Stat3 (Tyr705) Polyclonal Antibody, Cell Signaling Cat No#9131

16.5 APPENDIX E: Multicenter Data and Safety Monitoring Plan

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1. INTRODUCTION

The Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (DF/HCC DSMP) outlines the procedures for conducting a DF/HCC Multi-Center research protocol. The DF/HCC DSMP should serve as a reference for any sites external to DF/HCC that will be participating in the research protocol.

1.1 Purpose

To establish standards that will ensure that a Dana-Farber/Harvard Cancer Center Multi-Center protocol will comply with Federal Regulations, Health Insurance Portability and Accountability Act (HIPAA) requirements and applicable DF/HCC Standard Operating Procedures.

1.2 Multi-Center Data and Safety Monitoring Plan Definitions

DF/HCC Multi-Center Protocol: A research protocol in which one or more outside institutions are collaborating with Dana-Farber/Harvard Cancer Center where a DF/HCC investigator is the sponsor. DF/HCC includes Dana-Farber/Partners Cancer Care (DF/PCC) Network Clinical Trial Affiliates.

Lead Institution: Dana-Farber Cancer Institute (DFCI) is responsible for the coordination, development, submission, and approval of a protocol as well as its subsequent amendments per the DFCI IRB and applicable regulatory guidelines (Food and Drug Administration (FDA) etc.). The Lead Institution is typically the home of the DF/HCC Sponsor. The Lead Institution also typically serves as the Coordinating Center for the DF/HCC Multi-Center Protocol.

DF/HCC Sponsor: The person sponsoring the submitted Multi-Center protocol. Within DF/HCC, this person is the Overall Principal Investigator who takes responsibility for initiation, management and conduct of the protocol at all research locations. In applicable protocols, the DF/HCC Sponsor will serve as the single liaison with any regulatory agencies (i.e. FDA, etc.). The DF/HCC Sponsor has ultimate authority over the protocol and is responsible for the conduct of the study at DF/HCC and all Participating Institutions. In most cases the DF/HCC Sponsor is the same person as the DF/HCC Overall Principal Investigator; however, both roles can be filled by two different people.

Participating Institution: An institution that is outside the DF/HCC and DF/PCC consortium that is collaborating with DF/HCC on a protocol where the sponsor is a DF/HCC Investigator. The Participating Institution acknowledges the DF/HCC Sponsor as having the ultimate authority and responsibility for the overall conduct of the study.

Coordinating Center: The entity (i.e. Lead Institution or Project Manager) that provides administrative support to the DF/HCC Sponsor in order that he/she may fulfill the responsibilities outlined in the protocol document and DSMP, and as specified in applicable regulatory guidelines. In general, the Lead Institution is the Coordinating Center for the DF/HCC Multi-Center Protocol.

DF/HCC Office of Data Quality (ODQ): A group within DF/HCC responsible for ensuring high-quality standards are used for data collection and the ongoing management of clinical trials, auditing, and data and safety monitoring. ODQ also coordinates quality assurance efforts related to multi-center clinical research.

DF/HCC Research Informatics Office (RIO): A group within DF/HCC responsible for providing a comprehensive data management platform for managing clinical trial data.

2. GENERAL ROLES AND RESPONSIBILITIES

For DF/HCC Multi-Center Protocols, the DF/HCC Sponsor, the Coordinating Center, and the Participating Institutions are expected to adhere to the following general responsibilities:

2.1 DF/HCC Sponsor

The DF/HCC Sponsor, Filipa Lynce, MD, will accept responsibility for all aspects of conducting a DF/HCC Multi-Center protocol which includes but is not limited to:

- Oversee the coordination, development, submission, and approval of the protocol as well as subsequent amendments.
- Ensure that the investigators, study team members, and Participating Institutions are qualified and appropriately resourced to conduct the protocol.
- Include the Multi-Center Data and Safety Monitoring Plan as an appendix to the protocol.
- Ensure all Participating Institutions are using the correct version of the protocol.
- Ensure that each participating investigator and study team member receives adequate protocol training and/or a Site Initiation Visit prior to enrolling participants and throughout trial's conduct as needed.
- Ensure the protocol will be provided to each participating site in a language understandable to all applicable site personnel when English is not the primary language.
- Monitor progress and overall conduct of the study at all Participating Institutions.
- Ensure all DFCI Institutional Review Board (IRB), DF/HCC and other applicable (i.e. FDA) reporting requirements are met.
- Review data and maintain timely submission of data for study analysis.
- Act as the single liaison with FDA (investigator-held IND trials).
- Ensure compliance with all requirements as set forth in the Code of Federal Regulations, applicable DF/HCC requirements, HIPAA requirements, and the approved protocol.
- Commit to the provision that the protocol will not be rewritten or modified by anyone other than the DF/HCC Sponsor.
- Identify and qualify Participating Institutions and obtain accrual commitments prior to extending the protocol to that site.
- Monitor accrual and address Participating Institutions that are not meeting their accrual requirements.

2.2 Coordinating Center

The general responsibilities of the Coordinating Center may include but are not limited to:

- Assist in protocol development.
- Maintain FDA correspondence, as applicable.
- Review registration materials for eligibility and register participants from Participating Institutions in the DF/HCC clinical trial management system (CTMS).
- Distribute protocol and informed consent document updates to Participating Institutions as needed.
- Oversee the data collection process from Participating Institutions.
- Maintain documentation of Serious Adverse Event (SAE) reports and deviations/violation submitted by Participating Institutions and provide to the DF/HCC Sponsor for timely review.
- Distribute serious adverse events reported to the DF/HCC Sponsor that fall under the DFCI IRB Adverse Event Reporting Policy to all Participating Institutions.
- Provide Participating Institutions with information regarding DF/HCC requirements that they will be expected to comply with.
- Carry out plan to monitor Participating Institutions either by on-site or remote monitoring.
- Maintain Regulatory documents of all Participating Institutions which includes but is not limited to the following: local IRB approvals/notifications from all Participating Institutions, confirmation of Federalwide Assurances (FWAs) for all sites, all SAE submissions, Screening Logs for all sites, IRB approved consents for all sites
- Conduct regular communications with all Participating Institutions (conference calls, emails, etc) and maintain documentation all relevant communications.

2.3 Participating Institution

Each Participating Institution is expected to comply with all applicable federal regulations and DF/HCC requirements, the protocol and HIPAA requirements.

The general responsibilities for each Participating Institution may include but are not limited to:

- Document the delegation of research specific activities to study personnel.
- Commit to the accrual of participants to the protocol.
- Submit protocol and/or amendments to their local IRB.
- Maintain regulatory files as per sponsor requirements.
- Provide the Coordinating Center with regulatory documents or source documents as requested.
- Participate in protocol training prior to enrolling participants and throughout the trial as required (i.e. teleconferences).
- Update Coordinating Center with research staff changes on a timely basis.
- Register participants through the Coordinating Center prior to beginning research related activities.
- Submit Serious Adverse Event (SAE) reports to local IRB per local requirements and to the Coordinating Center, in accordance with DF/HCC requirements.

- Submit protocol deviations and violations to local IRB per local requirements and to the DF/HCC Sponsor in accordance with DF/HCC requirements.
- Order, store and dispense investigational agents and/or other protocol mandated drugs per federal guidelines and protocol requirements.
- Have office space, office equipment, and internet access that meet HIPAA standards.
- Participate in any quality assurance activities and meet with monitors or auditors at the conclusion of a visit to review findings.
- Promptly provide follow-up and/or corrective action plans for any monitoring queries or audit findings.

3. DF/HCC REQUIREMENTS FOR MULTI-CENTER PROTOCOLS

The following section will clarify DF/HCC Requirements and further detail the expectations for participating in a DF/HCC Multi-Center protocol.

3.1 Protocol Distribution

The Coordinating Center will distribute the final DFCI IRB approved protocol and any subsequent amended protocols to all Participating Institutions.

3.2 Protocol Revisions and Closures

The Participating Institutions will receive notification of protocol revisions and closures from the Coordinating Center. It is the individual Participating Institution's responsibility to notify its IRB of these revisions.

- **Non life-threatening revisions:** Participating Institutions will receive written notification of protocol revisions regarding non life-threatening events from the Coordinating Center. Non-life-threatening protocol revisions must be IRB approved and implemented within 90 days from receipt of the notification.

- **Revisions for life-threatening causes:** Participating Institutions will receive immediate notification from the Coordinating Center concerning protocol revisions required to protect lives with follow-up by fax, mail, e-mail, etc. Life-threatening protocol revisions will be implemented immediately followed by IRB request for approval.

- **Protocol closures and temporary holds:** Participating Institutions will receive notification of protocol closures and temporary holds from the Coordinating Center. Closures and holds will be effective immediately. In addition, the Coordinating Center, will update the Participating Institutions on an ongoing basis about protocol accrual data so that they will be aware of imminent protocol closures.

3.3 Informed Consent Requirements

The DF/HCC approved informed consent document will serve as a template for the informed consent for Participating Institutions. The Participating Institution consent form must follow the consent template as closely as possible and should adhere to specifications outlined in the DF/HCC Guidance Document on Model Consent Language for PI-Initiated Multi-Center Protocols. This document will be provided separately to each Participating Institution.

Participating Institutions are to send their version of the informed consent document and HIPAA authorization, if a separate document, to the Coordinating Center for review and approval prior to submission to their local IRB. The approved consent form must also be submitted to the Coordinating Center after approval by the local IRB for all consent versions.

The Principal Investigator (PI) at each Participating Institution will identify the physician members of the study team who will be obtaining consent and signing the consent form for therapeutic protocols. Participating institutions must follow the DF/HCC requirement that only attending physicians obtain informed consent and re-consent for all interventional drug, biologic, or device research.

3.4 IRB Documentation

The following must be on file with the Coordinating Center:

- Initial approval letter of the Participating Institution's IRB.
- Copy of the Informed Consent Form(s) approved by the Participating Institution's IRB.
- Participating Institution's IRB approval for all amendments.
- Annual approval letters by the Participating Institution's IRB.

3.5 IRB Re-Approval

Verification of IRB re-approval from the Participating Institutions is required in order to continue research activities. There is no grace period for continuing approvals.

The Coordinating Center will not register participants if a re-approval letter is not received from the Participating Institution on or before the anniversary of the previous approval date.

3.6 Participant Confidentiality and Authorization Statement

In 1996, congress passed the first federal law covering the privacy of health information known as the Health Insurance Portability and Accountability Act (HIPPA). Any information, related to the physical or mental health of an individual is called Protected Health Information (PHI). HIPAA outlines how and under what circumstances PHI can be used or disclosed.

In order for covered entities to use or disclose protected health information during the course of a study, the study participant must sign an authorization statement. This authorization statement may or may not be separate from the informed consent document. The Coordinating Center, with the approval from the DFCI IRB will provide a consent template, with information regarding authorization for the disclosure of protected health information.

The DF/HCC Sponsor will use all efforts to limit its use of protected health information in its trials. However, because of the nature of these trials, certain protected health information must be collected. DF/HCC has chosen to use authorizations, signed by the participant in the trial, rather than limited data sets with data use agreements.

3.6.1 DF/HCC Multi-Center Protocol Confidentiality

All documents, investigative reports, or information relating to the participant are strictly confidential. Whenever reasonably feasible, any participant specific reports (i.e. Pathology Reports, MRI Reports, Operative Reports, etc.) submitted to the Coordinating Center should be de-identified. It is recommended that the assigned protocol case number (as described below) be used for all participant specific documents. Participant initials may be included or retained for cross verification of identification.

3.7 DF/HCC Multi-Center Protocol Registration Policy

3.7.1 Participant Registration and Randomization

Refer to protocol section 4.3 and 4.4 of the protocol for the participant registration process.

3.7.2 Initiation of Therapy

Participants must be registered with the DF/HCC CTMS before receiving treatment. Treatment may not be initiated until the Participating Institution receives confirmation of the participant's registration from the Coordinating Center. The DF/HCC Sponsor and DFCI IRB must be notified of any violations to this policy.

3.7.3 Eligibility Exceptions

No exceptions to the eligibility requirements for a protocol without DFCI IRB approval will be permitted. All Participating Institutions are required to fully comply with this requirement. The process for requesting an eligibility exception is defined below.

3.8 DF/HCC Protocol Case Number

At the time of registration, the following identifiers are required for all subjects: initials, date of birth, gender, race and ethnicity. Once eligibility has been established and the participant successfully registered, the participant is assigned a unique protocol case number. This number is unique to the participant on this trial and must be used for CRF/eCRF completion and correspondence. Participating Institutions should submit all de-identified subsequent communication and documents to the Coordinating Center, using this case number to identify the subject.

3.8.1 Protocol Deviations, Exceptions and Violations

Federal Regulations require an IRB to review proposed changes in a research activity to ensure that researchers do not initiate changes in approved research without IRB review and approval, except when necessary to eliminate apparent immediate hazards to the participant. DF/HCC requires all departures from the defined procedures set forth in the IRB approved protocol to be reported to the DF/HCC Sponsor, who in turn is responsible for reporting to the DFCI IRB.

For reporting purposes, DF/HCC uses the terms "violation", "deviation" and "exception" to describe departures from a protocol. All Participating Institutions must adhere to these requirements for reporting to the DF/HCC Sponsor and will follow their institutional policy for reporting to their local IRB.

3.8.2 Definitions

Protocol Deviation: Any departure from the defined procedures set forth in the IRB-approved protocol which is *prospectively approved* prior to its implementation.

Protocol Exception: Any protocol deviation that relates to the eligibility criteria, e.g. enrollment of a participant who does not meet all inclusion/exclusion criteria.

Protocol Violation: Any protocol deviation that was not *prospectively approved* by the IRB prior to its initiation or implementation.

3.8.3 Reporting Procedures

DF/HCC Sponsor: is responsible for ensuring that clear documentation is available in the medical record and/or regulatory documents to describe all protocol exceptions, deviations and violations. The DF/HCC Sponsor will also be responsible for ensuring that all protocol violations/deviations are promptly reported per DFCI IRB guidelines.

Participating Institutions: Protocol deviations require prospective approval from the DFCI IRB. The Participating Institution must submit the deviation request to the Coordinating Center who will then submit the deviation request to the DFCI IRB. Upon DFCI IRB approval the deviation is submitted to the Participating Institution IRB, per institutional policy. A copy of the Participating Institution's IRB report and determination will be forwarded to the Coordinating Center within 10 business days after the original submission.

All protocol violations must be sent to the Coordinating Center in a timely manner.

Coordinating Center: Upon receipt of the violation/deviation report from the Participating Institution, the Coordinating Center will submit the report to the DF/HCC Sponsor for review. Subsequently, the Participating Institution's IRB violation/deviation report will be submitted to the DFCI IRB for review per DFCI IRB reporting guidelines.

3.9 Safety Assessments and Toxicity Monitoring

The study teams at all participating institutions are responsible for protecting the safety, rights and well-being of study participants. Recording and reporting of adverse events that occur during the course of a study help ensure the continuing safety of study participants.

All participants receiving investigational agents and/or other protocol mandated treatment will be evaluated for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical examination findings, and spontaneous reports of adverse events reported by participants. All toxicities encountered during the study will be evaluated according to the NCI criteria specified in the protocol. Life-threatening toxicities must be reported immediately to the DF/HCC Sponsor via the Coordinating Center.

Additional safety assessments and toxicity monitoring will be outlined in the protocol.

3.9.1 Guidelines for Reporting Serious Adverse Events

Guidelines for reporting Adverse Events (AEs) and Serious Adverse Events (SAEs) are detailed in protocol section 11.0.

Participating Institutions must report the SAEs to the DF/HCC Sponsor and the Coordinating Center following the DFCI IRB Adverse Event Reporting Policy.

The Coordinating Center will maintain documentation of all Participating Institution Adverse Event reports and be responsible for communicating to all participating investigators, any observations reportable under the DFCI IRB Reporting Requirements. Participating Institutions will review and submit to their IRB according to their institutional policies and procedures

3.9.2 Guidelines for Processing IND Safety Reports

The DF/HCC Sponsor will review all IND Safety Reports per DF/HCC requirements, and ensure that all IND Safety Reports are distributed to the External Sites as required by DF/HCC Policy. External Sites will review/submit to the IRB according to their institutional policies and procedures.

3.10 Data Management

The DF/HCC RIO develops case report forms (CRF/eCRFs), for use with the protocol. These forms are designed to collect data for each study. DF/HCC RIO provides a web based training for all eCRF users.

3.10.1 Data Forms Review

Data submissions are monitored for timeliness and completeness of submission. If study forms are received with missing or questionable data, the submitting institution will receive

a written or electronic query from the DF/HCC Office of Data Quality, Coordinating Center, or designee.

Responses to all queries should be completed and submitted within 14 calendar days.

Responses may be returned on the written query or on an amended paper case report form, or in the case of electronic queries, within the electronic data capture (eDC) system. In the case of a written query for data submitted on a paper case report form, the query must be attached to the specific data being re-submitted in response.

If study forms are not submitted on schedule, the Participating Institution will periodically receive a Missing Form Report from the Coordinating Center noting the missing forms.

4. REQUISITIONING INVESTIGATIONAL DRUG

Ruxolitinib will be ordered from [redacted] The ordering of investigational agent is specified in the protocol section 7.1.8.

Paclitaxel, Cyclophosphamide, and Doxorubicin are [redacted] available. Check with the local Director of Pharmacy and/or the Research Pharmacy to ensure that the agent is in stock. If the agent is not stocked, ensure that the agent can be ordered once the protocol is approved by the local IRB.

5. MONITORING: QUALITY CONTROL

The quality control process for a clinical trial requires verification of protocol compliance and data accuracy. As the Coordinating Center, the DF/HCC Lead Institution with the aid of the ODQ provides quality control oversight for the protocol.

5.1 Ongoing Monitoring of Protocol Compliance

The Participating Institutions will be required to submit participant source documents to the DF/HCC Lead Institution or designee for monitoring. Participating Institutions may also be subject to on-site monitoring conducted by the DF/HCC Lead Institution.

The DF/HCC Lead Institution will implement on-site and virtual monitoring activities to ensure that Participating Institutions are complying with regulatory and protocol requirements, data quality, and subject safety. At minimum, the DF/HCC Lead Institution, or designee, will monitor each participating site twice a year while patients are receiving study treatment. Should a Participating Institution be monitored once and then not accrue any additional participants or participant visits, then a second monitoring visit may not be necessary.

Monitoring practices may include but are not limited to: source verification, review and analysis of the following: eligibility requirements of all participants, informed consent procedures, adverse events and all associated documentation, study drug administration / treatment,

regulatory records and site trial master files, protocol deviations, pharmacy records, response assessments, and data management. Additionally, ongoing communication will occur through regularly scheduled teleconferences and by email. Source documents from Participating Institutions, will be collected at specific timepoints to support the primary and/or secondary endpoints.

On-Site Monitoring: On-site monitoring will occur on an as-needed basis. Participating Institutions will be required to provide access to participants' complete medical record and source documents for verification during on-site visits. Upon request, Participating Institutions should provide access to regulatory documents, pharmacy records, local policies related to the conduct of research, and any other trial-related documentation maintained by the Participating site. If there are protocol compliance concerns, issues that impact subject safety or the integrity of the study, or trends identified based on areas of need, additional monitoring visits may occur.

Virtual Monitoring: On-site monitoring visits will be supplemented with virtual monitoring visits. The DF/HCC Lead Institution will request source documentation from Participating Institutions as needed to complete virtual monitoring activities. Participating Institutions will be asked to forward de-identified copies of participants' medical record and source documents to the DF/HCC Lead Institution to aid in the source documentation verification process.

In addition to monitoring performed by the Coordinating Center, the DF/HCC ODQ may monitor data for timeliness of submission, completeness, and adherence to protocol requirements. The DF/HCC Lead Institution or designee and, if applicable, the ODQ Data Analysts will perform ongoing protocol data compliance monitoring with the support of the Participating Institution's Coordinators, the Principal Investigators, and the Protocol Chair.

5.2 Monitoring Reports

The DF/HCC Sponsor will review all monitoring reports for on-site and remote monitoring of Participating Institutions to ensure protocol compliance. The DF/HCC Sponsor may increase the monitoring activities at Participating Institutions that are unable to comply with the protocol, DF/HCC Sponsor requirements or federal and local regulations. Participating Institutions may also be subject to an audit as determined by the DF/HCC Sponsor.

5.3 Accrual Monitoring

Prior to extending a protocol to an external site, the DF/HCC Sponsor will establish accrual requirements for each participating institution. Accrual will be monitored for each participating institution by the DF/HCC Sponsor or designee. Sites that are not meeting accrual expectations may be subject to termination. Sites are expected to accrue at least 2 patients per year.

6. AUDITING: QUALITY ASSURANCE

Auditing is a method of Quality Assurance. Its main focus is to measure whether standards and procedures were followed. Auditing is the systematic and independent examination of all trial related activities and documents. Audits determine if evaluated activities were appropriately

conducted and whether data was generated, recorded and analyzed, and accurately reported per the protocol, Standard Operating Procedures (SOPs), and the Code of Federal Regulations (CFR).

6.1 Audit Plan: DF/HCC Sponsored Trials

All Participating Institutions are subject to audit by the DF/HCC Office of Data Quality (ODQ). Typically, approximately 3-4 participants would be audited at the site over a 2 day period. If violations which impact participant safety or the integrity of the study are found, more participant records may be audited.

6.2 Audit Notification

It is the Participating Institution's responsibility to notify the Coordinating Center of all scheduled audit dates (internal or external) and re-audit dates (if applicable), which involve this protocol. All institutions will forward a copy of final audit and/or re-audit reports and corrective action plans (if applicable) to the Coordinating Center, within 12 weeks after the audit date.

6.3 Audit Reports

The DF/HCC Sponsor will review all final audit reports and corrective action plans if applicable. The Coordinating Center must forward these reports to the DF/HCC ODQ per DF/HCC policy for review by the DF/HCC Audit Committee. Based upon the audit assessments the DF/HCC Audit Committee could accept or conditionally accept the audit rating and final report. Conditional approval could require the DF/HCC Sponsor to implement recommendations or require further follow-up. For unacceptable audits, the DF/HCC Audit Committee would forward the final audit report and corrective action plan to the DFCI IRB as applicable.

6.4 Participating Institution Performance

The DF/HCC Sponsor and the DFCI IRB are charged with considering the totality of an institution's performance in considering institutional participation in the protocol.

6.4.1 Corrective Actions

Participating Institutions that fail to meet the performance goals of accrual, submission of timely and accurate data, adherence to protocol requirements, and compliance with state and federal regulations, may be recommended for a six-month probation period. Such institutions must respond with a corrective action plan and must demonstrate during the probation period that deficiencies have been corrected, as evidenced by the improved performance measures. Participating Institutions that fail to demonstrate significant improvement will be considered by the DF/HCC Sponsor for revocation of participation. A DF/HCC Sponsor and/or the DFCI IRB may terminate a site's participation if it is determined that a site is not fulfilling its responsibilities as described above.