A Randomized Phase II Window-of-opportunity Trial of Ruxolitinib in Patients with High Risk and Premalignant Breast Conditions

Protocol Number	TBCRC 042
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- Protocol Revision Record -

Protocol Version	Version Date	Summary of Significant Changes
Original Protocol	11/29/2016	N/A
Amendment 1	02/17/2017	• Revised Study Drug Diary (Appendix A)
Amendment 2	05/14/2018	• Clarified that trial is double-blinded • Clarified that patients with microinvasive disease are eligible (Inclusion Criterion 4.1.1) • Details added regarding ordering study drug (Section 9.2)
Amendment 3	12/09/2019	• Addition of Inclusion Criterion (4.1.6): If patient undergoes germline genetic testing, results must be received prior to randomization. • Addition of Exclusion Criterion (4.2.1): Prior treatment with SERMs or AIs within past year. • Revision of Exclusion Criterion 4.2.3 (previously 4.2.2): Previous history of invasive breast cancer diagnosed within last 5 years is an exclusion. • *Toxicity Management* (Section 8.2): If participant has ≥ gr.3 toxicity considered related to study drug, treatment will be discontinued.
Amendment 4	01/25/2022	• Removed limit to enrollment of DCIS patients (approved by TBCRC Steering Committee December 2021); removed all references made to limiting enrollment of DCIS patients to 15 per arm (Inclusion Criterion 4.1.1, and Section 16.2). • Updated *Study Agent* (Section 3.1) to reflect FDA approval of ruxolitinib for treatment of aGvHD and cGvHD. • Removed *Criteria for Removal from Study* (previously Section 7.6) due to redundancy. • Revised *Anticipated Toxicities* (Section 8.1): Removed extraneous text; updated existing risks per current ruxolitinib Investigator's Brochure and added most common risks reported from GvHD clinical trials. • Added summary of significant changes to Protocol Revision Record.

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Table of Contents

SCHEMA **5**

1. STUDY DESIGN/SUMMARY **5**

2. OBJECTIVES **6**

2.1 PRIMARY OBJECTIVE 6
2.2 SECONDARY OBJECTIVES 6

3. BACKGROUND **7**

3.1 STUDY AGENT 7
3.2 STUDY DISEASE 7
3.3 RATIONALE 8

4. PARTICIPANT SELECTION **12**

4.1 INCLUSION CRITERIA 12
4.2 EXCLUSION CRITERIA 12
4.3 INCLUSION OF UNDERREPRESENTED POPULATIONS 13

5. REGISTRATION PROCEDURES **14**

5.1 GUIDELINES FOR LEAD INSTITUTION 14
5.2 GUIDELINES FOR OTHER TBCRC INSTITUTIONS 14
5.3 RANDOMIZATION 14

6. STUDY CALENDAR **15**

7. TREATMENT PLAN **16**

7.1 PRE-TREATMENT CRITERIA 16
7.2 AGENT ADMINISTRATION 16
7.3 DURATION OF THERAPY 16
7.4 UNBLINDING 16
7.5 DURATION OF FOLLOW-UP 17
7.6 DISCONTINUATION OF TREATMENT 17
7.7 WITHDRAWAL FROM STUDY 17

8. EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS **17**

8.1 ANTICIPATED TOXICITIES 17
8.2 TOXICITY MANAGEMENT 18
8.3 DOSE MODIFICATIONS/DELAYS 18

9. DRUG FORMULATION/STORAGE/SUPPLY **19**

9.1 RUXOLITINIB 19
9.2 ORDERING 19
9.3 COMPATIBILITY 19
9.4 DRUG ACCOUNTABILITY 19
9.5 DESTRUCTION AND RETURN 19

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10. CORRELATIVE/SPECIAL STUDIES 20

10.1 BLOOD SAMPLES 20
10.2 TISSUE SAMPLES 20

11. SPECIMEN BANKING 22

12. ADVERSE EVENT REPORTING REQUIREMENTS 23

12.1 GENERAL 23
12.2 DEFINITIONS 23
12.3 REPORTING PROCEDURES 24

13. DATA AND SAFETY MONITORING 26

13.1 DATA MANAGEMENT AND REPORTING 26
13.2 MEETINGS 26
13.3 MONITORING 26

14. REGULATORY CONSIDERATIONS 28

14.1 PROTOCOL REVIEW AND AMENDMENTS 28
14.2 INFORMED CONSENT 28
14.3 ETHICS AND GCP 28

15. MULTICENTER GUIDELINES 29

15.1 STUDY DOCUMENTATION 29
15.2 RECORDS RETENTION 29
15.3 PUBLICATION 29

16. STATISTICAL CONSIDERATIONS 30

16.1 PRIMARY ENDPOINT AND ANALYTIC PLAN 30
16.2 SAMPLE SIZE/JUSTIFICATION 30
16.3 SECONDARY ENDPOINTS AND ANALYTIC PLAN 30
16.4 REPORTING AND EXCLUSIONS 31

17. REFERENCES 32

APPENDIX A: STUDY DRUG DIARY 35

APPENDIX B: PROHIBITED MEDICATIONS 37

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SCHEMA



1. STUDY DESIGN/SUMMARY

This is a phase II, double-blind, placebo-controlled clinical trial. Our target population consists of both men and women (pre- and post-menopausal) with premalignant breast lesions scheduled for surgical excision. Participants will be randomized 1:1 to receive either ruxolitinib 20 mg twice daily or placebo for 15 days +/- 5 days after which they will proceed with standard of care surgical excision within 12 hours of the last dose. This dose has been shown to suppress Jak1 and Jak2 activity and is well tolerated by healthy volunteers[24,25,26] and by myelofibrosis patients[22,23]. Participants must have platelet levels > 200 x 10^9/L to enroll on this trial and a CBC will be checked at baseline and at completion of therapy. Blood will also be drawn at baseline and completion of therapy to look at CMP, CRP and prolactin, with collection of one additional tube for exploratory analysis. Tissue from the diagnostic biopsy and from surgical excision will be stained for biomarkers and assessed for changes (CC3 for apoptosis, pSTAT5, pSTAT3, TUNEL, Ki67, BclXL, Mcl1, Bcl2, and PrlR). Our sample size is 100 participants.

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2. OBJECTIVES

2.1 Primary Objective

To determine the difference in change in apoptosis between diagnosis and surgery as a function of ruxolitinib treatment versus placebo, among participants with high-risk or premalignant breast conditions

2.2 Secondary Objectives

2.2.1 To determine the difference in change in pSTAT5 levels between diagnosis and surgery as a function of ruxolitinib treatment versus placebo

2.2.2 To determine if pre- and post-treatment pSTAT5 levels are inversely correlated with baseline apoptosis

2.2.3 In patients treated with ruxoloitinib, to determine the difference in change in apoptosis between diagnosis and surgery as a function of lesion size (i.e., DCIS > 2 cm compared to others)

2.2.4 To perform an exploratory analysis of other biomarkers (TUNEL, Ki67, BclXL, Mcl1, Bcl2, PRLR, pSTAT3, and serum prolactin levels)

2.2.5 To determine if, at baseline and at surgery, differences in menstrual cycle are associated with differences in pSTAT5

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3. BACKGROUND

3.1 Study Agent

Ruxolitinib is a kinase inhibitor which selectively inhibits the Janus Associated Kinases (JAKs), JAK1 and JAK2. JAK1 and JAK2 mediate signaling of cytokine and growth factors responsible for hematopoiesis and immune function; JAK mediated signaling involves recruitment of STATs (signal transducers and activators of transcription) to cytokine receptors which lead to modulation of gene expression.

Pharmacokinetics and Pharmacodynamics are as follows[23,24]:

Absorption: Rapid
Distribution: V_d: 53-65 L
Protein binding: ~97%; primarily to albumin
Metabolism: Hepatic, primarily via CYP3A4; forms active metabolites responsible for 20% to 50% of activity
Half-life elimination: Ruxolitinib: 2.8 to 3 hours (hepatic impairment: 4 to 5 hours); Ruxolitinib + metabolites: ~6 hours
Time to peak: Within 1 to 2 hours
Excretion: Urine (74%, <1% as unchanged drug); feces (22%, <1% as unchanged drug)

Ruxolitinib was approved in 2011 by the FDA for treating myelofibrosis, based on two phase 3 clinical trials[20,21]. When given for several months, potential side effects include headache, dizziness, anemia, thrombocytopenia, or ecchymosis[20,21]; however, the likelihood of any serious complication in this 15 days +/- 5 only trial of healthy participants is very low evidenced by the studies of an anti-Jak inhibitor (ruxolitinib) in healthy volunteers[27-29]. Ruxolitinib was also approved for the treatment of polycythemia vera in December 2014, for the treatment of acute graft-versus-host disease in May 2019, and for the treatment of chronic graft-versus-host disease in September 2021. For a comprehensive list of potential side effects,refer to ruxolitinib's package insert and the current Investigator's Brochure.

In animal models it may prevent progression of premalignant breast lesions to invasive breast cancer (Section 3.3.2). The only other FDA approved JAK2 inhibitor is tofacitinib and it is approved for use in rheumatoid arthritis.

3.2 Study Disease

The overall goal of this proposal is to test a novel concept in breast cancer prevention. Breast cancer is the most common tumor in women in the Western world. In the U.S., more than 200,000 women will be diagnosed with breast cancer every year, and approximately 50,000 women will eventually die of the disease. While screening strategies have improved early detection and increased cured rates, the ultimate goal is to prevent the disease from ever occurring. Reducing breast cancer incidence can theoretically have a profound impact on saving lives and reducing the huge cost of treatment.

Preventive therapy must be inexpensive and very well tolerated. Currently, there are several models (i.e. Gail model, IBIS model) to calculate risk of breast cancer that help physicians decide if a woman is at high risk for breast cancer. For women who are at high risk for breast cancer (such as those with strong family history, known BRCA gene mutation, or personal history of premalignant breast lesion) medications also known as chemoprevention should be offered to reduce the risk of breast cancer. Current medications approved by the FDA to prevent breast cancer are the antiestrogens tamoxifen and raloxifene which can prevent half of ER+ breast cancer. However, because they can have significant side effects and require 5 years of continuous use to achieve preventive benefit, women

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who are yet cancer-free are often reluctant to take this type of drug. Furthermore, antiestrogens do not prevent ER-negative breast cancer. Therefore, it is urgent that we develop alternative prevention drugs that require only a short-term treatment, and are suitable for preventing both ER+ and ER- cancer. No prevention strategies have been developed for ER- or HER2+ subtypes, the most virulent forms of the disease.

Premalignant lesions are known to significantly increase risk of invasive breast cancer and include atypical ductal hyperplasia (ADH), atypical lobular hyperplasia (ALH), lobular carcinoma in situ (LCIS) and DCIS (ductal carcinoma in situ). What causes a small subset of these premalignant lesions to progress is not yet known, but understanding the mechanism could provide new molecular targets for prevention.

3.3 Rationale

3.3.1 Introduction

Invasive breast cancer evolves from early lesions of the breast (such as ADH, ALH, LCIS, or DCIS). However, most early lesions do not progress to invasive cancer. Studies in several human tissue types suggest that there are anticancer "barriers," namely apoptosis and senescence, which are erected in early lesions to prevent the progression to cancer. STAT5 may provide such a mechanism by suppressing apoptosis during breast cancer initiation. It belongs to the STAT family of seven transcriptional factors that are latent in the cytoplasm[1]. STATs are activated by phosphorylation on a tyrosine residue near the C-terminus, usually by a member of the JAK (Janus kinase) family, which is in turn activated by a ligand-bound receptor (such as cytokine receptors and the prolactin receptor). STATs may also be phosphorylated by other tyrosine kinases through growth factor signaling, as well as by Src. After phosphorylation, STATs form homodimers as well as heterodimers through reciprocal phosphotyrosine-SH2 domain interactions, translocate to the nucleus, and activate their targets, which include Bcl2, BclXL, and Mcl1. STAT5 is the principal STAT expressed in the mammary gland at all stages of mammary development except during involution[2,3,4]. The activated and phosphorylated STAT5, pSTAT5, is detected in a subset of mammary epithelial cells, varying in percentage during the menstrual cycle and reaching nearly 100% during late pregnancy and lactation[4,5,6]. Activated STAT5 regulates cell survival, proliferation, and differentiation into alveoli, as we[7] and others[1,8,9] have reported. Of note, there are two closely related STAT5 proteins: STAT5a and STAT5b, but their phosphorylated products cannot be easily distinguished by the commonly used commercial antibodies.

STAT5 may be involved in human breast cancer. While not mutated, pSTAT5 can be detected in many human tumors[10-12], including between 20 to 70% of human breast cancers[6,13,14] pSTAT5 is found primarily in ER+ tumors, but also in a subset of Her2+ tumors as well as tumors lacking ER, PR, and HER2 (triple-negative)[6,13,14]. Jak2 has also been implicated in human breast cancer. In addition, serum prolactin has been reported to vary among women, and is associated with increased risk of breast cancer[15].

3.3.2 Studies

To address these questions, the laboratory of Dr. Li at Baylor College of Medicine has looked at several murine models to determine if STAT5 is involved in the progression of premalignant breast lesions to invasive breast cancer.

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STAT5 lowers the rate of apoptosis:
In a MMTV-tva transgenic model of breast cancer, dual targeted introduction of ErbB2 and STAT5a into mammary epithelial cells produced a lower apoptosis rate than ErbB2 alone (p=0.005). Furthermore, co-infected cells evolved into tumors more rapidly than cells infected only by ErbB2 (Fig. 1). These data establish that STAT5 activation lowers the apoptosis barrier in oncogene-activated mammary cells and promotes their progression to cancer. This was confirmed in a second transgenic WAP[1]-tva model where STAT5 transcriptionally activates the WAP promotor, more pSTAT5+ cells were detected in the early lesions from WAP+ mice (p<0.01).

STAT5 and pregnancy:
STAT5 is broadly activated in the mammary epithelium during late pregnancy and lactation[8,9]. The Li group found that compared to infected age-matched virgin controls, pSTAT5+ cells were abundant in the early lesions at pregnancy day 7.5, at lactation day 12, and even at involution day 10, at which time point pSTAT5 was absent in the surrounding normal involuting mammary cells. Li also found that by involution day 10, early lesions from parous mice contained fewer apoptotic cells and were larger than the early lesions from the age-matched virgin controls. Accordingly, these parous mice developed tumors faster. Therefore, STAT5 activation is correlated with the increased cell survival and tumorigenesis following a pregnancy.

The Li group also evaluated whether STAT5a ablation eliminates the increased risk of breast cancer in parous mice. ErbB2-induced early lesions in *STAT5a*$^{-/-}$ mice exhibited significantly lower pSTAT5 levels than did lesions in *STAT5a*$^{+/+}$ mice, confirming the predominance of STAT5a in the mammary gland. These STAT5a-/- early lesions could no longer be stimulated by parity: when *STAT5a*$^{-/-}$ mice were bred to MMTV-*tva* mice, infected with *caErbB2*, the resultant early lesions exhibited a level of apoptosis both comparable to that of early lesions in the age-matched virgin control mice and significantly higher than that of early lesions of *STAT5a*$^{+/+}$ mice . The number and size of early lesions were also similar in parous vs. virgin *STAT5a*$^{-/-}$ mice. Importantly, both tumor incidence and tumor latency were similar in parous vs. virgin *STAT5a*$^{-/-}$ mice.

Together, these data demonstrate that STAT5 activation is responsible for the reduced apoptosis anticancer barrier in the genesis of breast cancer, and that *STAT5a* is necessary for pregnancy-mediated promotion of preneoplastic cell survival, lesion progression, and tumorigenesis. Of note, these mouse model data are also consistent with the clinical observation that pregnancy leads to a transient increase in breast cancer risk, that pregnancy in older women leads to an overall increase in breast cancer risk, and that breast cancer progresses faster when associated with pregnancy[19].

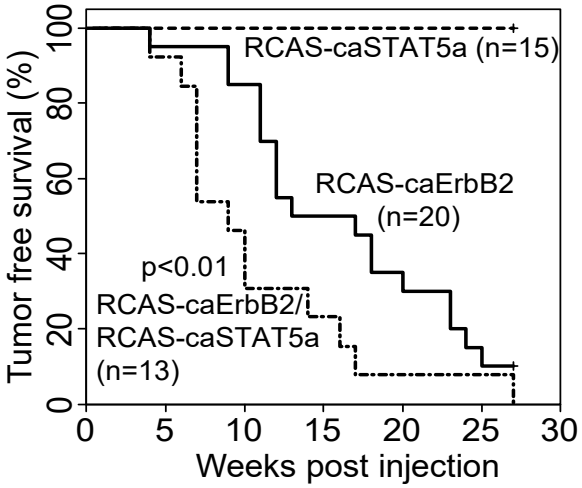


Figure 1. caSTAT5 activation increases tumorigenesis of ErbB2-activated mammary epithelial cells. Kaplan-Meier tumor-free survival curves of mice injected with the indicated viruses.

STAT5 inhibition prevents the progression of

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premalignant breast lesions

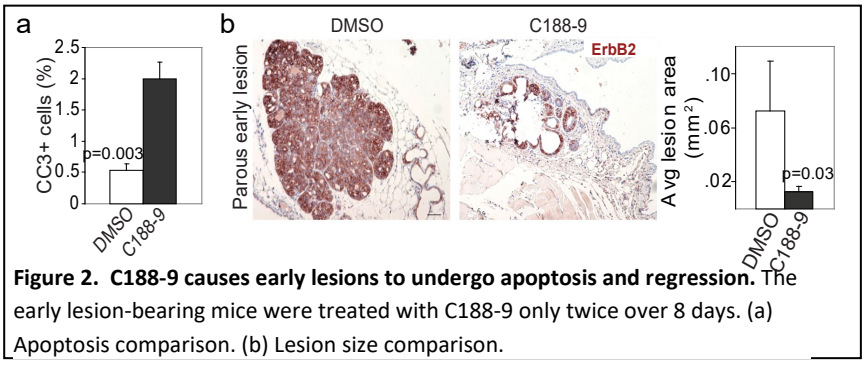


Figure 2. C188-9 causes early lesions to undergo apoptosis and regression. The early lesion-bearing mice were treated with C188-9 only twice over 8 days. (a) Apoptosis comparison. (b) Lesion size comparison.

STAT blockade lowers the risk of breast cancer in parous mice. To test this possibility in a preclinical setting, we tested whether transient blockade of STAT5 can induce apoptosis in preexisting early lesions and lower the risk of breast cancer in these parous mice. Dr. David Tweardy at Baylor had developed C188-9[46,47] which inhibits ligand-induced phosphorylation of all STATs that had been tested. In collaboration with him, we found that treatment of parous early lesions with this drug led to dramatic induction of apoptosis and regression of early lesions (Fig. 2). Importantly, treatment of mice bearing early lesions for only 4 weeks eliminated the increased breast cancer risk caused by pregnancy. These experiments demonstrate that even a transient blockade of STAT5 can lower breast cancer risk in parous mice. Of note, the drug-treated mice eventually developed mammary tumors after a longer delay, but a significant delay is what matters in cancer prevention.

The Li Lab then tested the feasibility of blocking STAT5 signaling for preventing human breast cancer with ruxolitinib which is an FDA approved drug for the treatment of myelofibrosis. Early lesion-bearing parous mice were treated with ruxolitinib for two weeks and this treatment resulted in suppressed pSTAT5, induced apoptosis, and caused regression (Fig. 3). These preclinical data suggest that short-term ruxolitinib may also reduce the load of precancerous cells in humans and may prevent human breast cancer.

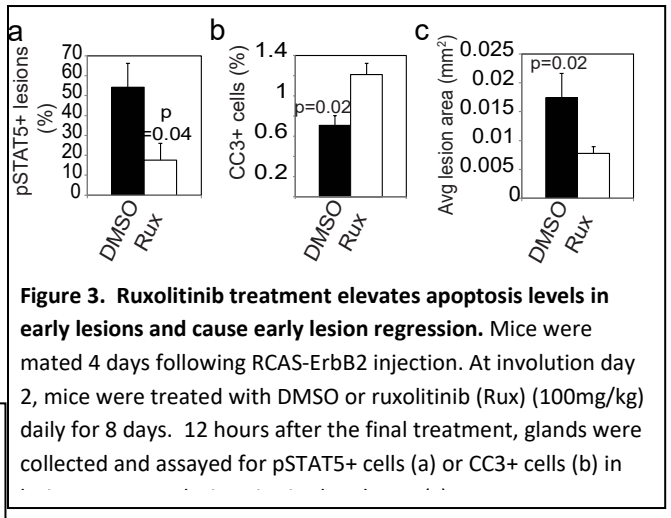


Figure 3. Ruxolitinib treatment elevates apoptosis levels in early lesions and cause early lesion regression. Mice were mated 4 days following RCAS-ErbB2 injection. At involution day 2, mice were treated with DMSO or ruxolitinib (Rux) (100mg/kg) daily for 8 days. 12 hours after the final treatment, glands were collected and assayed for pSTAT5+ cells (a) or CC3+ cells (b) in

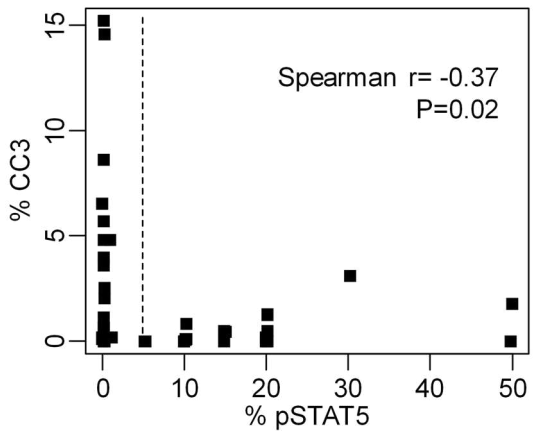


Figure 4. pSTAT5 is inversely correlated with apoptosis in human DCIS.

Finally, the Li Lab wanted to see if pSTAT5 in human DCIS is correlated with lower apoptosis by surveying pSTAT5 and apoptosis in 43 human DCIS cases. As shown in Fig. 4, 18 of the 43 cases (42%) were scored positive for pSTAT5 (cut-off = 5%), and these have lower apoptosis levels than the pSTAT5-negative subset. Of note, pSTAT5+ positivity was detected in both ER+ cases (9 out of 20) and ER- cases (9 out of 23). In addition, using a different cohort of DCIS that have parity information, we have found that pSTAT5 was significantly higher in parous DCIS cases than in nulliparous cases, further corroborating our preclinical work.

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3.3.3 Current Study Rationale

We have reported that the apoptosis anticancer barrier is especially important in suppressing progression from early lesions to breast cancer in mouse models, and that overcoming such a barrier is a critical step in the progression from early lesions to cancer. Based on our extensive mouse model data above, we suggest that STAT5 is "hijacked" in some early lesions to weaken the apoptosis barrier and to promote progression to malignancy. Importantly, in our preliminary studies, we found that transient inhibition of this STAT5-regulated survival pathway can devitalize early lesions and lower the risk of breast cancer. While STAT5 has key roles in embryonic development, we have found that in adult mice short-term suppression of STAT5 has little toxicity, suggesting that an anti-STAT5 prevention strategy would be safe.

We hypothesize that the JAK2-STAT5 pathway in human premalignant lesions promotes the progression of these lesions to malignancy by lowering the apoptosis anticancer barrier; therefore, inhibition of this pro-survival pathway could reduce the load of premalignant lesions in the breast and thus lower breast cancer risk. We propose to conduct a randomized phase II window-of-opportunity trial to test whether short-term ruxolitinib treatment (which inhibits the JAK2-STAT5 pathway) of participants with premalignant lesions will result in increased apoptosis. This clinical trial is designed as a biomarker trial to determine if ruxolitinib treatment for approximately 2 weeks induces apoptosis as it does in mouse models in our preliminary studies. If the predicted data are generated, the result of this trial will provide strong evidence for a future large-scale prevention trial to test the efficacy of short-term or intermittent ruxolitinib for preventing breast cancer in women with a premalignant lesion and in healthy women at increased risk of breast cancer.

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4. PARTICIPANT SELECTION

4.1 Inclusion Criteria

Participants must meet the following criteria to be eligible to participate in the study:

4.1.1 Have a breast biopsy showing ADH (atypical ductal hyperplasia), ALH (atypical lobular hyperplasia), LCIS (lobular carcinoma in situ), or DCIS (ductal carcinoma in situ) requiring surgical excision. Microinvasive disease is allowed.

NOTE: Tissue from the diagnostic biopsy must be accessible/available for research correlates (i.e., a tissue block or 10 unstained slides). Due to the nature of the study, fewer slides may be accepted with prior permission from the Protocol Chair if there is insufficient tissue.

4.1.2 Women and men age 18 and older.

4.1.3 Adequate hematologic and organ function, defined as follows:
- Absolute neutrophil count ≥ 1500/mm^3
- Hemoglobin ≥ 9.0 g/dL
- Platelets > 200 x 10^9/L
- Total bilirubin ≤ 1.5 x institutional upper limit of normal (ULN)
- AST and ALT ≤ 2.5 x institutional ULN
- Alkaline phosphatase ≤ 5 x institutional ULN
- Creatinine clearance > 50 mL/min as calculated by the Cockroft-Gault method

4.1.4 Willing to not use concomitant strong CYP3A4 inhibitors as this could interfere with the metabolism of ruxolitinib (i.e., azole antifungals, clarithromycin, conivaptan, grapefruit juice, nefazodone, protease inhibitors, telithromycin). See Appendix B for a complete list.

4.1.5 Women of childbearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

4.1.6 If the patient undergoes germline genetic testing, the results must be received prior to randomization, as the results may affect the surgical approach and, in turn, the date of surgical excision.

4.1.7 Patient understands the study regimen, its requirements, risks, and discomforts, and is able and willing to sign a written informed consent document.

4.2 Exclusion Criteria

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

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4.2.1 Treatment with selective estrogen receptor modulators (SERMs) or aromatase inhibitors for breast cancer prevention within 1 year prior to starting study treatment

4.2.2 Treatment with any other investigational agents within 30 days of starting study treatment

4.2.3 Current diagnosis of invasive breast cancer (current microinvasive disease is allowed), or previous history of invasive breast cancer diagnosed within the last 5 years.

NOTE: If previous history of ER+ invasive breast cancer diagnosed > 5 years ago, patient must be off endocrine therapy for at least 1 year prior to starting study treatment.

4.2.4 Uncontrolled intercurrent illness including, but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, end stage renal disease (ESRD), or psychiatric illness/social situations that would limit compliance with study requirements.

4.2.5 Women who are pregnant or nursing

4.2.6 HIV-positive participants on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with ruxolitinib.

4.2.7 Prior or current treatment with a JAK inhibitor for any indication

4.2.8 Known active Hepatitis B or C

4.3 Inclusion of Underrepresented Populations

Individuals of all races and ethnic groups are eligible for this trial. There is no bias towards age or race in the clinical trial outlined. This trial is open to the accrual of men and women.

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5. REGISTRATION PROCEDURES

5.1 Guidelines for Lead Institution

All consented patients will be registered in the online collaborative research environment (OnCore), a Clinical Research Management System available at BCM Dan L. Duncan Cancer Center. Prior to enrollment in the study, the eligibility data must be reviewed and verified by the study coordinator and a Breast Center physician, in accordance with Lester & Sue Smith Breast Center standard operating procedures. After the eligibility status is verified, the participant will be assigned a unique study sequence number and be randomized to either the ruxolitinib or placebo arm. Because the study is double-blinded, the randomization assignment will only be viewable by the Baylor Biostatistics Core and Investigational Pharmacy staff.

5.2 Guidelines for Other TBCRC Institutions

Participants MUST be registered with the Lead Institution prior to the start of protocol treatment. Following registration, participants should begin protocol treatment within 28 days. Issues that would cause treatment delays should be discussed with the Principal Investigator.

All consented patients will be registered in OnCore. Once the patient has completed all required screening procedures and is considered eligible, the responsible study coordinator will fax or email all supporting source documents (including lab results, progress notes, surgical pathology report, etc.) to the Coordinating Center Study Liaison, who will register the participant in OnCore and confirm eligibility. After the eligibility status is verified, the participant will be assigned a unique subject identification number by the Study Liaison and will be randomized to either the ruxolitinib or placebo arm. Because the study is double-blinded, the randomization assignment will only by viewable by the Baylor Biostatistics Core and the designated Investigational Pharmacy staff at the site enrolling the participant. Any requests for eligibility exceptions and/or deviations must be approved by the lead institution prior to execution.

5.3 Randomization

Participants will be randomized in a 1:1 ratio to receive either ruxolitinib or placebo. Randomization will be double-blinded, use permuted block method and be stratified by type of lesion (non-DCIS vs. DCIS). Randomization will be performed centrally at the Lester & Sue Smith Breast Center, Baylor College of Medicine. Upon randomization, the site's designated unblinded pharmacy staff will be notified via email of subject treatment assignment. Randomization information will be stored on a secure, access-restricted server at Baylor College of Medicine.

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6. STUDY CALENDAR

Procedure	Pre-Study[1]	On Study			Off Study
		Day 1	Pre-Surgery (-3 days)	Surgery[6]	(Post-Op Visit)
History (including menopausal status)	X				
Physical Exam (including Ht, Wt, VS)	X				X
CBC/differential, CMP, CRP, Prolactin[2]	X		X		
Pregnancy Test (urine or serum)[3]	X				
Ruxolitinib or Placebo[4]		X X			
Compliance Assessment					X
Research blood draw[9]	X		X		
Archival tissue[5,6]	X			X	
Concurrent Medications[7]	X	X X			
Adverse Event Evaluation[8]		X X			

1. Pre-Study assessments required within 28 days prior to starting ruxolitinib/placebo, unless otherwise noted.
2. Blood samples for CBC (complete blood count, including platelets), CMP (complete metabolic panel, including sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, total bilirubin, calcium, total protein, albumin, AST, ALT, and alkaline phosphatase), CRP (C-reactive protein) and prolactin to be analyzed locally. NOTE: Blood samples prior to the surgical procedure should occur after at least 10 days of ruxolitinib/placebo and no sooner than 3 days prior to surgery.
3. Only for women of childbearing potential; must be done within 7 days prior to starting treatment
4. Ruxolitinib/placebo will be given for 15 days (+/- 5 days) prior to surgery (last dose preferred within 12 hours prior to surgery).
5. A representative paraffin-embedded tissue block or ~10 unstained slides from both the diagnostic breast biopsy and breast surgical excision are required. This should be submitted to the Coordinating Center within 30 days after surgery. NOTE: On an individual basis, fewer slides will be accepted with approval from the Protocol Chair due to insufficient tissue.
6. Surgical excision is preferred on Day 15 (+/- 5 days) within 12 hours after last dose of ruxolitinib/placebo; however, if surgery occurs within 48 hours of last dose, it will not be considered a deviation. Tissue will still be collected for the study in the event that surgery is delayed beyond 48 hours after last dose.
7. Concurrent medication collection should continue until the standard post-operative visit; generally within 2-4 weeks post-operatively. If visit is delayed beyond 4 weeks, medications only need to be collected until 30 days following the last dose of ruxolitinib/placebo.
8. Adverse events will be collected until 30 days following the last dose of ruxolitinib/placebo. Participants with ongoing, study-drug related events should be followed until resolution of the event or until the event is deemed irreversible (time intervals for follow-up will be at the discretion of the local PI).
9. Collect one (1) 10-mL EDTA tube for each research blood draw.

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7. TREATMENT PLAN

7.1 Pre-treatment Criteria

7.1.1 Must meet the eligibility criteria within 28 days prior to starting treatment.

7.1.2 Women of childbearing potential must have a negative pregnancy test within 7 days prior to starting treatment.

7.1.3 Blood draw is required at baseline prior to starting treatment.

7.2 Agent Administration

Treatment will be administered on an outpatient basis. Expected toxicities and potential risks for ruxolitinib are described in Section 8 (Expected Toxicities). Treatment will start on Day 1. All participants will be randomized to either ruxolitinib 20 mg orally, twice daily (i.e., PO BID; given as four [4] 5 mg tablets) or matched placebo for 15 days (+/- 5 days). If a dose is missed, it can be taken if it is within 2 hours after the regularly scheduled time; otherwise it should be skipped. No additional dose should be taken if a participant vomits—return to the usual dosing schedule and do not administer an additional dose.

To document drug compliance, participants will be instructed to bring back all dispensed bottles of study treatment to the post-op visit, and any remaining pills will be counted by a member of study staff. A Study Drug Diary (Appendix A) may be used, but is not required.

7.3 Duration of Therapy

Duration of therapy will be for 15 days (+/- 5 days) and start at least 10 days prior to planned surgical excision of premalignant breast lesion, with dosing not to exceed 20 days. The last dose of ruxolitinib/placebo is preferred within 12 hours prior to surgical excision, however the last dose within 48 hours prior to surgical excision will not be considered a deviation.

Treatment may continue for 15 days (+/- 5 days) or until one of the following criteria applies:

- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Participant demonstrates an inability or unwillingness to comply with the oral medication regimen and/or documentation requirements
- Participant decides to withdraw from the study, or
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the opinion of the treating investigator.

7.4 Unblinding

Participants may be unblinded in the event of an SAE or other significant medical event, where knowledge of the participant's treatment assignment would influence management. To unblind a participant, the site Principal Investigator or treating physician should contact the Protocol Chair or her designee. Requests for unblinding must be made in writing via email, and should include a description of the event that necessitates unblinding, along with an explanation of how unblinding would influence the care of the participant. Upon approval, the Baylor Biostatistics Core will unblind the participant. The pharmacy staff at each site will also have access to the participant's treatment assignment, and may unblind the participant in the event the Baylor Biostatistics Core is not available.

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However, pharmacy staff should not unblind participants without prior written approval from the Protocol Chair.

7.5 Duration of Follow-Up

Participants will be followed for 24-48 days (from start of treatment until post-surgical follow-up in clinic, which is typically 2-4 weeks post-op). Participants removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

7.6 Discontinuation of Treatment

The reasons for discontinuation or protocol treatment include:

- Non-compliance with the study protocol. The Protocol Chair will determine when non-compliance should lead to removal from study.
  Note: These participants will still be included in the overall evaluation of response (intent-to-treat analysis).
- Unacceptable major toxicity.
  Note: These participants will still be included in the overall evaluation of response (intent-to-treat analysis).
- Intercurrent illness or condition that would, in the judgment of the treating investigator, affect assessment of clinical status to a significant degree or require discontinuation of study treatment.
- At participant's own request.
  Note: The reason for discontinuation must be documented. These participants will be included in the overall evaluation of response (intent-to-treat analysis) if any study treatment was administered prior to withdrawal.
- Study is terminated for any reason (e.g., new information shows that the participant's welfare would be at risk if study treatment continued).
- General or specific changes in the participant's condition which render the participant unacceptable for further treatment in the opinion of the treating investigator.

7.7 Withdrawal from Study

The reasons for withdrawal from the study include:

- Participant withdraws consent.
- Participant is lost to follow-up.
- Study is terminated for any reason.

8. EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

8.1 Anticipated Toxicities

Ruxolitinib is FDA-approved for the treatment of myelofibrosis and polycythemia vera[20,21], as well as the treatment of graft-versus-host disease (both acute and chronic).

The most common side effects in clinical studies of ruxolitinib for the treatment of myelofibrosis and polycythemia vera include anemia, thrombocytopenia, urinary tract infection, weight gain, dizziness, headache, and ecchymosis.

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The most common side effects in clinical studies of ruxolitinib for the treatment of both acute and chronic graft-versus-host disease include anemia, thrombocytopenia, neutropenia, cytomegalovirus (CMV) infection, hypertension, and nausea.

For a comprehensive list of potential side effects, refer to ruxolitinib's package insert and the current Investigator's Brochure.

8.2 Toxicity Management

If a participant experiences a grade 3 or higher toxicity that is considered related to the study treatment by the treating physician and the Protocol Chair, treatment will be discontinued and the participant will be withdrawn from the study. Management of grade 1 and grade 2 treatment-related toxicities will be at the discretion of the participant's treating physician.

8.3 Dose Modifications/Delays

There will be no dose modifications or delays in treatment for this study.

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9. DRUG FORMULATION/STORAGE/SUPPLY

9.1 Ruxolitinib

Ruxolitinib is an investigational agent and will be supplied free-of-charge by Incyte in 5 mg tablets. Ruxolitinib should be stored at room temperature, in accordance with manufacturer guidelines listed in the investigator's brochure.

NOTE: Incyte will also provide identical placebo tablets that should be handled in the same way as ruxolitinib. All references to the study drug/investigational product in this section refer to handling of both ruxolitinib and placebo.

9.2 Ordering

The initial request for investigational product will be generated by the Coordinating Center. Subsequent shipment requests for investigational product will be generated by designated, unblinded pharmacy staff at each site and submitted via email to Incyte's vendor (Catalent). Investigational product will be shipped directly to each site. The Coordinating Center will provide each site with a shipment request form once the site is approved to begin enrolling subjects. The completed form should be e-mailed to the contacts listed at the top of the form.

9.3 Compatibility

Ruxolitinib may be taken either with food or without food. Concomitant use of CYP3A4 inhibitors must be avoided, as this could interfere with the metabolism of ruxolitinib (i.e., azole antifungals, clarithromycin, grapefruit juice, protease inhibitors). Refer to Appendix B for a complete list.

9.4 Drug Accountability

The investigational pharmacy at each institution must maintain a careful record of the inventory and disposition of the agent. This record must document the number of tablets dispensed, as well as the number returned.

9.5 Destruction and Return

At the end of the study, unused supplies of ruxolitinib/placebo should be destroyed and documented according to institutional policies.

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10. CORRELATIVE/SPECIAL STUDIES

10.1 Blood Samples

In order to address the secondary objectives in Section 2.2, prolactin and CRP levels will be collected and analyzed locally at the Pre-Study and Pre-Surgery time points.

In addition to the above, one 10-mL EDTA tube will be collected at both time points. This additional blood will be used for exploratory analysis, as described in Sections 10.2.3 and 10.2.4. Blood samples should be processed and shipped as described in the laboratory manual. All samples should be shipped to the following location:

Breast Center Tissue Bank (Attn: Bryant McCue)
One Baylor Plaza, N1110.08
Houston, TX 77030

10.2 Tissue Samples

In order to address the primary and secondary objectives in Sections 2.1 and 2.2, archival tissue will be requested from participant's baseline breast biopsy as well as from the surgical excision. This tissue is collected as standard of care and does not require any extra procedures for the participant.

For each time point, sites should send the following:
- Representative paraffin block (preferred) ***OR***
- 10 unstained slides

Archival tissue from the diagnostic biopsy and from surgical excision should be submitted as described in the laboratory manual to the following address, preferably within 30 days after surgical excision:

Breast Center Tissue Bank (Attn: Bryant McCue)
One Baylor Plaza, N1110.08
Houston, TX 77030

If there is no residual ADH, ALH, DCIS or LCIS at the time of surgical excision, archival tissue does not need to be submitted for this time point.

Markers described below will be assessed at the end of the study, when all tissues and bloods are available and can be run as a single batch.

10.2.1 Induction of apoptosis

Apoptosis or programmed cell death will be assessed by direct counting of positively staining cells for cleaved caspase 3 (CC3). TUNEL is another assay to detect apoptotic cells. Increase in apoptosis is expected to be associated with ruxolitinib use.

STAT5 in breast early lesions blocks the apoptosis barrier and promotes progression to cancer. Suppression of STAT5 signaling restores apoptosis to premalignant mammary cells. It is expected that with the use of ruxolitinib STAT5 signaling will be decreased which would restore apoptosis. Both of these markers were looked at in the preliminary work in mouse models described in Section 3.3.2 and we expect to see similar results in humans.

10.2.2 Proliferation markers

Proliferation will be measured by Ki-67. It is anticipated that treatment will decrease Ki-67 staining.

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10.2.3 Other markers

Exploratory analysis of other markers in this pathway will also be performed including BclXL, Mcl1, Bcl2, PRLR.

10.2.4 Genetics research

Although there are currently no plans for genetic studies on the collected samples, there is a possibility that these samples may be used for genetic studies at a later date. Whether or not these samples are used for genetics research may depend on available technologies, future discovery of predictive markers, etc.

Participants will be given information as part of the informed consent process that samples may be used for research tests that will include genetic studies and testing. The intent is not to give participants (or their medical providers) the results of any testing done for research purposes; however, incidental germline (heritable) mutations may be identified of which a participant may or may not already be aware. In the case that an incidental genetic finding is identified, the Protocol Chair of this project will be notified. The possible decisions for handling incidental findings may include notification of the participant (and provider); recommendation for genetic counseling, which may or may not include genetic testing (e.g., if the finding was not done in a CLIA-certified laboratory); or, neither. In general, a member of the participant's treating team will be given the information to help with notification. In all cases, the current policy of the Baylor College of Medicine, and local/participating site IRBs, as applicable, will be followed and any additional approvals that may be required prior to participant notification will be secured in advance.

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11. SPECIMEN BANKING

Any leftover study blood and tissue samples may be stored for future research studies. Study participants will indicate in the consent form whether or not they agree to the future use of samples. All samples will be stored indefinitely in the Lester & Sue Smith Breast Center Tumor Bank, located at One Baylor Plaza, Houston TX 77030. Samples will not contain any traditional patient identifiers; however, samples will be coded such that specimens are traceable back to the participant. The link between the specimen code and the participant will be maintained in a secure area, accessible only to the Tissue Bank Coordinator. Samples will only be released for use in future studies after approval by the Principal Investigator and the TBCRC, 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 biospecimens for new endpoints must be submitted to the TBCRC Central Office for possible review by the TBCRC Correlative Science Review Committee.

In the event that a study participant withdraws consent, including for the future use of samples, any remaining tissue or blood will be discarded. No future data will be collected from the participant's samples; however, previously collected data will not be deleted.

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12. ADVERSE EVENT REPORTING REQUIREMENTS

12.1 General

Adverse event collection and reporting is a routine part of every clinical trial. Adverse events will be assessed according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. The CTCAE severity Grade 5 (death) will not be used in this study; rather, information about deaths will be collected as an outcome of the event. The occurrence of AEs should be sought by nondirective questioning of the subject at each visit during the study. Adverse events may also be detected when they are reported by the participant, directly observed or detected by physical examination, laboratory test, or other assessments. As far as possible, each AE should be evaluated to determine:

- The severity grade (CTCAE Grade 1 to 4).
- Attribution in relation to study treatment (e.g., definite, probable, possible, unlikely, unrelated)
- Start and end dates
- Action taken with respect to study drug (e.g., none, permanently discontinued, unknown, not applicable).
- Outcome (e.g., not recovered/not resolved, recovered/resolved, recovering/resolving, recovered/resolved with sequelae, fatal, unknown).

Adverse events experienced by participants will be collected and reported from initiation of study medication, throughout the study, and within 30 days of the last dose of study medication. Participants who experience an ongoing adverse event considered possibly, probably or definitely related to a study procedure and/or study medication beyond 30 days will continue to be contacted by a member of the study team until the event is resolved, stabilized, or determined to be irreversible by the participating investigator.

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. The investigator should notify the IRB and any other applicable regulatory agency of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

12.2 Definitions

12.2.1 Adverse Event (AE)

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

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

12.2.2 Serious adverse event (SAE)

A serious adverse event is an undesirable sign, symptom, or medical condition which:

- is fatal or life-threatening;
- requires or prolongs inpatient hospitalization;
- results in persistent or significant disability/incapacity;
- constitutes a congenital anomaly or birth defect; or

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- jeopardizes the participant and requires medical or surgical intervention to prevent one of the outcomes listed above;
- Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

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

12.2.3 Expectedness

- Expected: 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: 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

12.2.4 Attribution

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

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

12.3 Reporting Procedures

12.3.1 General

All adverse events will be captured on the appropriate study-specific case report forms (CRFs).

12.3.2 Serious Adverse Events

All serious adverse events, regardless of causality to study drug, will be reported to the Principal Investigator and/or the Study Coordinator at each institution, and also to the Coordinating Center and Incyte Corporation.

Participating sites should report all serious adverse events to the Coordinating Center, within 1 business day after the investigator becomes aware of the event. Events should be reported using a

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MedWatch form (FDA Form 3500A) as available on the FDA website. Follow-up information must also be reported within 1 business day of receipt of the information by the investigator.

The Coordinating Center will disseminate information regarding serious adverse events to the participating sites within 5 days of review of the information by the Protocol Chair (or her designee in the event of extended absence) only in the case that the event(s) is believed to be related (i.e., possibly, probably, or definitely) to the study medication. The Coordinating Center will be responsible for reporting of events to the FDA and supporters, as appropriate (outlined below).

12.3.3 Institutional Review Board

All adverse events and serious adverse events should be reported to the IRB in accordance with the local guidelines at each participating institution. If an adverse event requires modification of the informed consent, these modifications will be provided to the IRB with the report of the adverse event. If an adverse event requires modification to the study protocol, these modifications will be provided to the IRB as soon as is possible.

12.3.4 Food and Drug Administration (FDA)

In this trial, unexpected adverse events believed to be definitely, probably, or possibly related to the medications will be reported to the Food and Drug Administration. The Coordinating Center will be responsible for correspondence regarding adverse events with the FDA for all participating sites.

12.3.5 Incyte Corporation

The Coordinating Center will report all Serious Adverse Events (SAEs) to Incyte within 24 hours of learning of an event, regardless of suspected causality. This notification should be provided on a Serious Adverse Event (SAE) Form (provided by Incyte), a CIOMS-I or MedWatch Form FDA 3500A. Medical records will not be provided to Incyte unless specifically requested.

The SAE reporting period for each subject begins the day the informed consent is signed by the patient and ends 30 days after the subject has taken the last dose of study drug. SAEs occurring using Incyte study drug are reported in accordance with the effective protocol. SAEs occurring with any other commercial drug are reported to the manufacturer of that drug in accordance with regulations and protocol. Initial SAEs and/or subsequent follow-up reports should be reported via email to SafetyReporting@Incyte.com or via fax to 1-866-981-2057. SAE reports should be for a single subject and should be sent with a cover sheet and any additional attachments.

Copies of IND safety reports submitted to the FDA by the Coordinating Center will be shared with Incyte Corporation so that these reports can be evaluated and included in investigator brochure or Incyte IND safety submissions as required to ensure safety of other patients who are receiving the product from Incyte for sponsored trials.

An initial pregnancy report must be completed on the Report of Pregnancy or Drug Exposure Via Breastmilk form (provided by Incyte) and emailed to SafetyReporting@Incyte.com or faxed to 1-866-981-2057 within 24 hours of discovery of the pregnancy of a subject who has taken the Incyte study drug or the pregnancy of the partner of a subject who has taken the Incyte study drug. A follow-up report must be completed on the same form and emailed to SafetyReporting@Incyte.com or faxed to 1-866-981-2057 within 30 days after delivery, so that Incyte is provided with information regarding the outcome of the pregnancy. If the pregnancy results in any events which meet the serious critieria (i.e., miscarriage or termination), the SAE reporting process needs to be followed and the timelines associated with SAE reporting should be followed.

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13. DATA AND SAFETY MONITORING

13.1 Data Management and Reporting

The trial will be conducted by the Lester & Sue Smith Breast Center (LSSBC) at the Dan L. Duncan Comprehensive Cancer Center (DLDCCC) at Baylor College of Medicine (BCM), which has in place the infrastructure of personnel and services in biostatistics, regulatory affairs, study coordination, data management, quality assurance, and administrative support required for clinical investigations. At each site once participants are referred for entry on the protocol, the Principal Investigator and/or sub-investigators will be responsible for assessing their eligibility, for their clinical care and for monitoring their progress. The Coordinating Center will confirm that the participant is eligible prior to randomization. At each site, the research coordinator integrates the care of participants enrolled in the study and is responsible for the timely collection of all protocol-specified data. The regulatory affairs coordinator at the Coordinating Center prepares and submits required documents to the IRB and will distribute these to all sites once approved by the IRB. The Coordinating Center statistician ensures the statistical validity of data collected in clinical research studies.

13.2 Meetings

We will utilize the Data and Safety Monitoring Plan of the DLDCCC at BCM. During the clinical trial, clinical data on all participants enrolled on the study will be reviewed in our monthly breast cancer program Data and Safety Monitoring Meeting. The lead PI will review all adverse events as they occur to evaluate causality and severity. This will ensure that any unanticipated risks or changes to the risk/benefit ratio are identified and reported in a timely manner. All BCM research staff – including PIs, statisticians, study coordinators, and regulatory personnel – are in attendance at the breast cancer program Clinical Research and Data Review meeting.

Initially, the trial will be reviewed by the DLDCCC Data Review Committee (DRC) at BCM. After the first participating site is activated to begin enrolling, the trial will be reviewed by the DLDCCC Data Safety and Monitoring Board (DSMB) at BCM moving forward. Both committees will review accruals, serious adverse events, and cumulative toxicity data, and will make recommendations about safety and tolerability.

13.3 Monitoring

The DLDCCC conducts first participant audits on investigator-initiated trials, and regular audits as the trial progresses. The Data and Safety Monitoring Plan for this trial follows the requirements of the DLDCCC's NCI-approved data and safety monitoring plan.

The DRC/DSMB reviews all toxicities at a minimum of once per year. All reportable toxicities are included in the report, along with CTCAE grade and causality. Such review and evaluation will ensure the safety of all study participants and the integrity of study data collected. The confidentiality of study participants and study data will be maintained at all times. The review and monitoring activities of the DRC/DSMB include, but are not limited to, the following:

1. Review of the study protocol, including plans for data and safety monitoring.
2. Evaluation of study progress, including risk/benefit outcomes and consideration of new scientific or therapeutic developments that may impact the risks/benefits of the study.
3. Review of summaries of cumulative toxicity data in order to determine if the study should continue as designed, be revised, or be terminated.

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4. Review of major proposed study modifications prior to implementation (for example, terminating one arm of a study based on toxicity results, or increasing the sample size).

Data are submitted in a standardized format to the DRC/DSMB. The information includes (but is not limited to): protocol title, PI, expected number of patients to be treated, number of participants currently enrolled, interim analyses planned (y/n), date of interim analyses, data from interim analyses, toxicity, and the next monitoring date (as specified in the protocol). In most circumstances, annual submission of data will be adequate, although the DRC/DSMB may request more frequent submission. The DRC/DSMB and audit results are reported to the Duncan Cancer Center's Clinical Research Leadership.

As the Coordinating Center, Baylor College of Medicine will monitor all participating sites at least once and at regular intervals as needed.

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14. REGULATORY CONSIDERATIONS

14.1 Protocol Review and Amendments

Information regarding study conduct and progress will be reported to the Institutional Review Board (IRB) per the current institutional standards of each participating center.

Any changes to the protocol will be made in the form of an amendment and must be reviewed by Incyte and approved by the IRB of each institution prior to implementation.

The Protocol Chair (or her designee) is responsible for the coordination and development of all protocol amendments, and will disseminate this information to the participating centers.

14.2 Informed Consent

The investigator (or his/her designee) will explain to each participant the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each participant will be informed that participation in the study is voluntary, that s/he may withdraw from the study at any time, and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician(s) or institution. The informed consent will be given by means of a standard written statement, written in non-technical language, which will be IRB approved. The participant should read and consider the statement before signing and dating it, and will be given a copy of the document. No participant will be enrolled on the study or have study-specific procedures done before his/her informed consent has been obtained.

In accordance with the Health Information Portability and Accountability Act (HIPAA), the written informed consent document (or a separate document to be given in conjunction with the consent document) will include a participant authorization to release medical information to the study sponsor and supporting agencies and/or allow these bodies, a regulatory authority, or Institutional Review Board access to participants' medical information that includes all hospital records relevant to the study, including participants' medical history.

14.3 Ethics and GCP

This study will be carried out in compliance with the protocol and Good Clinical Practice, as described in:

1. ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996.
2. US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).
3. Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996).

The investigator agrees to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice that it conforms to.

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15. MULTICENTER GUIDELINES

15.1 Study Documentation

Each participating site is responsible for submitting copies of all relevant regulatory documentation to the Coordinating Center. The required documents include, but are not limited to the following: local IRB approvals (i.e., protocol, consent form, amendments, patient brochures and recruitment material, etc.), IRB membership rosters, summary of unanticipated problems or protocol deviations, and documentation of expertise of the investigators. The Coordinating Center will provide each participating site with a comprehensive list of the necessary documents. It is the responsibility of the participating sites to maintain copies of all documentation submitted to the Coordinating Center.

15.2 Records Retention

Following closure of the study, each participating center will maintain a copy of all site study records in a safe and secure location. The Coordinating Center will inform the investigator at each site at such time that the records may be destroyed.

15.3 Publication

It is understood that any manuscript or releases resulting from the collaborative research must be approved by the Protocol Chair and will be circulated to applicable participating sites/investigators prior to submission for publication or presentation.

Additionally, any publication of study data and results must conform to the publications policy as stated the Translational Breast Cancer Research Consortium's (TBCRC) "Policies and Procedures."

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16. STATISTICAL CONSIDERATIONS

16.1 Primary Endpoint and Analytic Plan

The primary endpoint is change in apotosis, as measured by CC3 staining, in surgical samples relative to baseline biopsy. Lesions of different types will be characterized separately, and the participant's most advanced lesion present at baseline will be used for the primary analysis. The difference in changes between groups will be evaluated using the Wilcoxon rank sum test.

16.2 Sample Size/Justification

We expect to have to enroll 100 participants in order to obtain 70 evaluable subjects. Based on pSTAT5 & CC3 data from 43 DCIS cases (Section 3.3.2, Fig. 4), we expect about 50% of cases to exhibit high pSTAT5 (≥ 5% positive cells) at baseline. In the ruxolitinib arm, we expect that 90% of cases with high pSTAT5 will convert to a low-pSTAT5 state and will exhibit apoptosis rates like cases with low pSTAT5 (< 5%), while apoptosis in cases with low pSTAT5 at baseline will not change. We have used empirical cumulative distributions from the observed data, and the hypothesized ruxolitinib-associated transitions, to generate simulated post-treatment data. Based on this, we expect to see a post-treatment median apoptosis rate of 0.35% in the placebo group compared to 1.67% in the ruxolitinib group. Under these conditions, the proposed sample size of 70 (35/group) evaluable patients will provide about 80% power (alpha = 7.5%, one-tailed) to detect such an difference in post-treatment apoptosis. The apoptosis data are highly skewed and this estimate is based on a simulation with 2000 replications of a two-sample Wilcoxon rank sum test. Assuming at least a modest correlation between baseline and post-treatment, using "change in apoptosis" rather than "post-treatment apoptosis" should reduce variability and thus increase power. We will stratify randomization by type of lesion (non-DCIS vs. DCIS). We expect that approximately 30% of surgical samples will be inevaluable because of no residual ADH, ALH, LCIS or DCIS at the time of surgical excision and thus have increased the sample size from 70 to 100.

16.3 Secondary Endpoints and Analytic Plan

The secondary objectives are mostly concerned with the effects of ruxolotinib treatment on biomarkers. The secondary endpoints include pre and posttreatment pSTAT5, TUNEL, Ki67, BclXL, Mcl1, Bcl2, PRLR, pSTAT3, and serum prolactin levels.

Tissue sections from pre- and post-treatment specimens will be stained by IHC for key biomarkers (pSTAT5, CC3, TUNEL, Ki67, BclXL, Mcl1, Bcl2, and PRLR). Changes in these markers will be compared between groups using the Wilcoxon rank sum test. Change within group will be tested using the Wilcoxon signed rank test.

Correlations between baseline CC3 and pSTAT5, and between changes in CC3 and pSTAT5 will be assessed using Spearman correlation coefficients. Correlations will be done separately for each treatment group, and exploratorily by lesion type.

Serum prolactin levels will be correlated with pSTAT5 levels using Spearman correlation. These correlations will be done separately at baseline and at end of treatment to explore a potential link between serum prolactin and pSTAT5 in breast lesions.
In the pre-clinical mice studies, there was a lack of efficacy of ruxolitinib in large DCIS lesions so we will look at DCIS 2-5 cm versus all other lesions (ADH, ALH, LCIS, DCIS ≤ 2 cm) using a Wilcoxon rank sum test to see if there is any difference in baseline or change in CC3 staining in the ruxolitinib treated group.

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We will compare pSTAT5 levels as a function of menstrual cycle, using the Kruskal-Wallis test.

Further, exploratory analysis of the primary outcome, change in apoptosis as measured by CC3 levels, will be undertaken to look for and adjust for possible effects due to duration of ruxolitinib treatment and/or delay in surgery and collection of the post-treatment sample. This will likely require exploration of appropriate transformations and application of parametric modeling.

16.4 Reporting and Exclusions

Evaluation of toxicity. All participants who received at least 1 dose of ruxolitinib/placebo will be evaluable for toxicity.

Evaluation of response. All participants who received at least 1 dose of ruxolitinib/placebo and have residual ADH, ALH, LCIS or DCIS at the time of surgery will be assessed for response to treatment.

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17. REFERENCES

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APPENDIX A: STUDY DRUG DIARY

Instructions:
You will use this diary to record each dose of study drug (ruxolitinib/placebo) that you take. You should also use this diary to record any side effects that you experience and medications that you take other than the study drug. Please be sure to bring this diary with you to your next clinic visit.

Study Drug – Ruxolitinib/Placebo:

- Ruxolitinib/placebo is taken twice a day for up to 20 days prior to your surgery.
- The study drug may be taken with or without food.
- Take the study drug at about the same times every day, about 12 hours apart.
- If you forget to take a dose and it is within 2 hours of when it should have been taken, you should take the dose. If it has been more than 2 hours, you should skip that dose and take the next dose at the next regular time.
- If you vomit after taking a dose of study drug, you should not make up the dose or take additional pills, you should take the next dose at the next regular time.
- If not collected from you on the day of your surgery, bring your pill bottles and this diary with you to the first clinic visit after your surgery.

If you have any questions, please ask a study team member or your doctor.

Subject Number: _____

Subject Initials: _____

Start Date: _____

STUDY STAFF USE ONLY

Staff member reviewing diary at end of cycle:

_____ _____

Staff initials Date reviewed

Number of pills to take at each dose: 4 pills

Other information/Comments:

_____

_____

_____

_____

_____

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Subject Number/ Initials: _____

Study Drug Diary					
Day	**Date**	**AM/ PM**	**Time**	**# of Pills Taken**	**Comments** (Side effects, complaints, other medications)
Ex	*7/1/2016*	*AM*	*8:00am*	*4*	*Headache (took Tylenol)*
1		AM			
		PM			
2		AM			
		PM			
3		AM			
		PM			
4		AM			
		PM			
5		AM			
		PM			
6		AM			
		PM			
7		AM			
		PM			
8		AM			
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APPENDIX B: PROHIBITED MEDICATIONS

Drug Class	Agent
Antibiotics	clarithromycin, telithromycin
Antifungals	ketoconazole, itraconazole, fluconazole, voriconazole, miconazole, posaconazole
Antidepressants	nefazodone
Antiretrovirals, Protease Inhibitors	atazanavir, darunavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, tipranavir, delavirdine, telaprevir, danoprevir, elvitegravir, boceprevir
Diuretics	conivaptan
Foods	grapefruit, grapefruit juice
Other	midazolam, alfentanil

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