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Phase II Short-term Adjuvant Therapy and Biomarker studies with targeted agents in Women with Estrogen Receptor Negative Breast Cancer

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SCHEMA

Phase II Short-term Adjuvant Therapy and Biomarker studies with targeted agents in Women with **Estrogen Receptor Negative Breast Cancer**

Study Population: Women history of ER negative breast cancer

↓

Medical history, (physical exam), blood

↓

Sign consent

↓

Baseline random periareolar breast fine needle aspiration (RPFNA), research blood

↓

Randomize, on study for 3 months (+/- 7 days)

↓

Month 3 periareolar breast fine needle aspiration, research blood

Endpoints: Primary: Changes in Ki-67
Secondary: Changes in cytology, proliferation, apoptosis in breast tissue (p-Y418-Src, p-Y-118-Paxillin, (and markers identified in Aims 1 and 3 of the grant), CC3, bcl-2, EGFR, P-EGFR, IGF pathway in serum)

Toxicity assessment

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1. OBJECTIVES

Overall objective is to evaluate the chemopreventive potential of dasatinib in a phase II short term adjuvant and biomarker study, by evaluating its biomarker modulatory efficacy in breast tissue and serum of women at increased risk for second primary breast cancer.

1.1. Primary Objective

To evaluate the ability of treatment with daily dasatinib to decrease proliferation of high risk breast epithelial cells on contralateral random periareolar fine needle aspiration (RPFNA) in patients with breast cancer that do not overexpress estrogen receptor (ER). We will evaluate pre- and post dasatinib treatment Ki-67 in samples obtained via FNA from breast tissue of women at high risk for second primary breast cancer. This specific aim tests the hypothesis that treatment with dasatinib will induce a decrease in proliferation.

1.2. Secondary Objectives:

1) To evaluate dasatinib induced modulation of cytology, other proliferation and apoptosis markers in high risk breast tissue (p-Y418-Src, p-Y-118-Paxillin, epidermal growth factor receptor (EGFR), phosphorylated EGFR (P-EGFR), b cell lymphoma 2 (bcl-2), cleaved caspase 3 (CC3,)) by immunohistochemistry (IHC) and by direct proteomic profiling methods (as well as novel markers identified in Aims 1 and 3 of the grant: IGF-1, IGFBP1 and 3).

2) To evaluate dasatinib induced modulation of biomarker in the serum including insulin like growth factor (IGF) pathway

3) To assess toxicity of dasatinib in this patient population

2. BACKGROUND AND RATIONALE

2.1 Breast Cancer Chemoprevention and Chemoprevention Agents

Tamoxifen is currently the only FDA approved agent for the prevention of breast cancer in high risk women (1).The National Adjuvant Breast and Bowel Project (NSABP) conducted a large-scale, double blind, phase III breast cancer prevention trial P1 (BCPT-P1) using tamoxifen versus placebo in 13,388 women at high risk for breast cancer (2). After a median follow-up of 54 months, a 49% reduction in the incidence of invasive breast cancer (p□0.00001) occurred among those receiving tamoxifen (1). Side effects seen with tamoxifen use included increased risk of thromboembolic events, uterine cancer, and menopausal symptoms. Furthermore, reduction seen in breast cancer incidence was only in ER positive tumors; tamoxifen did not reduce the incidence of ER negative breast cancer. ER negative breast cancers occur more in pre-menopausal women and are associated with aggressive behavior and poor prognostic features (3), (4). Unfortunately, currently there are no non-hormonal agents available and approved for the prevention of ER negative cancer. Furthermore, there is a need for less toxic agents. Several classes of commonly used drugs and novel agents, such as NSAIDs, retinoids, ornithine decarboxylase inhibitors, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins), and src inhibitor, are potential agents for investigation for breast cancer prevention

2.2 Dasatinib as a Chemopreventive Agent for Breast Cancer

Preclinical studies:

The steroid receptor co-activator (Src) pathway shares a common pathway with AMPK via mammalian target of rapamycin (mTOR). c-Src (Src) belongs to the Src family non-receptor tyrosine kinases. All Src family members share structural similarities: 1) a c-terminal tail containing a negative-regulatory Y residue (Y527); 2) Src homology domains (SH2 and SH3) and SH1 kinase domain containing an autophosphorylation site (Y416) for maximal kinase activity; and 3) a N-terminal domain (SH4) for myrisylation and membrane binding (5). EGFR and HER2, similar to other growth factor receptors, can bind to and activate Src (6). Src is involved in multiple tumorigenesis processes (7). Src overexpression (Src+) and activation was detected in > 80% of ductal carcinoma in situ (DCIS) lesions and correlated with HER2 expression (7), (8). In our preliminary data, Src+ and activation was detected in 10% (1/10) patients with breast hyperplasia, 50% (3/6) patients with high-grade atypia, 80% (4/5) patients with ER- high grade atypia. Src+ also correlated with HER2+, and Src phosphorylation on Y416 (p-Y-416, indicating activation) predicted ER negativity/Tamoxifen resistance (TamR). The reverse relationship between Src and ER is consistent with previous reports that Src promoted estrogen dependent ERalpha degradation (9). Src activation has also been observed in TamR breast cancer cells and Src inhibitor (AZD0530) suppressed the invasion of TamR cells (10). These findings highlight a key role for Src in receptor tyrosine kinase (RTK) signaling, and in ER- breast cancer development. Therefore, inhibiting src pathway may present an effective way to prevent breast cancer.

Dasatinib is a potent, broad spectrum ATP-competitive inhibitor of 5 critical oncogenic tyrosine kinase/kinase families: BCR-ABL, SRC, c-KIT, PDGF receptor β (PDGFRβ), and ephrin (EPH) receptor kinases, each of which has been linked to multiple forms of human malignancies. Drug discovery and nonclinical pharmacology studies showed that dasatinib (Sprycel (dasatinib) BMS-354825 B-MSIB, version #5, 2006) inhibit proliferation of cancer cell lines that express activated src and c-kit.

Clinical Experience with dasatinib:

Four single-arm multicenter studies were conducted to determine the efficacy and safety of dasatinib in patients with CML or Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) resistant to or intolerant of treatment with imatinib. Resistance to imatinib included failure to achieve a complete hematologic response (within 3–6 months) or major cytogenetic response (by month 12) or progression of disease after a previous cytogenetic or hematologic response. Imatinib intolerance included inability to tolerate 400 mg or more of imatinib per day or discontinuation of imatinib because of toxicity. The studies are ongoing. The results are based on a minimum of 6 months follow-up after the start of dasatinib therapy. Most patients had long disease histories with extensive prior treatment, including imatinib, cytotoxic chemotherapy, interferon, and stem cell transplant. The maximum imatinib dose had been 400–600 mg/day in about one-half of the patients and >600 mg/day in the other half (SPRYCEL® (dasatinib) Tablets Prescribing Information.Bristol-Myers Squibb Company P, NJ. June 2006). All patients were treated with dasatinib 70 mg BID on a continuous basis. The median durations of treatment was between 2.8 - 5.6 months SPRYCEL® (dasatinib) Tablets Prescribing Information. Bristol-Myers Squibb Company P, NJ. June 2006).

The primary efficacy endpoint in chronic phase CML was major cytogenetic response (MCyR), defined as elimination (complete cytogenetic response, CCyR) or substantial diminution (by at least 65%, partial cytogenetic response) of Ph+ hematopoietic cells. The primary endpoint in accelerated phase, myeloid blast phase, and lymphoid blast phase CML, and Ph+ ALL was major hematologic response (MaHR), defined as either a complete hematologic response or no evidence of leukemia as defined in Table 1.

Most cytogenetic responses occurred after 12 weeks of treatment, when the first cytogenetic analyses were performed. Hematologic and cytogenetic responses were stable during the 6-month follow-up of patients with chronic phase, accelerated phase, and myeloid blast phase CML. The median durations of major hematologic response were 3.7 months in lymphoid blast CML and 4.8 months in Ph+ ALL. There were no age- or gender-related response differences (SPRYCEL® (dasatinib) Tablets Prescribing Information. Bristol-Myers Squibb Company P, NJ. June 2006)

Table 1: Efficacy in Dasatinib Clinical Studies (All Treated Populations)[a]

	Chronic (n=186)	Accelerated (n=107)	Myeloid Blast (n=74)	Lymphoid Blast (n=42)	Ph+ ALL (n=36)
Hematologic Response Rate[b] (%)					
MaHR (95% CI)	n/a	**59 (49–68)**	**32 (22–44)**	**31 (18–47)**	**42 (26–59)**
CHR (95% CI)	**90 (85–94)**	33 (24–42)	24 (15–36)	26 (14-42)	31 (16–48)
NEL (95% CI)	n/a	26 (18–36)	8 (3–17)	5 (0.6–16)	11 (3.1–26)
Cytogenetic Response[c] (%)					
MCyR (95% CI)	**45 (37–52)**	31 (22–41)	30 (20–42)	50 (34–66)	58 (41–74)
CCyR (95% CI)	33 (26–40)	21 (14–30)	27 (17–39)	43 (28–59)	58 (41–74)

[a] Numbers in bold font are the results of primary endpoint.

[b] Hematologic response criteria (all responses confirmed after 4 weeks):
Major hematologic response: (MaHR) = complete hematologic response (CHR) + no evidence of leukemia (NEL).
CHR (chronic CML): WBC ≤ institutional ULN, platelets <450,000/mm^3, no blasts or promyelocytes in peripheral blood, <5% myelocytes plus metamyelocytes in peripheral blood, basophils in peripheral blood ≤ institutional ULN, and no extramedullary involvement.
CHR (advanced CML/Ph+ ALL): WBC ≤ institutional ULN, ANC ≥ 1000/mm^3, platelets ≥ 100,000/mm^3, no blasts or promyelocytes in peripheral blood, bone marrow blasts ≤ 5%, <5% myelocytes plus metamyelocytes in peripheral blood, basophils in peripheral blood ≤ institutional ULN, and no extramedullary involvement.
NEL: same criteria as for CHR but ANC ≥ 500/mm^3, <1000/mm^3 and/or platelets ≥ 20,000/mm^3 and ≤ 100,000/mm^3.

[c] Cytogenetic response criteria: complete (0% Ph+ metaphases) or partial (>0%–35%). MCyR (0%–35%) combines both complete and partial responses.

n/a = not applicable.

Safety of dasatinib in clinical studies

The below data reflect exposure to dasatinib in 911 patients with leukemia from 1 Phase I and 5 Phase II clinical studies. The median duration of therapy was 6 months (range 0–19 months).

The majority of dasatinib -treated patients experienced adverse drug reactions at some time. Drug was discontinued for adverse drug reactions in 6% of patients in chronic phase CML, 5% in accelerated phase CML, 11% in myeloid blast phase CML, and 6% in lymphoid blast phase CML or Ph+ ALL.

The most frequently reported serious adverse events (SAEs) included pyrexia (9%), pleural effusion (8%), febrile neutropenia (7%), gastrointestinal bleeding (6%), pneumonia (6%), thrombocytopenia (5%), dyspnea (4%), anemia (3%), cardiac failure (3%), and diarrhea (2%).

All treatment-emergent adverse events (excluding laboratory abnormalities), regardless of relationship to study drug, that were reported in at least 20% of the patients in dasatinib clinical studies are shown in Table 2.

Table 2: Adverse Events Reported ≥ 20% in Clinical Studies

	All Patients (n=911)		Chronic Phase (n=488)	Accelerated Phase (n=186)	Myeloid Blast Phase (n=132)	Lymphoid Blast Phase and Ph+ ALL (n=105)
	All Grades	Grades 3/4	Grades 3/4	Grades 3/4	Grades 3/4	Grades 3/4
Preferred Term	**Percent (%) of Patients**					
Fluid Retention	50	9	6	6	23	9
Superficial Edema	36	1	0	2	3	2
Pleural Effusion	22	5	3	3	14	8
Diarrhea	49	5	3	10	8	6
Headache	40	2	2	2	4	6
Hemorrhage	40	10	3	18	23	17
Musculoskeletal Pain	39	4	2	3	6	13
Pyrexia	39	5	1	5	13	9
Fatigue	39	3	2	4	4	8
Skin Rash[a]	35	1	1	1	1	4
Nausea	34	1	<1	0	5	2
Dyspnea	32	6	5	7	11	9
Cough	28	<1	<1	1	1	0
Infection (including bacterial, viral, fungal, non-specified)	34	7	4	8	15	13
Infection/Inflammation	26	1	1	1	5	1
Abdominal Pain	25	2	1	2	4	6
Pain	26	2	<1	1	5	4
Vomiting	22	1	1	2	2	2
Febrile Neutropenia	9	8	2	11	17	20

Myelosuppression was commonly reported in all patient populations. The frequency of Grade 3 or 4 neutropenia, thrombocytopenia, and anemia was higher in patients with advanced CML or Ph+ ALL than in chronic phase CML. Myelosuppression was reported in patients with normal baseline laboratory values as well as in patients with pre-existing laboratory abnormalities (Table 3).

In patients who experienced severe myelosuppression, recovery generally occurred following dose interruption and/or reduction; permanent discontinuation of treatment occurred in 1% of patients. Grade 3 or 4 elevations of transaminases or bilirubin and Grade 3 or 4 hypocalcemia and hypophosphatemia were reported in patients with all phases of CML but were reported with an increased frequency in patients with myeloid or lymphoid blast CML and Ph+ ALL. Elevations in transaminases or bilirubin were usually managed with dose reduction or interruption. Patients developing Grade 3 or 4 hypocalcemia during the course of dasatinib therapy often had recovery with oral calcium supplementation. (Table 3).

Table 3: CTC Grades 3/4 Laboratory Abnormalities in Clinical Studies

	Chronic Phase (n=488)	Accelerated Phase (n=186)	Myeloid Blast Phase (n=132)	Lymphoid Blast Phase and Ph+ ALL (n=105)
	Percent (%) of Patients			
Hematology Parameters				
Neutropenia	49	74	83	81
Thrombocytopenia	48	83	82	83
Anemia	18	70	70	51
Biochemistry Parameters				
Hypophosphatemia	11	13	23	21
Hypocalcemia	2	9	20	15
Elevated SGPT (ALT)	1	4	7	11
Elevated SGOT (AST)	1	2	5	8
Elevated Bilirubin	<1	1	5	8
Elevated Creatinine	0	2	1	1

CTC grades: neutropenia (Grade 3 ≥0.5–1.0 × 10^9/L, Grade 4 <0.5 × 10^9/L); thrombocytopenia (Grade 3 ≥10–50 × 10^9/L, Grade 4 <10 × 10^9/L); anemia (hemoglobin ≥65–80 g/L, Grade 4 <65 g/L); elevated creatinine (Grade 3 >3–6 × upper limit normal range (ULN), Grade 4 >6 × ULN); elevated bilirubin (Grade 3 >3–10 × ULN, Grade 4 >10 × ULN); elevated SGOT or SGPT (Grade 3 >5–20 × ULN, Grade 4 >20 × ULN); hypocalcemia (Grade 3 <7.0–6.0 mg/dL, Grade 4 <6.0 mg/dL); hypophosphatemia (Grade 3 <2.0–1.0 mg/dL, Grade 4 <1.0 mg/dL).

Myelosuppression

Treatment with dasatinib B is associated with severe (NCI CTC Grade 3 or 4) thrombocytopenia, neutropenia, and anemia. Their occurrence is more frequent in patients with advanced CML or Ph+ ALL than in chronic phase CML. Complete blood counts should be performed weekly for the first 2 months and then monthly thereafter, or as clinically indicated. Myelosuppression was generally reversible and usually managed by withholding dasatinib temporarily or dose reduction (SPRYCEL® (dasatinib) Tablets Prescribing Information. Bristol-Myers Squibb Company P, NJ. June 2006.).

In addition to causing thrombocytopenia in human subjects, dasatinib caused platelet dysfunction *in vitro*. Severe CNS hemorrhages, including fatalities, occurred in 1% of patients receiving dasatinib. Severe gastrointestinal hemorrhage occurred in 7% of patients and generally required treatment interruptions and transfusions. Other cases of severe hemorrhage occurred in 4% of patients. Most bleeding events were associated with severe thrombocytopenia.

Patients were excluded from participation in dasatinib clinical studies if they took medications that inhibit platelet function or anticoagulants. Caution should be exercised if patients are required to take medications that inhibit platelet function or anticoagulants.

Fluid Retention

Dasatinib is associated with fluid retention, which was severe in 9% of patients, including pleural and pericardial effusion reported in 5% and 1% of patients, respectively. Severe ascites and generalized edema were each reported in 1%. Severe pulmonary edema was reported in 1% of patients. Patients who develop symptoms suggestive of pleural effusion such as dyspnea or dry cough should be evaluated by chest X-ray. Severe pleural effusion may require thoracentesis and oxygen therapy. Fluid retention events were typically managed by supportive care measures that include diuretics or short courses of steroids SPRYCEL® (dasatinib) Tablets Prescribing Information. Bristol-Myers Squibb Company P, NJ. June 2006.

QT Prolongation

In vitro data suggest that dasatinib has the potential to prolong cardiac ventricular repolarization (QT interval). In single-arm clinical studies in patients with leukemia treated with dasatinib, the mean QTc interval changes from baseline using Fridericia's method (QTcF) were 3–6 msec; the upper 95% confidence intervals for all mean changes from baseline were <8 msec. Nine patients had QTc prolongation reported as an adverse event. Three patients (<1%) experienced a QTcF >500 msec.

Dasatinib should be administered with caution to patients who have or may develop prolongation of QTc. These include patients with hypokalemia or hypomagnesemia, patients with congenital long QT syndrome, patients taking anti-arrhythmic medicines or other medicinal products that lead to QT prolongation, and cumulative high-dose anthracycline therapy. Hypokalemia or hypomagnesemia should be corrected prior to dasatinib administration (SPRYCEL® (dasatinib) Tablets Prescribing Information. Bristol-Myers Squibb Company P, NJ. June 2006).

Phase I experience in Solid Tumors

In a Phase I study (CA180003) conducted by Bristol Myers Squibb (BMS), dasatinib was administered on a BID schedule to 42 subjects with refractory solid tumor. To date, doses up to 160 mg BID on a 5-day on/2-day off schedule have been administered. A dose of 120 mg BID continuous daily schedule is currently under investigation.

No severe clinical toxicity has been encountered. Gastrointestinal symptoms were reported in most subjects, fatigue was reported in 17 subjects (40%) and rash in 10 subjects (24%). Edema, lethargy and headache were uncommon, and appear to be dose-related. Grade 3 asymptomatic hypocalcemia was considered dose-limiting in one subject, Grade 2 rash was considered dose-limiting in two other subjects, and Grade 2 nausea and vomiting (with dysarthria, lightheadedness and lethargy in a 49 kg subject taking concurrent diazepam) was considered dose-limiting in one subject.

In another Phase I study (CA180021), dasatinib was administered on a QD schedule to 24 subjects at doses up to 180 mg. Pleural effusions were observed in three subjects at the 180 mg dose level (one with pneumonia and two with malignant effusion). A dose of 250 mg QD is currently under consideration. Hypocalcemia, GI symptoms and skin rash have been mild and infrequent.

To date, the safety profile in solid tumor subjects has been similar to that in CP CML subjects with the exception of severe myelosuppression, which has not been observed in solid tumor subjects and is considered related to efficacy against the leukemia as noted above, and severe bleeding which is secondary

to thrombocytopenia in most instances.

2.3 Tissue Acquisition Method Fine Needle Aspiration of Breast Tissue and Biomarker Evaluation

Intermediate biomarkers of cancer are phenotypic, genotypic, and molecular changes that occur during carcinogenesis. Currently there are no validated surrogate endpoint biomarkers for breast cancer in the context of chemoprevention trials with invasive cancer as the definitive end points; therefore the identification and validation of intermediate biomarkers as surrogate endpoints for subsequent large clinical chemoprevention trials for breast cancer is very important. In our current proposal, breast tissue and serum biomarker modulation that will reflect dasatinib activity will be evaluated. These will include, in breast tissue, cytologic changes, markers of proliferation, apoptosis and changes in proteomic profiling; in serum markers of IGF pathway (IGFBP1, IGFBP-3, IGF-I).

Studies using periareolar fine needle aspiration (FNA) of the breast as a tissue acquisition method in phase I and II breast cancer prevention trials have been pioneered and successfully completed by Fabian et. al (11) and is now considered to be an acceptable method, in early phase breast cancer prevention studies (12) We have successfully used and are currently using this method in our prevention trials (13), (14). In our experience, this method is highly acceptable from the patient point of view, as all of our patients returned for their second FNAs and have not complained about the procedure. Therefore, we will use this method in our proposal.

Conclusion and Clinical Relevance:

Phase III breast cancer prevention trials require large numbers of patients, a long duration of follow-up, and are costly. As an alternative strategy, short-term chemoprevention trials utilizing surrogate markers of drug activity may allow for a small sample size and brief duration of exposure to assess the efficacy of potential chemopreventive agents. There is an immediate need to evaluate agents that can reduce the risk of ER negative breast cancer development. Finally, potential preventive agents should have a favorable safety profile. Taken all these and the preclinical information provided above, we are proposing a short-term phase II study evaluating the effects of dasatinib in women who are at increased risk to develop a second primary breast cancer. Our aim is to understand the pathway that is involved in ER negative breast cancer development and progression so that further studies with agents that target the src pathway (example p70S6K inhibitors) could be undertaken that will ultimately lead to the development of prospective phase III studies targeting ER negative breast cancer prevention.

3. SUMMARY OF STUDY PLAN

This is a phase II, prospective biomarker modulation study in women with a prior history of ER negative breast cancer who have currently no active disease and have completed all adjuvant therapy. Women with a previous history of breast cancer have a 0.5-0.8%/year risk to develop a contralateral primary breast cancer (15), (16) and hence represent a high risk study cohort. Sixty six evaluable patients after signing informed consent will undergo baseline periareolar FNA of the breast and blood collection for the analysis of the proposed markers. Participants will take no drug, or the study drug dasatinib at a dose of 40mg or 80mg daily for 3 months (+/- 7 days) and at the end of the 3 months (+/- 7 days) will undergo a repeat FNA and blood collection for the same marker analyses. The study will be undertaken at the University of Texas, M.D. Anderson Cancer Center and Duke University. Patients will return to clinic at month 1 for evaluation and again at month 3 and will be followed-up with a phone call at month 2. Assuming a screening rate of approximately 20 participants per month and a conservatively estimated accrual rate of 2 patients per months we will be able to complete accrual in 30 months.

4. PARTICIPANT SELECTION

4.1 Inclusion Criteria

1. Histological confirmation of ER negative (defined as less than 10%) breast carcinoma, stage I, II, or III
2. Completed all adjuvant therapy including (if indicated) endocrine, trastuzumab, radiation therapy.
3. At least 18 years of age.
4. Female:

   A female is eligible to enter and participate in the study if she is of:

   a. *Non-childbearing potential* (i.e., women with functioning ovaries who have a current documented tubal ligation, hysterectomy alone, hysterectomy and bilateral salpingo-oophorectomy, bilateral salpingo-oophorectomy alone, or women who are post-menopausal); or

   b. *Childbearing potential* (i.e., women with functioning ovaries and no documented impairment of oviductal or uterine function that would cause sterility. This category includes women with oligomenorrhoea (severe), women who are perimenopausal, and young women who have begun to menstruate), has a negative urine or serum pregnancy test at screening, and agrees to one of the following where considered acceptable to the local IRB/IEC:

      - Double-barrier contraception (condom with spermicidal jelly, foam suppository, or film; diaphragm with spermicide; or male condom and diaphragm).
      - Abstinence from sexual intercourse from 2 weeks prior to administration of the investigational product, throughout the active study treatment period.
      - Male partner who is sterile prior to the female subject's entry into the study and is the sole sexual partner for that female subject.
      - Any intrauterine device (IUD).
      - Barrier methods including diaphragm or condom with a spermicide.
5. Able to swallow and retain oral medication.
6. ECOG (Eastern Cooperative Oncology Group) performance status 0 to 2.
7. Provide written informed consent.
8. Adequate bone marrow function
   - Hemoglobin $\geq$ 9 gm/dL.
   - Absolute granulocyte count $\geq$ 1,500/mm^3 (1.5 x 10^9/L).
   - Platelets $\geq$ 75,000/mm^3 (100 x 10^9/L).
9. Serum creatinine < 1.4 mg/dL or calculated creatinine clearance (CrCl) $\geq$ 30 mL/min
10. Total bilirubin $\leq$ 1.5 times the upper limit of the reference range
11. Aspartate and alanine transaminase (AST or ALT) $\leq$ 2 times the upper limit of the reference range
12. Patients must have a baseline ECG with QTcF within the normal range within 28 days prior to registration.
13. Normal mammogram of unaffected breast within 12 months prior to study entry.

4.2 Exclusion Criteria

1. Unwillingness to undergo RPFNA.
2. Contraindication to RPFNA including breast implant(s), bilateral radiation, anticoagulation (excluding those on 81mg aspirin).
3. Concurrent medical condition that would increase drug toxicity: Pleural or pericardial effusion, coagulation or platelet function disorder, ongoing or recent (less than 3 months gastrointestinal bleeding)
4. Uncontrolled angina, congestive heart failure, MI (within last 6 months), congenital long QT syndrome, history of clinically significant ventricular arrhythmia, prolonged QTcF interval on pre- entry EKG (greater than normal range)
5. Hypokalemia or hypomagnesemia if it cannot be corrected
6. Is a pregnant or lactating female.
7. Has evidence of recurrent or metastatic (Stage IV) breast cancer.
8. Is considered medically unfit for the study by the investigator as a result of the medical interview, physical exam, or screening investigations.
9. Has a known immediate or delayed hypersensitivity reaction or idiosyncrasy to dasatinib
10. Has received treatment with any investigational drug in the previous 4 weeks.
11. Has received chemotherapy, immunotherapy, biologic therapy or endocrine therapy within the past 12 weeks.
12. Is currently receiving oral steroid treatment (inhaled steroids are permitted)
13. Oral estrogen, progesterone, testosterone therapy within last 3 months.
14. Concomitant Medications: Drugs that are considered category D (Consider therapy modification) and X (Avoid combination) using the Lexicomp database are prohibited. Concomitant drugs that fall into categories A (No known interaction), B (no action needed) and C (monitor therapy) are allowed.

4.3 Inclusion of Women and Minorities

Since our research topic, breast cancer, is relevant to women, all of our participants will be women of all races and ethnic groups. Male breast cancer is seen very rarely, therefore, we will not include male participants. The research topic to be studied, breast cancer, mainly effects the adult population and is not relevant to children. Therefore, we will exclude individuals who are under 18 years of age. Our research proposal will study women who are at increased risk to develop a second primary breast cancer.

4.4 Recruitment and Retention Plan

Pre-initiation Recruitment

During the pre-initiation recruitment phase, physicians within or outside the Institution working with patient populations that include potential participants will be contacted, the specific inclusion/exclusion

criteria reported, and the importance of accruing participants who are likely to be compliant and retainable will be emphasized. Contact with outside referring physicians will mainly take place by e-mail, and Newsletters, or by face-to-face meeting during outreach seminars, which is organized by the M. D. Anderson Physician Referral Service and the Physicians Network. Information about the study and eligibility criteria will be placed on our website. Investigators at each site will present the study to potential referring physicians within the Institution to explain the goals for the study, describe the protocol, discuss enrollment procedures, and provide contact information. Beside accrual from UT M.D. Anderson Cancer Center, a major source for minority referral for the study will be referrals from Lyndon B. Johnson (LBJ) Hospital, Houston, which primarily serves the minority and underserved population and is staffed by Faculty members from UT M.D. Anderson Cancer Center. A recent search of the LBJ Hospital database indicated that in the last 5 years, 42% of the patients who were seen at LBJ Hospital were African American and 28% were of Hispanic origin. We believe that LBJ Hospital will serve as an ideal referral source for minority accrual for our proposed study.

UT M.D. Anderson Cancer Center recently opened a new center "Center for Research on Minority Health". This center provides an opportunity for this trial to be publicized. Specifically, the PI will have an initial meeting with the Director of that Center, Dr. Jones, and brief him and his staff about the study and provide them with an eligibility summary card. Our study coordinator will then do bi-weekly follow- ups with that team to evaluate potential eligible participants. These can be done via e-mail or telephone.

Other sources for minority recruitment will include working together with community and local organizations such as: Sisters' Network, Inc., One Hundred Black Women, National Association for the Advancement of Colored People, Inc., Top Ladies of Distinction, Inc. and the African Methodist Episcopal Church. We conservatively estimate that 10-15 % of the study participants will belong to the minority population. Besides these promising 5 community partnering programs, our program engages in a variety outreach programs, such as local churches, local shopping centers. Brochures about the study will be taken to any events or functions the above mentioned organizations are doing.

Active Recruitment Phase

We have a very well developed web based communication system at UT MD Anderson Center which will assist us in posting newsletters and advertising the trial on our website for the public as well as for local and nationwide oncologists.

MD Anderson Cancer Center serves as a resource through outreach programs such as MD Anderson Information Line, The Anderson Network, and the Information Service. Through these programs we will alert the community and health care professionals about this study. We also have periodic publications addressing various health related issues, including prevention of cancer. "CancerWise" for example is monthly publication with reports on advances in cancer prevention, treatment and research. Finally, local representatives of the American Cancer Society and the Susan G. Komen Breast Cancer Foundation, with which we have already close relationships, will help us advertising the study. The outreach programs will help us accruing minorities as well, such as MD Anderson Information Line, The Anderson Network, and CancerWise Community Speakers Bureau. In addition, to increase minority accrual and increase awareness about this study will be working together with some of the local organizations such as: Sisters' Network, Inc., One Hundred Black Women, National Association for the Advancement of colored People, Inc., Top Ladies of Distinction, Inc. African Methodist Episcopal Church.

Retention Plan

In our study, participants will return to clinic in 1 and 3 months; month 3 is usually the follow-up interval for our adjuvant patients during their initial 2 years after completing adjuvant therapy. Patients beyond these 2 years usually are seen every 6 months, therefore, for these patients there may be 2 extra visits (month 1 and 3) which would be sponsored by the study.

We will also strive to retain participants by offering well-trained, friendly staff, consistent monitoring of

recruitment and adherence; and specific efforts early in the study to enthusiastically engage compliant participants in the trial.

5. AGENT ADMINISTRATION

Intervention will be administered on an outpatient basis. Reported adverse events and potential risks are described in section 6.2.

5.1 Dose Regimen and Dose Groups

Participants will receive dasatinib at 40mg, or 80mg once a day or no drug. Dasatinib will be taken for 3 months (+/- 7 days). If a dose is missed by more than 12 hours, the dose should be omitted. Tablets should not be crushed and be swallowed as a whole. Dasatinib can be taken with or without a meal.

5.2 Study Agent Administration

- The participant will self-administer dasatinib by mouth.

5.3 Run-in Procedures

N/A

5.4 Prohibited Therapies

Drugs that are considered category D (Consider therapy modification) and X (Avoid combination) using the Lexicomp database are prohibited. Concomitant drugs that fall into categories A (No known interaction), B (no action needed) and C (monitor therapy) are allowed.

5.5 Restricted Therapies

Drugs that are considered category D (Consider therapy modification) and X (Avoid combination) using the Lexicomp database are prohibited. Concomitant drugs that fall into categories A (No known interaction), B (no action needed) and C (monitor therapy) are allowed.

5.6 Concomitant Medications

- No other investigational therapy will be given to patients.
- The use of anticancer agents (other than hormonal therapy) or radiation therapy is not allowed (an exception would be the use of megestrol acetate for the treatment of anorexia).
- Patients enrolled in the Phase I portion of the trial may not receive GCSF or GMCSF during cycle 1 of therapy. The use of hematologic growth factors for the treatment of anemia is allowed at any time during protocol therapy. Patients enrolled in the Phase II portion of the trial or those who have completed >1 cycle of therapy in the phase I portion of the trial may receive growth factor support as per ASCO guidelines.
- Drugs that are considered category D (Consider therapy modification) and X (Avoid combination) using the Lexicomp database are prohibited. Concomitant drugs that fall into categories A (No known interaction), B (no action needed) and C (monitor therapy) are allowed.

5.7 Dose Modification

For grade 1 toxicity or less, no dose modifications will be made. For grade 2 or greater toxicity probably, possibly, or definitely related to dasatinib, dasatinib will be held until recovery at least to grade 1 and patient will re-start at one lower dose level if applicable (80 mg to 40). For grade 3 or 4 toxicity the study

drug will be discontinued.

5.8 Adherence/Compliance

- Participants who are randomized to the treatment group will be evaluable if they received at least 75% of their assigned dasatinib dose. No imputation will be performed on missing data

- The following 2 methods will be used to monitor each participant's agent compliance:
  1) Participants will be given a pill diary and instructed to initial it each time a dose is taken
  2) At the end of the treatment period, the actual quantity of unused drug will be compared to the anticipated amount of unused drug and participant pill diary.

6. PHARMACEUTICAL INFORMATION

6.1 Study Agent

Dasatinib tablets are white to off-white, biconvex, film-coated tablets containing dasatinib, with the following inactive ingredients: lactose monohydrate, microcrystalline cellulose, croscarmellose sodium, hydroxypropyl cellulose, and magnesium stearate. The tablet coating consists of hypromellose, titanium dioxide, and polyethylene glycol.

Preclinical Toxicology

Single or repeated oral administration of dasatinib principally affected the gastro-intestinal (GI) tract, including the liver, the hematopoietic, and lymphoid systems in rats and monkeys. Other prominent effects after single oral administration of dasatinib included renal and cardiac toxicity in rats at lethal doses, and cutaneous hemorrhage in monkeys. Dasatinib can also affect the immune system and bone turnover.

Dasatinib *in vitro* activity in the HERG/IKr and Purkinje-fiber assays indicated a moderate liability for prolongation of cardiac ventricular repolarization (QT interval) in the clinic. However, there were no dasatinib -related changes observed in electrocardiograms, nervous system function, respirations and heart rate, blood pressure, or arterial oxygen saturation in single-dose, 10-day, or 1-month oral toxicity studies in monkeys.

Dasatinib was found to exhibit a profile of broad-spectrum platelet inhibition best typified by anti-platelet agents such as the GPIIb/IIIa antagonists, integrelin and abciximab.

Finally, modulation of SRC kinase activity could also affect osteoclast morphology and function and bone remodeling. This effect could potentially result in an increase in bone mineral density and a phenotype analogous to osteopetrosis(15).

Clinical Pharmacokinetics

The pharmacokinetics of dasatinib have been evaluated in 229 healthy subjects and in 137 patients with leukemia.

Absorption

Maximum plasma concentrations (C_{max}) of dasatinib are observed between 0.5 and 6 hours (T_{max}) following oral administration. dasatinib exhibits dose proportional increases in AUC and linear elimination characteristics over the dose range of 15 mg to 240 mg/day. The overall mean terminal half-life of dasatinib is 3–5 hours(14).

Data from a study of 54 healthy subjects administered a single, 100-mg dose of Dasatinib 30 minutes

following consumption of a high-fat meal resulted in a 14% increase in the mean AUC of dasatinib. The observed food effects were not clinically relevant.

Distribution

In patients, dasatinib has an apparent volume of distribution of 2505 L, suggesting that the drug is extensively distributed in the extravascular space. Binding of dasatinib and its active metabolite to human plasma proteins *in vitro* was approximately 96% and 93%, respectively, with no concentration dependence over the range of 100–500 ng/mL(14).

Metabolism

Dasatinib is extensively metabolized in humans, primarily by the cytochrome P450 enzyme 3A4 (see section 7.6). CYP3A4 was the primary enzyme responsible for the formation of the active metabolite. Flavin-containing monooxygenase 3 (FMO-3) and uridine diphosphate-glucuronosyltransferase (UGT) enzymes are also involved in the formation of dasatinib metabolites. In human liver microsomes, dasatinib was a weak time-dependent inhibitor of CYP3A4.

The exposure of the active metabolite, which is equipotent to dasatinib, represents approximately 5% of the dasatinib AUC. This indicates that the active metabolite of dasatinib is unlikely to play a major role in the observed pharmacology of the drug. dasatinib also had several other inactive oxidative metabolites.

Elimination

Elimination is primarily via the feces. Following a single oral dose of [^{14}C]-labeled dasatinib, approximately 4% and 85% of the administered radioactivity was recovered in the urine and feces, respectively, within 10 days. Unchanged dasatinib accounted for 0.1% and 19% of the administered dose in urine and feces, respectively, with the remainder of the dose being metabolites(14).

6.2 Rationale for Dose Selection:

Since our aim in this pilot biomarker prevention study is to identify the minimal dose required to induce biomarker modulation by dasatinib in high risk breast epithelium and not to identify the maximum tolerated dose for antitumor efficacy, we will not perform a traditional phase I dose escalation study that is utilized in metastatic phase I studies (as MTD for anticancer treatment has already been defined). Previous studies have shown considerable toxicity with 100 mg of dasatinib, therefore, in our pilot biomarker modulation study, we will randomly assign patients to 0,40, or 80 mg daily dasatinib and perform ongoing evaluation of toxicity (please see toxicity evaluation section (12).

6.3 Availability

Dasatinib will be requested from BMS. As an alternative plan, dasatinib will be purchased using other funding mechanisms.

6.4 Agent Accountability

The PI, or a responsible party designated by the investigator, will maintain a careful record of receipt, disposition, and return of all study drugs on the Investigational Agent Accountability Record. All study drug supplies will be kept in a locked, limited access area. The study drug will not be used outside the context of the protocol. Under no circumstances will the investigator or other site personnel supply study drug to other patients, or clinics, or allow supplies to be used other than directed by this protocol. Study agent will not be transferred from one patient to another, or from one protocol to another. All other transfers (*i.e.* patient or PI moves) must be approved in advance by the study PIs.

The investigator will maintain records documenting the receipt, use, loss or other disposition of the investigational product, including batch or code numbers, and account for it's disposition on a subject-by-subject basis, including specific dates and quantities. The source document, documenting the subject's

participation in this randomized clinical trial, must be documented in the medical and research records. Destruction will be documented in accordance with institutional SOPs.

6.5 Packaging and Labels

Dasatinib will be packaged in bottles as follows:

- Dasatinib 20 mg film-coated tablets, 30 tabs/bottle

Each bottle will be labeled in an open label. Labels will contain, at a minimum, the following information: product name, tablet strength, batch number, directions for use, storage conditions, and appropriate caution statements.

Storage-Handling and Disposal Dasatinib tablets should be stored in a secure area at 25°C (77°F); excursions permitted between 15–30°C (59–86°F).

Procedures for proper handling and disposal of anticancer drugs should be considered. There is no general agreement that all of the procedures recommended in the guidelines are necessary or appropriate.

Dasatinib tablets consist of a core tablet (containing the active drug substance), surrounded by a film coating to prevent exposure of pharmacy and clinical personnel to the active drug substance. However, if tablets are crushed or broken, pharmacy and clinical personnel should wear disposable chemotherapy gloves. Personnel who are pregnant should avoid exposure to crushed and/or broken tablets

6.7 Registration

Participating institutions will register all participants per their IRB institutional policy. In addition, participants will be registered and randomized at MDACC. Registration will be completed in the MDACC Office of Research Administration computer system CORe (Clinical Oncology Research). CORe will assign the overall protocol accession number. The randomization program based on this method will developed by the Department of Biostatistics, and the program will be available via an intranet web site of MDACC.

6.8 Agent Administration

Upon randomization one month supply of study drug will be dispensed to the patients randomized to the treatment arms. Additional bottles will be dispensed at 1 month clinic visits, as necessary, to ensure that the subject has sufficient study drug to last until the next planned clinic visit. Follow-up schedule will be taken into consideration when drug is dispensed and those with the modified visit schedule will be provided an adequate supply to last until next visit.

During study participation, subjects will be asked to: self-administer the study medication; avoid certain medications/supplements and complete daily logs to record study agent intake, concomitant medications, and AEs. Subjects are expected to: maintain ≥75% compliance with study agent intake; comply with dietary, medication and supplement restrictions; and complete the daily log.

6.9 Destruction of Dasatinib

At the completion of investigation, all unused study agent will be destroyed according to institutional guidelines.

7. CLINICAL EVALUATIONS AND PROCEDURES

7.1 Schedule of Events

Please refer to Table 1 that lists baseline testing/pre-study evaluation, agent administration, study assessments, procedures and case report forms.

7.2 Baseline Testing/Pre-study Evaluation

7.2.1 If individual is eligible as outlined in section 4.1, she will sign consent and she will be assigned a participant identification number at the time of enrollment and the following procedures will be undertaken:

7.2.2 Data collection will include: medical history, demographic information including race, age, height, weight, cancer family history, reproductive history (age at menarche, age at first pregnancy, outcome of each pregnancy, lactation and duration of each episode of lactation, history of previous benign breast disease (age at each breast biopsy and histologies identified in each breast biopsy), hormonal medication use (age at first use and duration of use of oral contraceptives, hormone replacement or fertility drugs),and usual weekly alcohol consumption.

7.2.3 General physical exam, including a clinical breast examination, if these exams were not performed in the last 12 months. If the physical exam and a clinical breast exam were performed within the past12 months, do not need to repeat them for baseline. If a breast mass is found on initial physical exam, the participant will receive an appropriate evaluation, and will not participate in the study until the mass has been definitively classified as benign.

7.2.4 Gynecology evaluation within the last 5 years (as suggested by patient's gynecologist). Participants with a prior hysterectomy alone, hysterectomy and bilateral salpingo-oophorectomy, or bilateral salpingo-oophorectomy alone are exempt from this requirement.

7.2.5 Bilateral or unilateral mammogram within 12 months prior to registration.

7.2.6 Blood draw for CBC with differential, platelet count, electrolyte, Magnesium, liver panel (LFT) within 3 months prior to randomization, EKG (within 1 month), if normal (upper limit normal is sufficient for liver panel, creatinine), and if eligible, patient will proceed to 7.2.8

7.2.7. Urine pregnancy test (if not already completed within 14 days prior to randomization or start of study agent, order STAT for results to be available prior to drug administration) for women of childbearing potential.

7.2.8 Breast periareolar fine needle aspiration (FNA),(baseline)
(If participant is on aspirin (except those who take 81 mg aspirin), non-steroidal anti-inflammatory drugs and Vitamin E, she will be instructed to discontinue these and return in 7-10 days for the FNA) FNA procedure is described in section 7.5.6.

7.2.9 Blood draw for research biomarkers (Day 0)

7.2.10 The participant will be randomized to drug or no drug and the ones that are randomized to dasatinib will be provided with a drug kit with a 3 month supply, and a pill diary and will be instructed to return any unused drugs. The participant will start dasatinib within 7 days. Concomitant medications will be documented.

7.2.11 A 3-month follow-up (+/- 7 days) will be scheduled and participants will be instructed to call if any symptoms occur.

7.3 Evaluations During Study Intervention

At month 1 and 2: Study nurse will call the participant to document and grade the presence of adverse events, compliance and concomitant medications.

7.4 Evaluations at Completion of Study Intervention

Month 3 clinic visit will include: Vital signs, AE, concomitant medication, and review of study medication taken and pill counts. Participants will return agent diary and unused study medication. Blood sampling for repeat biomarkers studies, and breast FNA will be performed (The study drug can be taken on the biopsy day).

7.5 Methods for Clinical Procedures

7.5.1 Medical history: Research staff will obtain data from a prospectively maintained research database that all UT MDACC breast cancer patients are entered in.

7.5.2 Karnofsky performance status: The Karnofsky Performance Scale Index allows subjects to be monitored for basic functional status and to monitor any functional impairment. Although no changes in functional impairment are anticipated, we plan to utilize this scale to ensure optimum functional status to participate in this clinical trial. This will be recorded in the participant's CRF.

7**.5.3 Study agent intake log (pill diary):** Participants will be provided with a Study Agent Intake log (pill diary) which they will be required to complete daily. The pill diary will require the subject to check that study agent has been taken. Monthly, subjects will be contacted by a member of the research staff by phone for review of: i) pill diary, and; ii) use of concomitant medications; iii) Anticipated and unanticipated, grades of constitutional, dermatological, gastrointestinal (GI), metabolic and pain symptoms noted;

7.5.4 Adverse Events (AEs): Adverse Events (AEs): All AEs that are reported by the subject, detected during a visit, physical examination, or laboratory work-up will be recorded in the participant's study record and recorded on the CRF. All AEs that occur after the informed consent is signed will be recorded on the AE CRF whether or not related to study agent. The following information will be captured for each AE: date reported; verbatim term; Common Toxicity Criteria (CTCAE Term v 4.0); onset and resolution date; severity grade; attribution to study agent; whether or not the event was reported as an SAE; action taken; whether or not the subject dropped due to the AE; outcome; and comments;

7.5.5 Fine Needle Aspiration: In order to evaluate changes in biomarkers patients will undergo FNA at baseline and 3 month after taking dasatinib. The procedure is as follows:

The skin of the periareolar region (upper and lower outer quadrants) is cleansed with an alcohol prep pad infiltrated with 0.1m. of 1% Lidocaine mixed 9:1 within 8.3 sodium bicarbonate. We will obtain pre and post dasatinib treatment FNA from breast tissue (only 1 breast; the same breast will be used for the repeat FNA) from the most dense area, mostly at 3 o'clock position and 9 o'clock position, 4 passes at each position, 8 passes total. A 25-gauge needle attached to a 10 ml syringe in a syringe holder is used to perform the aspiration. The needle is inserted into the breast to a distance of 1 – 1 ½. Suction is applied by pulling on the plunger of the syringe, and the needle is moved back and forth rapidly in the same plane within the most dense breast tissue. Suction is maintained until some material appears in the clear plastic needle hub (this may require as many as 20 up and down motions of the needle with continuous suction). When material appears in the hub of the needle, the syringe plunger is released and the needle is withdrawn. The material obtained by fine needle aspiration is rinsed into the CytoLyt fixative solution (methanol based). The procedure is repeated, using a disposable needle and a syringe. Once cells are in CytoLyt, the cells will be fixed and the

vial can be left in room temperature until next step. The cells are spun down and an aliquot of the sediment is re-suspend in PreserveCyt solution and then thin-layered slides are prepared using the ThinPrep Processor. Thin-layer slide is fixed with 95% alcohol and stained with Papanicolaou and cellularity of this slide is evaluated. We then re-suspend the cells in the remaining sediment in PreserveCyt solution and prepare as thin-layer slides, fix them with 95% alcohol and air-dry and store them at -80C for the proposed markers. A preprinted label will be attached to identify all sample vials. Manual pressure is held over the biopsy site between each needle pass and for 10 minutes after the last pass; participants will then be given ice packs to put pressure over the biopsy site for 20 minutes.

7.5.6 Biomarkers to be assessed:

Cytomorphology: FNA Cytomorphology is a valid and modifiable risk biomarker and response indicator for early prevention trials and has been reported to be present about 25% (range 15-50%) of high risk individuals{Arun, #3399}, {Fabian, 1998 #3400}.Cytomorphology and Ki-67 expression are independent variables considered complementary (17).Cytomorphology will be assessed by a single expert breast cytopathologist who will assign a categorical assessment of nonproliferative, hyperplasia, borderline hyperplasia with atypia, or hyperplasia with atypia (18). The pathologist will be blinded as to the pre and post dasatinib samples.

Cell proliferation by Ki-67: The expression of the human Ki-67 protein is associated with cell proliferation, as this protein is present during all active phases of the cell cycle (G1, S, G2 and mitosis), but is absent from resting cells G0, making it an excellent marker for determining the growth fraction of a given cell population (19). Ki-67 has shown rapid modulation in previous studies, including 14-day treatment with Tamoxifen. It has also demonstrated correlation with subsequent clinical response (20). Recent studies (14), (21), have demonstrated that Ki-67 proliferation index is an established, valid, reliable marker of proliferation and that changes in Ki-67 in FNA is a valid IEB of chemoprevention efficacy. Slides in which more than 500 epithelial cells are visible by Papanicolaou staining will be further processed for Ki-67. After de-staining, antigen retrieval will be performed with a 10 nM/l citrate buffer (pH 6) in a Biocare (Walnut Creek, CA, USA) decloaking chamber (DC 2002) for 2 min at 120°C. Slides will be stained with a MIB-1 monoclonal antibody (M7240 Dako Cytomation; Dako, Carpenteria, CA, USA) at a 1:20 dilution using a Dako autostainer. Hyperplastic clusters will be preferentially assessed, and the number of cells with unequivocal nuclear staining out of a total of 500 cells will be assessed.

Apoptosis by the Caspase-3 Method: From Cell Signaling Technology (CST) we will obtain the Apoptosis Marker: Signal Stain Cleaved Caspase-3 (Asp 175) IHC Detection Kit. This is a "ready to use" system designed to detect the activation of caspase-3 in human cell preps by immunohistochemistry with which we have extensive experience.

Other markers: If samples permit slides that are prepared using the Thin Prep technique from Cytyc will be used for immunohostochemitry studies using previously described antibodies for EGFR, phosphorylated EGFR and bcl-2, p-Y418-Src, p-Y-118-Paxillin, and markers identified in Aims 1 and 3 of the grant (IGF-1, IGFBP1 and 3).

Proteomic Profiling: Markers of proliferation and apoptosis as well markers involved in ER negative breast cancer development pathway, including the src and mTOR pathway will be evaluated using the reverse phase protein microarray (RPPM) method: Epithelial cell clusters prepared from breast FNAs are microdissected using an AutoPix Automated Laser Capture Microscope. To ensure that we distinguish *1)* epithelial cells versus stromal cells, and *2)* identify the degree of cytological atypia, slides will be photographed before microdissection. As in preliminary data, 100 total and phosphoproteins will be profiled. Total protein concentrations will be assessed by Spyro Ruby fluorescent staining (22), (23). RPPM will be performed using MicroVigene™ image

analysis software (24). Arrays will be scanned, spot intensity normalized, and standard single value will be generated for each sample (Image Quant v5.2). Spot intensity will be integrated and background intensity calculated (JMP v5.0, SAS). Key technological components of RPPM offer unique advantages over tissue or antibody arrays(22), (23). RPPM uses denatured lysate so antigen retrieval, which is a limitation for tissue arrays, is not a problem. Secondly, each sample is printed on the array in serial dilution, providing an internal standard. Finally, RPPM does not require direct labeling of the sample, thus improving reproducibility, sensitivity, and robustness.

Serum IGF pathway markers: IGF-1 and IGFBP3 are potential intermediate biomarkers for short term breast cancer prevention trials (25), (26), {Arun, 2011 #3436}.

Non-fasting blood sample of approximately 10 ml will be drawn at the baseline visit and at 3 months, using sterile needles into a 10 ml speckled red top SST tube. The blood in speckled red top tubes will be processed at the clinic site by standing for 20-60 min to clot, then spinning for 20 minutes at 3200 rpm. Serum will be aliquotted into 3 tubes (1-2.0 ml each), and placed in a -70º C freezer.

Table 1. SCHEDULE OF EVENTS

Evaluation/Procedure	Registration	Baseline	Month 1[h]	Month 2[h]	Month 3 / Early Termination[h]
Informed Consent	X[g]				
Assess Eligibility	X	X			
Medical History		X			
Physical Exam		X[f]			
Vital Signs		X			
Height and Weight		X			
Laboratory Tests		X[a]			
EKG		X			
Mammogram		X[b]			
Gynecologic Evaluation		X[e]			
Pregnancy test		X[c]			
Breast FNA		X			X
Blood Biomarkers		X			X
Concomitant Medications		X	X[d]	X[d]	X
Dispense Study Agent		X			
Collect Study Agent					X
Review Diary		X	X[d]	X[d]	X
Adverse Events			X[d]	X[d]	X
Nurse telephone follow-up			X[d]	X[d]	

a = Normal CBC, platelet count, electrolyte, Magnesium, liver panel, creatinine within last 3 months and EKG within one month prior to randomization
b = Normal mammogram within last 12 months prior to randomization
c = Negative pregnancy test within 14 days prior to randomization or start of the study agent, if applicable.
d = During nurse phone call
e = Gynecology evaluation within the last 5 years prior to randomization. Participants with a prior history of hysterectomy alone, hysterectomy and bilateral salpingo-ooporectomy, or bilateral salpingo-ooporectomy alone are exempt from this requirement.
f = Normal physical exam within last 12 months prior to randomization
g = Informed consent is valid within 30 days only prior to the study drug; otherwise, a new informed consent should be obtained before reassessing the subject for protocol eligibility
h = A window of +/- 7days is allowed for these visits

8. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

Study endpoints in our study consist of biomarker changes in breast tissue and serum. Samples for the evaluation of these markers will be obtained pre study initiation and at 3 months (+/- 7 days). The specifics are described below.

8.1 Primary Endpoint

The primary goal is to evaluate Dasatinib induced reduction of proliferation. Ki-67 will be measured in baseline and month 3 contralateral breast FNA samples.

8.2 Secondary Endpoints

8.2.1 Cytology: Changes in cytology in baseline and month 3 FNA samples will be evaluated. The cytology criteria used for the interpretation of the smear are similar to the 1997 consensus criteria published by the National Cancer Institute: Inadequate cellular material for diagnosis, normal epithelial cells, atypical hyperplasia, suspicious and malignant.

8.2.2 Proliferation and apoptosis analysis of FNA samples: EGFR, P-EGFR, bcl-2, CC3 expression in FNA samples at baseline and at month 3 (+/- 7 days) will be evaluated by immunohistochemistry (IHC). Scoring will be performed by Dr. Yun Gong.

8.2.3 Proteomic profile analysis of FNA samples: Src, AMPK and M-TOR related pathways will be evaluated in FNA samples obtained at baseline and at 3 months after dasatinib treatment. Samples will be evaluated by Dr. Seewaldt.

8.2.4 Serum markers: IGF pathway (IGF-I, IGFBP-1, IGFBP-3) at baseline and at 3 months will be evaluated in Dr. Arun's laboratory.

8.3 Off Agent Criteria

Participants may stop taking study agent for the following reasons: completed the protocol-prescribed intervention, adverse event or serious adverse event, inadequate agent supply, noncompliance, concomitant medications, or medical contraindication. Participants will continue to be followed, if possible, for safety reasons and in order to collect endpoint data according to the schedule of events. In case participants will be off agent but have taken at least 1 dose of dasatinib and undergo a month 3 FNA, they will be statistically evaluable for biomarker endpoint.

8.4 Off Study Criteria

Participants may go 'off-study' for the following reasons: the protocol intervention and any protocol-required follow-up period is completed, adverse event/serious adverse event, lost to follow-up, non-compliance, concomitant medication, medical contraindication, recurrent disease, withdraw consent, or death.

9. SPECIMEN MANAGEMENT

9.1 Laboratories

9.1.1 Obtaining and processing of breast FNA samples and cytopathologic interpretation will be performed, evaluated and stored at the MD Anderson Cancer Center Cytopathology laboratory (Address: UT M.D. Anderson Cancer Center Department of Cytopathology Unit 53, 1515 Holcombe Blvd, Room G3.3628, Houston, Texas 77030-3721) FNA samples in CytoLyt

labeled with the study ID from DUKE will be sent to the MD Anderson Cancer Center Cytopathology laboratory (same address), for centralized cytology review by Dr. Yun Gong

9.1.2 KI-67, EGFR, P-EGFR, bcl-2, CC3 p-Y418-Src, p-Y-118-Paxillin expression in FNA samples will be performed by IHC analysis at MD Anderson Cancer Center, Pathology Immunohistochemistry Core Laboratory for centralized staining. Scoring will be performed by Dr. Yun Gong.

9.1.3 Proteomic profile analysis involving the src, AMPK and mTOR pathway in FNA samples that are labeled as in section 7.5.5 will be performed by Dr.Victoria Seewaldt at Duke University (Shipping address: Duke university Medical Center, 2301 Erwin Rd, Rm 221A MSRB, Durham, NC 27710)

9.1.4 IGF pathway (IGF-I, IGFBP-1, IGFBP-2), leptin, adiponektin at baseline and at 3 months will be evaluated in Dr.Arun's laboratory (1515 Pressler Street, Unit 1354, Houston, TX 77030).

9.1.5 Routine laboratory tests for eligibility at baseline (Section 7.2) will be performed at each Institution's standard laboratory.

9.2 Collection and Handling Procedures

Fine Needle Aspiration (FNA):

We will obtain pre and post dasatinib treatment FNA from breast tissue at baseline and at 3 months (+/- 7 days) from each participant as described in section 7.6. An average of 6-8 separate needle passes will be performed in 1 breast. The material obtained by fine needle aspiration is rinsed into the CytoLyt fixative solution. The procedure is repeated, using a disposable needle and a syringe. Once cells are in CytoLyt, the cells will be fixed and the vial can be left in room temperature until next step. Cells in the CytoLyt are spinned down and an aliquot of the sediment is re-suspend in PreserveCyt solution and then thin-layered slides are prepared using the ThinPrep Processor. Thin-layer slide is fixed with 95% alcohol and stained with Papanicolaou and cellularity of this slide is evaluated. The cells in the remaining sediment will be re-suspended in PreserveCyt solution and thin-layered slides will be prepared. Slides will be fixed with 95% alcohol and air-dried and stored at -80°C for the analysis of proposed markers. One FNA pass will be placed in saline solution and frozen at -80°C. A preprinted label will be attached to identify all sample vials.

Blood Collection:

Peripheral blood will be obtained by venipuncture at baseline and at 3 months (+/- 7 days). Twenty ml blood will be collected in 2 red top tubes for biomarkers analysis (baseline and at 3 months (+/- 7 days)) and be processed within 24hrs of collection. If blood is not processed within 24hrs, a new sample will need to be collected and processed correctly.

For the purpose of tracking samples: A pre-printed label will be attached to identify all sample vials. Blood will be stored at -20°C, serum will be stored at -80°C.

9.3 Tissue Banking

If the participants agree, unused FNA samples without participant identifiers (except study PID) will be placed into 1.5 ml RNA later (Ambion). This will be held at room temperature for 15 minutes then snap frozen and stored at - 80°C in our (MDACC) Institutional Tissue Bank Core Facility/Cytopathology Lab.

Unused serum will be stored at -80°C in Dr. Arun's Laboratory.

10. REPORTING ADVERSE EVENTS

DEFINITION: An adverse event (AE) is any untoward medical occurrence in a study participant. An AE does not necessarily have a causal relationship with the treatment or study participant. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with participation in a study, whether or not related to that participation. This includes all deaths that occur while a participant is on a study.

A list of adverse events that have occurred or might occur (Reported Adverse Events and Potential Risks) can be found in Section 6.2, Pharmaceutical Information as well as the Investigator Brochure or package insert.

10.1 Adverse Events

10.1.1 Reportable Adverse Events

All adverse events that occur after the informed consent is signed (including run-in) must be recorded on the adverse event CRF (paper and/or electronic) whether or not related to study agent.

10.1.2 AE Data Elements:

- AE reported date
- AE Verbatim Term
- CTCAE Category (v4.0)
- CTCAE Term (v 4.0)
- Event onset date and event ended date
- Severity grade
- Attribution to study agent (relatedness)
- Whether or not the event was reported as a Serious Adverse Event (SAE)
- Whether or not the participant dropped due to the adverse event
- Action taken with the study agent
- Outcome of the event
- Comments

10.1.3 Severity of AEs

10.1.3.1 Adverse event will be identified using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. The CTCAE provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTCAE can be found at http://ctep.cancer.gov.

AEs will be assessed according to the CTCAE grade associated with the AE term. AEs that do not have a corresponding CTCAE term will be assessed according to their impact on the participant's ability to perform daily activities as follows:

Grade	Severity	Description
1	Mild	• Barely noticeable, does not influence functioning • Causing no limitations of usual activities
2	Moderate	• Makes participant uncomfortable, influences functioning • Causing some limitations of usual activities
3	Severe	• Severe discomfort, treatment needed • Severe and undesirable, causing inability to carry out usual activities

4	Life threatening	• Immediate risk of death • Life threatening or disabling
5	Fatal	• Causes death of the participant

10.1.4 Assessment of relationship of AE to treatment

The possibility that the adverse event is related to study drug will be classified as one of the following: unrelated, unlikely, possible, probable, definite.

10.1.5 Follow-up of AEs

All AEs, including lab abnormalities that in the opinion of the investigator are clinically significant, will be followed according to good medical practices and documented as such.

10.2 Serious Adverse Events

10.2.1 DEFINITION: ICH Guideline E2A and Fed. Reg. 62, Oct. 7, 1997 define serious adverse events as those events, occurring at any dose, which meet any of the following criteria:

- Results in death
- Is life threatening (*Note: the term life-threatening refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe).*
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital abnormality/birth defect
- Events that may not meet these criteria, but which the investigator finds very unusual and/or potentially serious, will also be reported in the same manner

10.2.2 Participating organization will comply with applicable regulatory requirements related to reporting SAEs to their IRB.

11. STUDY MONITORING

Please refer to Appendix A.

12. STATISTICAL CONSIDERATIONS

12.1 Study Design/Endpoints

This is a phase I biomarker modulation study of dasatinib in breast tissue of women who are at increased risk. To have a total of 60 participants, we have built in a 10% attrition rate and will accrue 66 participants. Participants will be randomized into two treatment groups and one no treatment control group (N=15). The two treatment groups are 40 mg qd (N=30), and 80 mg qd (N=15) of dasatinib. The primary endpoint will be the change in Ki-67 before and after 3 months of dasatinib treatment in breast tissue of high-risk women. Ki-67 is measured as a continuous variable and a one-way ANOVA followed by Dunnett's multiple comparison test comparing the change of Ki-67 of each of the three treated groups with control. The purpose of this study is to determine the minimum biological effective dose, which is

defined as the lowest dose where the change of Ki-67 is significantly different from that in the control group. Based on the recent report by Fabian et al. (26), the baseline Ki-67 value (in % of cell positive) is about 6.5% with a range between 0% and 40%.

12.2 Sample Size/Accrual Rate

The primary endpoint is the change of Ki-67 between pre-treatment and post treatment. Relative difference of the Ki-67 change between groups is used to evaluate the treatment effect. We assume that the change of Ki-67 after the treatment is positively associated with the dose level. Monte Carlo simulations have been performed to analyze the power for the multiple comparisons using the Dunnett's two-sided test procedure, where the sample mean in each treatment group is compared with the sample mean in the control group. A one-way design with the sample size of 15 and 30 in the two treatment groups and 15 in the control group can achieve an any-pair power of 0.87 assuming the effect size of 1 and the significance level of 0.05. The any-pair power is defined as the probability of detecting the significant difference between any treatment groups and the control group. The effect size is the standardized mean difference between the treatment group and the control group, which is the ratio of detectable difference between the two groups and the common standard deviation within groups. Based on preliminary data (26), the standard deviation is about 10% for a single Ki-67 measure at the baseline or after treatment. Under a conservative assumption that the correlation coefficient between the before and after treatment Ki-67 is 0.5, the standard deviation of the Ki-67 modulation is also 10%. Therefore, an effect size of 1 corresponds to a Ki-67 change of 10% and an effect size of 1.2 corresponds to a Ki-67 change of 12%. With 30 and 15 participants in the two treatment groups, we will have at least 87% power to detect an effect size of 1.0 or larger. Power calculations were computed in PASS 2005.

Effect Size	Power to detect significance difference between any pairs
0.8	0.70
1.0	0.87
1.2	0.96

In addition to the above analysis, if the data deviate from Gaussian distribution, proper transformation will be sought and nonparametric methods will be applied to provide valid data analysis. Analysis of covariance (ANCOVA) adjusted by baseline covariates associated with Ki-67 will be used to increase the statistical power for identifying biologically effective treatments

12.3 Randomization and Stratification

No stratification factors will be used in this relative small phase I study (compared to phase III). A random permutated block design with a block size of 4 or 8 will be used to ensure balance in randomization.

12.4 Primary Endpoint

The primary endpoint will be the change in Ki-67 before and after 3 months of dasatinib treatment. As indicated in Section 13.1, a one-way ANOVA by Dunnett's multiple comparison test will be applied for analyzing the primary endpoint.

12.5 Secondary Endpoints

Secondary endpoints include evaluation of other proliferation and apoptosis markers (EGFR, CC3, bcl-2, ER, p21, p27), metabolites of the drug, and HMG-CoA reductase genotype. These will be exploratory endpoints performed and analyzed as samples permit. Descriptive statistics will be computed to estimate the distribution of the biomarkers and the change of biomarker values before and after treatment. Exploratory data analysis will be applied to check for outliers and to have a preliminary evaluation of the treatment effect by dose and association among various variables. Other standard statistical methods for

analyzing continuous and categorical data will be applied whenever appropriate.

12.6 Reporting and Exclusions

All evaluable participants in the study will be evaluated for toxicity and efficacy.

12.7 Evaluation of Toxicity

All participants will be evaluable for toxicity from the time of randomization.
An event with an attribution of possible, probably, or definite is considered related.

Bayesian toxicity monitoring schema will be used to monitor toxicity profile in the treatment arms. Each treatment arm will be monitored separately for toxicity. "Severe Toxicity" is defined as grade 3 or 4 toxicity related to the current dose level. The toxicity monitoring will start after enrolling at least 3 participants in each dose level. If a dose level is terminated due to the toxicity, the rest of the participants will be assigned to the lower dose levels only.
We denote the probability of toxicity for each dose level as θ_i, i=1, 2 and assume that no toxicity is observed in the control group. The treatment will be stopped early if

$$Pr[\theta_i > 0.10 \mid data\ ] > 0.85,\ i=1,\ 2$$

for dose level i and higher dose levels. In other words, the trial will be terminated if there is a greater than 85% chance to observe more than 10% toxicity at 40mg level. We assume that the prior distribution of θ_i follows a Beta (0.05, 0.95), which implies that the prior toxicity rate is 5% for each dose level. The choice of the parameters are based on prior data suggesting that about 5% of toxicity is expected in this drug and higher than 10% toxicity is considered unacceptable.

The stopping boundary and the probability of early stopping are presented in Tables 1 and 2 below.

The trial will be monitored for each dose level according to the following stopping boundaries for severe toxicity.

Table 1. Toxicity Monitoring Boundaries

Among number of Participants	Recommend stopping dose levels if number of severe toxicity observed >=
3-7	2
8-14	3
15-21	4
22-28	5
29-30	6

Table 2. Probability of early termination of a dose level due to severe toxicity

True Severe toxicity rate	Probability of early terminating 40mg level (therefore the trial)	Probability of early terminating 80mg level
5%	0.06	0.06
10%	0.29	0.22
15%	0.56	0.42
20%	0.78	0.63

12.8 Evaluability

Participants who are randomized to the treatment group will be evaluable if they received at least 75% of their assigned dasatinib dose. No imputation will be performed on missing data.

12.9 Interim Analysis

There will not be interim analysis on efficacy of the study. However, toxicity will be monitored closely.

13. ETHICAL AND REGULATORY CONSIDERATIONS

13.1 Institutional Review Board Approval

Prior to initiating the study the Protocol Lead Investigator at the Lead Organization must obtain written approval to conduct the study from the appropriate IRB. Should changes to the study become necessary, protocol amendments will be submitted to the IRB.

13.2 Informed Consent

All potential study participants will be given a copy of the IRB-approved Informed Consent to review. The investigator will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, he/she will be asked to sign the Informed Consent document. The informed consent document is considered valid for 30 days from the participant's signature date to begin treatment. If treatment has not begun within 30 days following the signature date listed on the informed consent document then the participant must be reassessed for protocol eligibility and a new consent document completed. The study agent(s) will not be released to a participant who has not signed the Informed Consent document. Participants who refuse to participate or who withdraw from the study will be treated without prejudice.

Participants must be provided the option to allow the use of blood samples, other body fluids, and tissues obtained during scheduled testing, operative procedures, or other standard medical practices for further research purposes. A separate signature area is required to allow participants to opt out of allowing tissue to be used for further research.

13.3 Other

This trial will be conducted in compliance with the protocol, Good Clinical Practice (GCP), and the applicable regulatory requirements.

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