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Phase I-II Study Of Dasatinib In Combination With Weekly Paclitaxel For Patients With Metastatic Breast Carcinoma
CA 180 194

MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

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Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

This trial is phase I-II study of dasatinib in combination with weekly paclitaxel for patients with advanced and metastatic breast carcinoma.

The phase I component of the study will be open to all patients with metastatic breast carcinoma, regardless of ER/PR/HER2 status and number and type of prior regimens.

The phase II component will be open to patients with HER2 negative metastatic breast carcinoma, with 0-2 prior chemotherapeutic regimen for metastatic disease. Patients with ER/PR positive disease will be allowed to enter the study.

Dasatinib [SPRYCEL®] 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. (1)

Drug discovery and non-clinical pharmacology studies showed that dasatinib:

- Kills BCR-ABL dependent leukemic cell lines, including a number that are resistant to imatinib due to kinase domain mutations or overexpression of SRC family kinases and is effective against all imatinib-resistant kinase domain mutations tested to date, except T315I
- Inhibited proliferation of cancer cell lines that express activated SRC or c-KIT
- Potently inhibits VEGF-stimulated proliferation and migration in HUVECs
- Has potent bone anti-resorptive activity

More specifically, recent data showed that dasatinib selectively inhibits growth of basal-type /ER/PR/HER2 negative breast cancer cell lines growing in vitro.

Two phase II trials are currently ongoing to assess activity and safety of single agent dasatinib in patients with metastatic breast carcinoma ER/PR/HER2 negative (CA 180059) and ER/PR positive or HER2 positive (CA 180088).

The combination of chemotherapy and dasatinib is currently being studied in patients with malignancies. This study will aim at defining the maximum tolerated dose (MTD), for the combination of weekly paclitaxel and dasatinib.

Once established the MTD, the phase II portion of the study will aim at defining efficacy and safety of the combination in patients with HER2 negative metastatic breast carcinoma.

Efficacy endpoints will include overall response rate based upon RECIST response criteria, clinical benefit (CR+PR+SD > 6 months), time to tumor progression, progression free survival and duration of response. Toxicity will be evaluated in terms of a toxicity rate; the type, frequency, severity, timing and relationship of each toxicity will be determined as per the NCI Common Toxicity Criteria, version 3. (Common Terminology Criteria for Adverse Events (CTCAE). Available at http://ctep.cancer.gov/forms/CTCAEv3.pdf)

Eligible patients must have pathologically confirmed breast cancer, which is known to be metastatic or locally advanced. Patients must have at least one site of measurable disease using RECIST criteria (**Appendix E**).

In the Phase I portion of the study, a standard three patient per cohort dose escalation scheme will be used, treating between 6 and 54 patients with dasatinib and a fixed dose of weekly paclitaxel. Dasatinib will be dose-escalated to determine the MTD using a standard, three-patient per cohort dose escalation schedule. Between 6 and 54 patients will be required to determine the MTD of dasatinib. There will be no intrapatient dose escalation. The starting dose of dasatinib is 70 mg DAILY, given continuously (Dose level 0). Two dose reductions levels have been included, if necessary. It is expected that the maximum of 54 patients will be enrolled in less than one year, between the two centers. All patients within a cohort will be observed for toxicity for 4 weeks (1 cycle) prior to entering patients at the next dose level.

Once the MTD for dasatinib has been identified, additional patients will be enrolled into the Phase II portion of the study to determine the efficacy and safety of dasatinib at the MTD when administered in combination with weekly paclitaxel. Efficacy will be measured in terms of response rate assessed after 2 cycles of therapy. Simon's two-stage optimal design will be used to test the null hypothesis of a 15% response rate against the alternative of a 30% response rate. Setting both the Type I and Type II errors at 10%, 23 patients will initially enter the study. If there are 3 or fewer responses observed, the study will be terminated early and declared to have a negative result. If 4 or more responses are observed, enrollment will be extended to 55 patients. If $\geq$ 12 responses are observed among the 55 patients, the study will be considered to have a positive result and this regimen would be considered worthy of further testing. For this design, the probability of early termination is 54% if the true response probability is $\leq$ 10%. Upon completion of the study, the true response rate will be estimated via the observed response rate and an exact confidence interval will be constructed.

The maximum of 55 patients possible for the Phase II portion of this study is estimated to take one year to accrue with an anticipated enrollment of 5 patients per month between the two centers.

2.0 OBJECTIVES AND SCIENTIFIC AIMS

The **primary** objectives of this trial are:

Phase I Portion: To determine the MTD of dasatinib when administered in combination with a fixed dose of weekly paclitaxel.

Phase II Portion: To estimate efficacy (objective response rate; ORR; complete response (CR) + partial response (PR)) of dasatinib when administered in combination with weekly paclitaxel at the MTD established during the phase I portion of this trial.

The **secondary** objectives of this trial are:

Phase I Portion:

1. To obtain preliminary data on the therapeutic activity of dasatinib when administered in combination with weekly paclitaxel.

Phase II Portion:

1. To obtain safety and tolerability of dasatinib when administered in combination with weekly paclitaxel;
2. To estimate secondary efficacy endpoints of this combination including clinical benefit (CR+PR+SD > 6 months), time to tumor progression (TTP), progression free survival (PFS) and duration of response.
3. To obtain exploratory tumor biomarker data: assays of p-SRC, VEGFR2 and Collagen Type IV in plasma, obtained at baseline and after 2 cycles of treatment (8 weeks), will be performed by enzyme-linked immunosorbent assay.
4. To perform analysis of mRNA expression on the tumor specimens, for a gene expression profiling of the responders versus non-responders, in order to identify potential predictors of response to dasatinib. If differentially interesting candidates emerge, immunohistochemistry assays including EphA2, IGFBP2, caveolin and phospho-caveolin and other potential predictive markers and SFK substrates as available will also be performed.
5. To collect circulating tumor cells (CTC) at baseline and after 2 cycles of treatment (8 weeks);

3.0 BACKGROUND AND RATIONALE

3.1 Metastatic Breast Cancer

Cancer of the breast is one of the most common life-threatening malignancies occurring in women. In 2007, an estimated 178,480 new cases of invasive breast cancer will be diagnosed in the United States and despite advances in early detection, surgery and adjuvant therapy, a significant number of women are expected to die of metastatic breast cancer. (2) Unlike early stage breast cancer, metastatic breast cancer is generally not curable with currently available therapy. (3) To date, the five-year survival rate for women with distant metastases is only 21%, underscoring the need for more efficacious therapeutic options for the advanced stages of this disease. When metastatic disease is diagnosed, the goals of treatment are multiple, including cure (<5%), palliation of symptoms and improved survival. Hormonal therapy is usually the first-line treatment of choice for those women with hormone receptor-positive cancer but eventually nearly all patients develop hormone refractory

disease. For these women, and for those with hormone receptor-negative disease, cytotoxic chemotherapy is utilized.
Today there are several classes of cytotoxic treatment available to patients with metastatic disease (eg, anthracyclines, taxanes, fluoropyrimidines, vinca alkaloids, topoisomerase inhibitors). A treatment paradigm of sequential single agent therapy is practiced in this disease of chronicity.

Newly introduced hormonal, cytotoxic and biologically targeted agents are hoped to improve upon the modest survival advantage with systemic therapy for these women.

3.2 Dasatinib

Dasatinib [SPRYCEL®] 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. (1)

Drug discovery and nonclinical pharmacology studies showed that dasatinib: (4)

- Kills BCR-ABL dependent leukemic cell lines, including a number that are resistant to imatinib due to kinase domain mutations or overexpression of SRC family kinases and is effective against all imatinib-resistant kinase domain mutations tested to date, except T315I
- Inhibited proliferation of cancer cell lines that express activated SRC or c-KIT
- Potently inhibits VEGF-stimulated proliferation and migration in HUVECs
- Has potent bone anti-resorptive activity

3.2.1 Pre-clinical Anti-tumor Activity: In Vitro Molecular Studies

Dasatinib potently inhibits: SRC kinases, BCR-ABL, c-KIT, PDGFRβ and EPHA and was less potent against 16 other unrelated protein tyrosine kinases (PTKs) and serine/threonine kinases. Imatinib is less potent against several key enzymes: for example, Dasatinib was 260-, 8-, 60-, and >1000-fold more potent than imatinib versus BCR-ABL, c-KIT, PDGFR®, and SRC kinases, respectively.[2]

In vitro, dasatinib was active in leukemic cell lines representing variants of imatinib mesylate sensitive and resistant disease. dasatinib inhibited the growth of chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL) cell lines overexpressing BCR-ABL. Under the conditions of the assays, dasatinib was able to overcome imatinib resistance resulting from BCR-ABL kinase domain mutations, activation of alternate signaling pathways involving the SRC family kinases (LYN, HCK), and multi-drug resistance gene overexpression. (1)

Dasatinib inhibits the BCR-ABL kinase with an *in vitro* IC_{50} of 3 nM, a potency 260-fold greater than that of imatinib mesylate (IC_{50} = 790 nM). In cellular assays, dasatinib killed or inhibited the proliferation of all BCR-ABL dependent leukemic cell lines tested to date. Dasatinib also demonstrated undiminished anti-tumor activity against several preclinically- and clinically-derived models of imatinib mesylate resistance. Evidence that SRC family kinase over expression may play a role in clinical resistance to imatinib mesylate was demonstrated in three CML cell lines established

from patients who failed imatinib mesylate therapy. These cells remained highly sensitive to the cell-killing effects of dasatinib.

These results demonstrate that dasatinib is effective in reducing the proliferation or survival of both imatinib mesylate-sensitive and resistant cells, and its inhibitory activity is not solely dependent on BCR-ABL.

In cells, dasatinib inhibited c-SRC, and other kinases in the SRC kinase family (including LCK, YES, FYN) at sub-nanomolar concentrations. Dasatinib inhibited the cellular SRC autophosphorylation in several cancer cell lines that highly express c-SRC, including the human prostate carcinoma cells PC3 and MDA-PCa-2b, and the human colon carcinoma cells WiDr and SW-480.

The concentrations required to inhibit SRC autophosphorylation in these cells (15 - 135 nM) approximate those needed for inhibition of cellular proliferation (range = 17 - 845 nM, median = 35 nM, n = 10).

Dasatinib demonstrated potent antiproliferative activity for VEGF- or bFGF-dependent human umbilical vein endothelial cells (HUVEC). An IC_{50} of 43 nM against VEGF-stimulated growth and of 248 nM under bFGF-driven growth conditions was demonstrated. Dasatinib also inhibited HUVEC cell migration with IC_{50} of < 5 nM in a standard *in vitro* cell migration assay (Boyden chamber).

SRC kinase plays a major role in osteoclast function. In short-term *in vitro* studies, dasatinib was a potent inhibitor of bone resorption. Dasatinib also potently inhibits KIT, the receptor tyrosine kinase for the natural cognate ligand stem-cell factor (SCF), also known as Steel factor (SLF); constitutive activation of KIT is a critical oncogenic contributing factor to the malignant phenotype of the sarcoma known as Gastrointestinal Stromal Tumor (GIST). In an *in vitro* biochemical assay, dasatinib inhibited KIT with an IC_{50} of 22 nM, while the IC_{50} for imatinib mesylate was 169 nM, an 8-fold difference.

In three small cell lung cancer cell lines (NCI-H59, H526 and H187) that are dependent on SCF for proliferation, dasatinib inhibited SCF-driven proliferation with IC_{50} in the range of 114 - 220 nM. In the same study, the IC_{50} for imatinib mesylate was 1150 - 2270 nM, a difference in potency of about 10-fold. The concentration range of dasatinib required to inhibit KIT phosphorylation in cells (10 - 1000 nM) was in agreement with that needed for inhibiting cellular proliferation.

3.2.2 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.

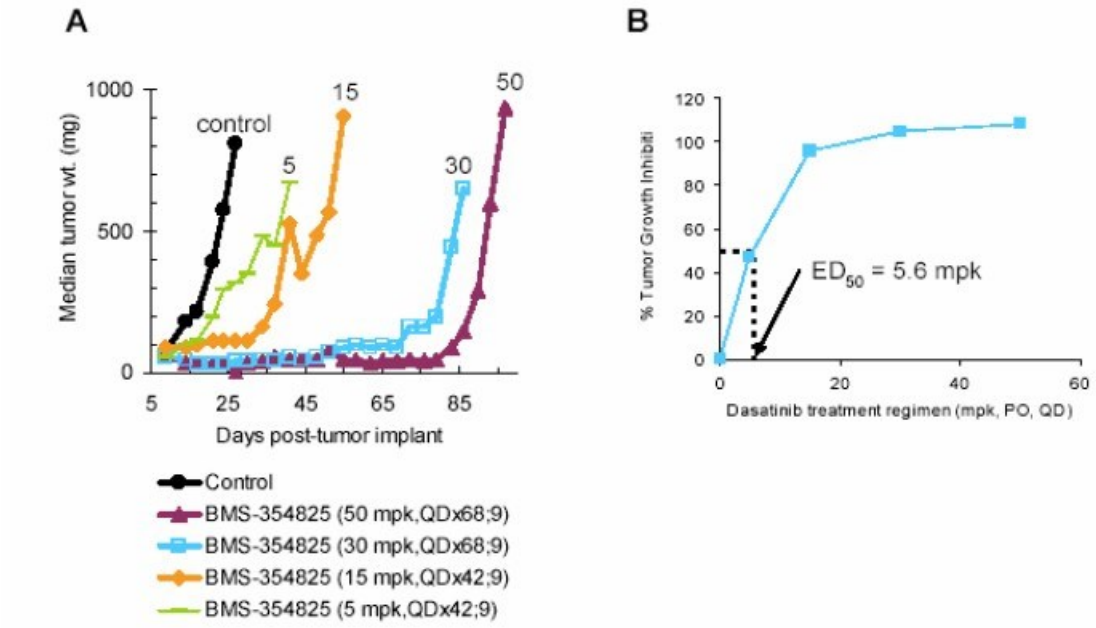
Dasatinib *in vitro* activity in the HERG/IKr and Purkinje-fiber assays indicated a moderate liability for prolongation of cardiac ventricular repolarization (QT interval) in the clinic. However, there were no dasatinib -related changes observed in electrocardiograms, nervous system function, respirations 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.(4)

3.2.3 In Vivo Studies of Dasatinib in Breast Cancer

An artificial Src-driven cell line "mamSRC" was developed which is sensitive to dasatinib *in vitro* with IC_{50} = 3.2 nM; sensitivity *in vivo* was striking:



Of 35 breast cancer cell lines, 11 were sensitive *in vitro* to dasatinib (defined as IC_{50} < 1μM); as will be indicated in Section 3.5.1, these were predominantly those of basal-like subtype. Well-tolerated regimens of oral dasatinib, typically administered BID on a 5-day

per week schedule to immunodeficient mice bearing xenograft tumors, provided growth inhibition of 32 - 87%, depending on the cell line. Additional studies are in progress.

3.2.4 Results of Clinical Investigations

Dasatinib was initially developed in patients with imatinib-resistant or –intolerant CML. A Phase I trial CA180002 was completed (see Section 3.2.7.1) and identified 70 mg twice daily (BID) as a safe starting dose for CML patients. (5) Subsequent Phase II trials were performed in all phases of imatinib-resistant and imatinib-intolerant CML to assess the efficacy and safety of dasatinib. (9-13) Data were submitted for regulatory review, leading to approval of dasatinib in the U.S. Two randomized phase III studies (CA1800034 and CA1800035) were performed to compare efficacy and safety of daily vs BID schedules in all phases of CML and of total daily dose in chronic phase CML . Two Phase I trials are in progress in patients with recurrent or progressive solid tumors, one (CA180003) using a BID and one (CA180021) using a daily schedule.

3.2.5 Pharmacokinetics of Dasatinib

The pharmacokinetics of dasatinib have been evaluated in 229 healthy subjects and in 137 patients with leukemia (CML or Ph+ALL) from a Phase I clinical study (CA180002). (4)

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. (1)

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.

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. (1)

Dasatinib is extensively metabolized in humans, primarily by the cytochrome P450 enzyme 3A4. CYP3A4 was the primary enzyme responsible for the formation of the active metabolite. Flavin-cotaining 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.

Dasatinib is a time-dependent inhibitor of CYP3A3. At clinically relevant concentrations, dasatinib does not inhibit CYP1A2, 2A6, 2B6, 2C9, 2C19, 2D6, or 2E1. Dasatinib is not an inducer of human CYP enzymes. (1)

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. (1)

3.2.6 Pharmacodynamics of Dasatinib

Src phosphorylation was studied in peripheral blood mononuclear cells (PBMC) and xenograft tumors (prostate cancer cell line PC3) of mice. Phospho-Src inhibition was > 90% both in PBMC and in tumor at doses ≥ 15 mg/kg, and correlated closely with tumor growth inhibition.

Doses of ~200 mg provide plasma concentrations in human subjects comparable to 15 mg/kg in mouse (Cmax ~150 - 200 ng/mL). The *in vitro* dose-response curve for phospho-Src inhibition is the same in PBMC of human and mouse. In human PBMC, 60 - 80% inhibition of phospho-Src persisted for at least 6 - 8 hours after doses of ≥ 70 mg; inhibition of tumor SFKs has not been measured in humans.

3.2.6.1 Somatic Mutations and Response to Dasatinib-Paclitaxel Drug Combination

In lung cancers models and patients, activating somatic mutations of the Discoidin Domain Receptor 2 (DDR2) gene were shown to be both oncogenic and associated with high sensitivity to dasatinib as single agent (Hammerman et al, Cancer Discov 2011). DDR2 is receptor tyrosine kinase that binds collagen as its endogenous ligand and is mutated in about 3% of squamous cell lung carcinomas. A paralog gene, DDR1, also exists. DDR2 mutations have been shown to be present in breast cancer at a frequency of 1 to 2%, according to data from The Cancer Genome Atlas (TCGA).

Somatic genomic analyses, based on next-generation sequencing (NGS) techniques applied to archival breast cancer tissue samples and/or on plasma (circulating tumor DNA) can unravel the somatic mutation(s) associated with sensitivity to the tested treatment regimen. This approach can be used not only to investigate the existence of both known (e.g. DDR2) mutations, but also for the identification of novel mutations related to sensitivity or resistance to a given therapy.

3.2.7 Experience in CML

Dasatinib has been administered to more than 2000 subjects, the majority with CML refractory or intolerant to imatinib. Dasatinib's activity has been investigated in eight Phase 1 studies (CA180009, CA180016, CA180019, CA180020, CA180022, CA180032, CA180037, and CA180002) and five Phase 2 studies (CA180005, CA180006,

CA180013, CA180015, and CA180017) that supported the approved indication and dosage of dasatinib at 70 mg BID in subjects with CML or Ph+ ALL.(4) Two Phase 3 studies (CA1800034 and CA180035) were designed to compare alternate doses and administration schedules in imatinib resistant or intolerant subjects with chronic, accelerated, or blast phase CML or Ph+ ALL.

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. 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.

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 the table below.

Additional information from all these studies may be found in the Investigator Brochure and in FDA-approved drug labeling. (4)

Table 1:

Efficacy in Dasatinib Clinical Studies in CML and Ph+ ALL (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 and <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.

3.2.7.1 Safety of Dasatinib in Clinical Studies in CML and Ph+ ALL

The data presented in Table 2 reflect exposure to dasatinib in 2182 patients with leukemia in clinical studies (starting dosage 100 mg once daily, 140 mg once daily, 50 mg twice daily, or 70 mg twice daily). The median duration of therapy was 11 months (range 0.03–26 months).

The majority of dasatinib-treated patients experienced adverse reactions at some time. Drug was discontinued for adverse reactions in 9% of patients in chronic phase CML, 10% in accelerated phase CML, 15% in myeloid blast phase CML, and 8% in lymphoid blast phase CML or Ph+ ALL. In a Phase 3 dose-optimization study in patients with chronic phase CML, the rate of discontinuation for adverse reaction was lower in patients treated with 100 mg once daily than in patients treated with 70 mg twice daily (4% and 12%, respectively).

The most frequently reported adverse reactions (reported in ≥20% of patients) included fluid retention events, diarrhea, headache, skin rash, nausea, hemorrhage, fatigue, and dyspnea.

The most frequently reported serious adverse reactions included pleural effusion (9%), pyrexia (3%), pneumonia (3%), infection (2%), febrile neutropenia (4%), gastrointestinal bleeding (4%), dyspnea (3%), sepsis (1%), diarrhea (2%), congestive heart failure (2%), and pericardial effusion (1%).

All adverse reactions (excluding laboratory abnormalities) that were reported in at least 10% of the patients in dasatinib clinical studies are shown in Table 2. (4)

Table 2: Adverse Reactions Reported in ≥10% of All Patients (All Grades) in Clinical Studies

	All Patients (n=2182)		Chronic Phase[a] (n=1150)	Accelerated Phase (n=502)	Myeloid Blast Phase (n=280)	Lymphoid Blast Phase and Ph+ ALL (n=250)
	All Grades	Grades 3/4	Grades 3/4	Grades 3/4	Grades 3/4	Grades 3/4
Preferred Term	Percent (%) of Patients					
Fluid Retention	37	8	6	7	13	7
Superficial localized edema	20	<1	<1	1	1	<1
Pleural effusion	22	5	4	5	10	6
Other fluid retention	10	3	3	3	6	2
Generalized edema	3	<1	<1	1	<1	1
Congestive heart failure/cardiac dysfunction[b]	2	1	2	<1	2	1
Pericardial effusion	3	1	1	1	2	0
Pulmonary edema	2	1	1	1	1	1
Ascites	<1	<1	0	0	1	<1
Pulmonary hypertension	1	<1	<1	0	1	1
Diarrhea	31	3	3	4	5	4
Headache	24	1	1	1	1	2
Skin Rash[c]	22	1	1	1	1	1
Nausea	22	1	1	1	2	2
Hemorrhage	21	6	2	11	12	8
Gastrointestinal bleeding	7	4	1	8	9	5
CNS bleeding	1	<1	0	<1	<1	2
Fatigue	21	2	2	3	1	2
Dyspnea	20	4	5	4	5	2
Musculoskeletal Pain	14	1	2	1	1	<1
Pyrexia	13	1	1	2	3	1

Table 2: Adverse Reactions Reported in ≥10% of All Patients (All Grades) in Clinical Studies

Preferred Term	All Patients (n=2182)		Chronic Phase[a] (n=1150)	Accelerated Phase (n=502)	Myeloid Blast Phase (n=280)	Lymphoid Blast Phase and Ph+ ALL (n=250)
	All Grades	Grades 3/4	Grades 3/4	Grades 3/4	Grades 3/4	Grades 3/4
	Percent (%) of Patients					
Vomiting	13	1	1	1	1	2
Abdominal Pain	10	1	1	<1	1	2

a The chronic phase data include patients prescribed any dose of SPRYCEL.

b Includes left ventricular dysfunction, cardiac failure, cardiac failure congestive, cardiomyopathy, congestive cardiomyopathy, diastolic dysfunction, ejection fraction decreased, and ventricular failure.

c Includes erythema, erythema multiforme, exfoliative rash, generalized erythema, heat rash, milia, rash, rash erythematous, rash follicular, rash generalized, rash macular, rash maculopapular, rash papular, rash pruritic, rash pustular, skin exfoliation, skin irritation, systemic lupus erythematosus rash, urticaria vesiculosa, and rash vesicular.

In the Phase 3 dose-optimization study in patients with chronic phase CML, the median duration of therapy was approximately 12 months (range <1-20 months). Selected adverse reactions are shown by dose regimen in Table 3. (4)

Table 3: Selected Adverse Reactions Reported in Phase 3 Dose-Optimization Study (Chronic Phase CML)

Preferred Term	100 mg daily (n=165)		140 mg daily[a] (n=163)		50 mg BID[a] (n=167)		70 mg BID[a] (n=167)	
	All Grades	Grades 3/4	All Grades	Grades 3/4	All Grades	Grades 3/4	All Grades	Grades 3/4
	Percent (%) of Patients							
Diarrhea	23	1	26	3	26	3	25	4
Fluid Retention	24	2	33	4	27	4	32	5
Superficial localized edema	14	0	14	1	14	0	16	0
Pleural effusion	10	2	20	2	16	3	18	2
Generalized edema	2	0	3	0	0	0	1	0
Congestive heart failure/cardiac dysfunction[b]	0	0	2	1	1	1	4	2

Table 3: Selected Adverse Reactions Reported in Phase 3 Dose-Optimization Study (Chronic Phase CML)								
	100 mg daily (n=165)		140 mg daily[a] (n=163)		50 mg BID[a] (n=167)		70 mg BID[a] (n=167)	
	All Grades	Grades 3/4	All Grades	Grades 3/4	All Grades	Grades 3/4	All Grades	Grades 3/4
Preferred Term	Percent (%) of Patients							
Pericardial effusion	1	1	4	1	2	1	2	1
Pulmonary edema	0	0	0	0	1	0	2	1
Pulmonary hypertension	0	0	0	0	0	0	1	1
Hemorrhage	10	1	12	1	9	2	14	2
Gastrointestinal bleeding	1	1	2	0	4	2	4	2

a Not a recommended starting dosage of Sprycel for chronic phase CML.

b Includes left ventricular dysfunction, cardiac failure, cardiac failure congestive, cardiomyopathy, congestive cardiomyopathy, diastolic dysfunction, ejection fraction decreased, and ventricular failure.

3.2.7.2 Laboratory Abnormalities

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 phase CML or Ph+ ALL than in chronic phase CML (Table 4). Myelosuppression was reported in patients with normal baseline laboratory values as well as in patients with pre-existing laboratory abnormalities.

In patients who experienced severe myelosuppression, recovery generally occurred following dose interruption or reduction; permanent discontinuation of treatment occurred in 1% of patients.

Grade 3 or 4 elevations of transaminase 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 phase CML and Ph+ ALL. Elevations in transaminase 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. In the Phase 2 randomized study, the frequency of Grade 3 or 4 neutropenia, thrombocytopenia, and anemia was 63%, 56%, and 19%, respectively, in the dasatinib group and 39%, 14%, and 8%, respectively, in the imatinib group. The frequency of Grade 3 or 4 hypocalcemia was 4% in the dasatinib group and 0% in the imatinib group. Laboratory abnormalities reported in the Phase 3 dose-optimization study in patients with chronic phase CML are shown in Table 5.

Table 4:	CTC Grades 3/4 Laboratory Abnormalities in Clinical Studies			
	Chronic Phase[a] (n=1150)	Accelerated Phase (n=502)	Myeloid Blast Phase (n=280)	Lymphoid Blast Phase and Ph+ ALL (n=250)
	Percent (%) of Patients			
Hematology Parameters				
Neutropenia	46	68	80	78
Thrombocytopenia	41	71	81	78
Anemia	18	55	75	45
Biochemistry Parameters				
Hypophosphatemia	10	12	19	20
Hypocalcemia	2	7	16	11
Elevated SGPT (ALT)	1	3	6	7
Elevated SGOT (AST)	1	1	4	5
Elevated Bilirubin	1	1	4	5
Elevated Creatinine	1	2	3	1

a The chronic phase data include patients prescribed any dose of SPRYCEL.

CTC grades: neutropenia (Grade 3 ≥0.5–1.0 × 109/L, Grade 4 <0.5 × 109/L); thrombocytopenia (Grade 3 ≥10–50 × 109/L, Grade 4 <10 × 109/L); anemia (hemoglobin ≥65–80 g/L, Grade 4 <65 g/L); elevated creatinine (Grade 3 >3–6 × upper limit of normal range (ULN), Grade 4 >6 × ULN); elevated bilirubin (Grade 3 >3–10 × ULN, Grade 4 >10 x ULN); elevated SGOT or SGPT (Grade 3 >5-20 x ULN, Grade 4 >20 x 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).

Table 5:	CTC Grades 3/4 Laboratory Abnormalities in Phase 3 Dose-Optimization Study (Chronic Phase CML)			
	100 mg daily (n=165)	140 mg daily[a] **(n=163)**	50 mg BID[a] **(n=167)**	70 mg BID[a] **(n=167)**
	Percent (%) of Patients			
Hematology Parameters				
Neutropenia	34	43	46	43
Thrombocytopenia	22	40	34	38
Anemia	10	19	18	17
Biochemistry Parameters				

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Table 5: CTC Grades 3/4 Laboratory Abnormalities in Phase 3 Dose-Optimization Study (Chronic Phase CML)				
	100 mg daily (n=165)	140 mg daily[a] **(n=163)**	50 mg BID[a] **(n=167)**	70 mg BID[a] **(n=167)**
	Percent (%) of Patients			
Hypophosphatemia	8	6	7	7
Hypocalcemia	2	3	1	2
Elevated SGPT (ALT)	0	1	1	1
Elevated SGOT (AST)	1	1	0	0
Elevated Bilirubin	1	2	0	1
Elevated Creatinine	0	1	0	1

a Not a recommended starting dosage of SPRYCEL for chronic phase CML.

CTC grades: neutropenia (Grade 3 ≥0.5–1.0 × 109/L, Grade 4 <0.5 × 109/L); thrombocytopenia (Grade 3 ≥10–50 × 109/L, Grade 4 <10 × 109/L); anemia (hemoglobin ≥65–80 g/L, Grade 4 <65 g/L); elevated creatinine (Grade 3 >3–6 × upper limit of normal range (ULN), Grade 4 >6 × ULN); elevated bilirubin (Grade 3 >3–10 × ULN, Grade 4 >10 x ULN); elevated SGOT or SGPT (Grade 3 >5-20 x ULN, Grade 4 >20 x 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).

3.2.8 Phase I-II 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. As of 10-Feb-2006, 33 subjects were treated for a full 4 weeks for evaluation of dose-limiting toxicities (DLTs). This study explored 8 dose levels with 2 different dosing schedules. The first schedule assessed 5 days of dasatinib followed by 2 days off-drug each week, and the second schedule assessed continuous daily dosing that was added in Amendment #3 (7-Mar-2005). In the 5 days on/2 days off schedule, the following dose levels were explored: 35 mg BID, 50 mg BID, 70 mg BID, 90 mg BID, 120 mg BID and 160 mg BID. Of the six subjects enrolled and evaluable for DLT determination at the dose level of 90 mg BID given on a continuous daily schedule, two episodes were considered possibly to represent DLT reported by 2 subjects. These were recurrent Grade 2 rash in 1 subject and removal from study for Grade 2 nausea and vomiting and lightheadedness in the other subject. Given the otherwise acceptable tolerability of this dose level with toxicities that are medically manageable with a maximum severity of Grade 2, further escalation to the next dose level of 120 mg BID continuous daily schedule is currently under investigation. No further escalation above 160 mg BID was explored on this schedule because, as per protocol, this represents the maximum tolerated dose (MTD). (4)

The preliminary safety results from CA180003 demonstrate that no severe clinical toxicity has been encountered. Dasatinib did not induce significant myelosuppression in this patient population Gastrointestinal symptoms including nausea and vomiting were reported in most subjects, fatigue was

reported in 17 subjects (40%) and rash in 9 subjects (21%). Edema, lethargy and headache were uncommon, and appear to be dose-related. Additionally, the incidence and severity of fluid retention and of pleural effusion, in particular, appear to be less in subjects with solid tumors than in those with leukemia receiving comparable doses of dasatinib. There were 4 subjects with drug-related AEs leading to discontinuation; none of these events were related to myelosuppression or fluid retention. (4)

In another Phase I study (CA180021), dasatinib was administered on a daily schedule to 24 subjects with solid tumors in a drug-drug interaction and multi-ascending dose (MAD) study at doses up to 180 mg. This ongoing study investigated the effect of ketoconazole, a potent inhibitor of CYP3A4, on the PK of dasatinib (Segment 1) and the effect of dasatinib on pharmacodynamic markers in subjects with advanced solid tumors that are refractory to standard therapies or for which no standard therapy exists (Segment 2).

Segment 1 includes 18 subjects, of which 16 subjects were treated with dasatinib as of 2-Jan-2006. Segment 2 includes approximately 30 additional subjects, of which 14 subjects were treated with dasatinib as of 2-Jan-2006. A total of 4 deaths, all related to disease progression, were reported. There were 4 subjects with drug-related AEs leading to discontinuation: 1 subject with Grade 1 amnesia, 1 with Grade 2 pleural effusion, 1 with Grade 3 dehydration, and 1 subject with Grade 3 dysphagia and Grade 3 dehydration. Hypocalcemia, GI symptoms and skin rash have been mild and infrequent. (4)

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. (4)

Two phase II trials are currently ongoing to assess activity and safety of single agent dasatinib in patients with metastatic breast carcinoma ER/PR/HER2 negative (CA 180059) and ER/PR positive or HER2 positive (CA 180088). Starting dose of dasatinib is 100 mg BID with continuous dosing, which was considered to be safe, based on data from the phase I experience.
In the ongoing Phase II trials however, suboptimal tolerance was observed at the dose of 100 mg BID, in terms of gastro-intestinal toxicity including nausea and vomit, and fluid retention, including pleural and pericardial effusions, prompting reduction to 70 mg BID with continuous dosing as starting dose. (Bristol Myers Squibb, data on file).

3.3 Paclitaxel

Paclitaxel is a taxane originally derived from the bark of the of the Pacific yew tree (*Taxus brevifolia)* and is one of the most active agents against breast cancer. Paclitaxel binds to the dimeric tubulin preventing microtubule disassembly by stabilizing microtubules so that the spindle cannot be dismantled. This microtubule disruption halts mitosis, interferes with other critical interphase functions and subsequently leads to cell death. (6)

Taxanes (docetaxel and paclitaxel) like many chemotherapy drugs are known to have anti-angiogenic activity. Paclitaxel has been shown to inhibit endothelial cell proliferation, migration and tubule formation at concentrations lower than required to induce tumor cell kill. (7) In vivo testing demonstrated that murine microvasculature was significantly decreased by treatment with paclitaxel.

Taxanes are indicated in several cancers but the most impressive activity of the taxanes has been seen in patients with ovarian and breast cancers. In metastatic breast cancer, paclitaxel is often indicated after failure of anthracycline based combination chemotherapy. The weekly schedule of paclitaxel has been proven to offer the best efficacy/tolerability ratio, when compared to the q 3-weekly schedule in randomized trials. (8) .

As monotherapy, paclitaxel showed to induce response rates ranging from 20 - 50 % in earlier studies in breast cancer patients. (8,9) However, more recent randomized trials have reported more modest response rates, in the range of 14-16%, likely due to the fact that nowadays patients are receiving overall more chemotherapy in the adjuvant setting, and the overall likelihood of response to therapy in the metastatic setting might result diminished. (10, 11)

Neutropenia and neuropathy are the two most common significant toxicities of paclitaxel. The onset of neutropenia usually occurs on days 8 to 10, with recovery generally complete by days 15 to 21 in every 3 week dosing regimens at doses of 175 to 200 mg/m$_2$ over 3 or 24 hours intravenous infusion. More frequent administration schedules, particularly weekly treatment with doses of 80 to 100 mg/m$_2$, are associated with less severe neutropenia and equivalent antitumor activity in a number of cancers, when compared to the every 3 week schedule. In the CALGB 9840 study (weekly paclitaxel given as 1-hour infusion v standard 3-hour infusion of paclitaxel every third week in the treatment of metastatic breast cancer, with trastuzumab for HER2 positive patients) the administration of paclitaxel in the weekly schedule caused less grade 3 neutropenia compared to the every 3 week administration regimen (8% v 15%, p = 0.013), but more grade 3 sensory/motor neuropathy (23/8% v 12/4%, p = 0.001/0.04). (8)

3.4 Study Rationale: Dasatinib in Combination with Paclitaxel

Taxanes, including paclitaxel, are very active agents in the treatment of breast cancer, and have shown clinical benefit both in the metastatic and adjuvant setting. (8, 12) The combination of taxanes with biologic agents is particularly appealing in the treatment of breast cancer and has shown significant results in a number of clinical trials. (13, 14)

Dasatinib has shown to inhibit proliferation of numerous solid tumor cell lines that expressed activated SRC or c-KIT, including breast, prostate, lung, colon carcinoma and rhabdomyosarcoma cell lines. In human prostate carcinoma xenografts, the combination of dasatinib and paclitaxel produced antitumor effects against that were significantly better than the effects of either single agent alone (P = 0.05). (15)

More specifically, recent data showed that dasatinib selectively inhibits growth of basal-type /ER/PR/HER2 negative breast cancer cell lines growing in vitro. (16) Bristol-Myers Squibb (BMS) has developed promising pharmacogenomic markers of response to dasatinib in preclinical models of cancer. Using *in vitro* assays, 7 of 23 well-characterized breast cancer cell lines were shown to be relatively sensitive to dasatinib (IC50 $\leq$ 1μM). It was noted that these were predominantly those known to be of 'basal-like' subtype. Pharmacogenomic analysis demonstrated a 161-gene subset that correlated with growth inhibition by dasatinib. Several genes in this subset were shown to be modulated *in vitro* by exposure to dasatinib. The existence of this expression-defined gene subset was demonstrated in a large number of patient samples and found to consist largely of 'triple-negative' cancers. Similar results, i.e. identification of the triple-negative subtype as that likely inhibited by dasatinib, were obtained independently and using somewhat different methodologies by investigators both at UCLA and at Duke. (16)

We identified patterns of gene expression from these human breast cancer specimens. Total RNA was isolated from these biopsies using TRIzol reagent, and double-stranded cDNA was synthesized by a chimeric oligonucleotide with oligo-dT and a T7 RNA polymerase promoter. Reverse transcription was carried out followed by biotin labeling and approximately 250-fold linear amplification by *in vitro* transcription followed by pre-hybridization, hybridization, washing, and staining with streptavidin-phycoerthyrin. The labeled cRNA was hybridized to the Affymetrix U133A GeneChip™ (Santa Clara, CA), which contains an array of 45,000 probe sets with more than 39,000 human genes. These confirmed differences in gene expression will be validated by relative quantitative reverse transcription polymerase chain reaction (q-RTPCR) assays. Over 100 ER-negative primary breast cancers has been molecularly profiled, and their gene expression profiles and DNA snp haplotypes compared to normal breast epithelial (n=50) profiles to accurately refine ER- tumors into clinical useful subtypes. Clinical molecular and histochemical assays will be developed and validated for the ER negative subtypes. The growth networks exploited by ER negative tumors that reflect pathways critical for proliferation and survival have been examined. Interestingly, in ER negative tumors, there was an increased differential expression of various tyrosine kinases, including EGFR, YES-1, KIT, EPH receptors B4, ABL. (Jenny Chang, Personal Communication)

Targeted agents have shown best results when combined with chemotherapy (13, 14), and therefore the combination of dasatinib and paclitaxel is appealing.

The combination of paclitaxel and dasatinib has never been tested in humans, although combination trials with other chemotherapeutic agents are ongoing. . This study will aim at defining the maximum tolerated dose (MTD), for the combination of weekly paclitaxel and dasatinib.

Once established the MTD, the phase II portion of the study will aim at defining efficacy and safety of the combination in patients with HER2 negative metastatic breast carcinoma

3.4.1 Dosing Rationale

Starting dose of dasatinib in the two ongoing phase II trials for patients with breast carcinoma (CA 180059 and CA 180088) was 100 mg BID with continuous dosing, which was considered to be safe, based on data from the phase I experience. However, suboptimal tolerance was observed at this dose-level, in terms of gastro-intestinal toxicity including nausea and vomit, and fluid retention, including pleural and pericardial effusions, prompting reduction to 70 mg BID with continuous dosing as starting dose. (Bristol Myers Squibb, data on file).

In large randomized studies in CML, daily dosing was better tolerated in long-term use. In chronic-phase CML, the efficacy of daily and BID schedules is equivalent. Theoretically, higher peak levels attained on daily could yield some benefit, but this rationale may be disease-specific. In the ongoing SWOG trial for bone-predominant disease, 100 daily vs 70 BID dosing arms are being studied.

The initial component of the Phase I trial with dasatinib on BID schedule in patients with solid tumors was performed with intermittent dosing, 5 days on and 2 days off. DLTs were observed at dasatinib dose of 90 and 120 mg BID given continuously, and (in subsequent expansion phase, data not yet available for distribution) at 100 BID, but not until 160 BID on 5 days out of 7 schedule. Although 5 days out of 7 seems to be better tolerated, it is not completely clear (due to limited patient numbers) whether this is really due to the 'rest break' or to a lower total dose (4).

Given the possibility of a combined treatment toxicity of dasatinib with paclitaxel, the data from the Phase I and II trials, and the fact that other trials of tyrosine kinase inhibitors in combination with chemotherapy have found better tolerance when dosing is omitted at the time of the chemotherapy pulse, we will plan to start the trial at a continuous dosing schedule of dasatinib, but then switch to the *alternate* schedule of 5 days per week if the MTD is exceeded at that particular dose-level. If tolerated, then dose-escalation will resume on the intermittent schedule. (see Section 4.3)

3.5 Correlative Studies:

3.5.1 Pharmacogenomic Identification

Validation of putative predictive markers remains a major challenge in oncology. Bristol-Myers Squibb (BMS) has developed promising pharmacogenomic markers of response to dasatinib in preclinical models of cancer. Using *in vitro* assays, 7 of 23 well-characterized breast cancer cell lines were shown to be relatively sensitive to dasatinib (IC50 $\leq$ 1μM). It was noted that these were predominantly those known to be of 'basal-like' subtype. Pharmacogenomic analysis demonstrated a 161-gene subset that correlated with growth inhibition by dasatinib. Several genes in this subset, including EphA2 (see Section 3.5.4) were shown to be modulated *in vitro* by exposure to dasatinib. The existence of this expression-defined gene subset was demonstrated in a large number of patient samples and found to consist largely of 'triple-negative' cancers.
Similar results, i.e. identification of the triple-negative subtype as that likely inhibited by

dasatinib, were obtained independently and using somewhat different methodologies by investigators both at UCLA and at Duke. (16)
Furthermore, a 6-gene subset was identified which correlated with *in vitro* response, and was then validated as a predictor in a separate set of 12 breast cancer cell lines, (thus 11 of 35 cell lines were sensitive *in toto*). The receiver operating characteristics of this predictor set were sensitivity 75%, specificity 100%, positive predictive value 100%,negative predictive value 89%. Similarly-small (and partly-overlapping) gene subsets were also identified independently by investigators at UCLA and at Duke. The 'triple-negative' subset with basal-like phenotype (cytokeratin-positive) was again identified by these expression subsets.

3.5.2 Role of Tyrosine Kinases in Breast Cancer

Dasatinib is a potent inhibitor of Src-family kinases, EphA2 and other tyrosine kinases strongly implicated in expression of the malignant phenotype. The IC_{50} of dasatinib for Src-family kinases is in the range of 1 nM and that for EphA2 is approximately 17 nM, depending on experimental conditions.

3.5.3 Src-family Kinases

Src-family kinases (SFKs) are a group of closely-related non-receptor tyrosine kinases which participate in numerous cellular signaling pathways. SRC is a non-receptor tyrosine kinase that plays a critical role in cellular proliferation, and SRC family kinases have been implicated in driving the development and progression of human malignancies. The role of Src-family kinases in tumor progression and expression of the metastatic phenotype has been extensively reviewed.(17, 18) Evidence has been developed for multiple specific activities of Src-family kinases, including mediation of growth factor signals, cell-cell and cell-substrate adhesion, functional membrane activation and motility, invasion and migration, and angiogenesis. As this literature is extensive, only a few representative lines of evidence bearing specifically on breast cancer will be summarized here.

It is well established that signaling via ErbB receptors (i.e., EGFR and Her2/neu) involves activation of c-Src.(19, 20) SFK activity was shown to synergize with anchorage-independent heregulin-mediated growth and mediate MAPK pathway activation. (21) A variety of mechanisms have been suggested, including pSrc stabilization as well as activation by Her2/neu (ErbB2), formation of a complex with STAT5b, or "reverse" activation of Her2/neu kinase by the CXCL-12 chemokine via SFK activity. (22, 23) Growth and survival signaling can also occur through direct Src activation by EstrogenReceptor (ER) or Progesterone Receptor (PgR). (24, 25) Other mitogenic pathways require
activation of Src-family kinases; for example, STAT3 signaling appears to be a major downstream effector of Src kinase activation in breast cancers.(26)

Altered structural and cell surface properties are key characteristics of the metastatic phenotype, and include membrane ruffling and podosome formation, loss of anoikis,

changes in cell-cell and cell-basement membrane adhesiveness, motility and invasiveness - all of which are “typical” Src-related phenomena. Although less well studied than mitogenic pathways, the focal adhesion proteins are essential membrane elements implicated in tumorigenesis. (27)
The cadherin/catenin system mediates cell-cell adhesion, loss of which is characteristic of epithelial malignancy. Inhibition of Src kinase activity restores E-cadherin/catenin expression and cell-cell adhesion in breast cancer cells, reducing metastasis.(28) In a series of experiments, Src activity was shown to stimulate phosphorylation of focal adhesion kinase (FAK) and paxillin, suppressing adhesion-dependent apoptosis and increasing expression of α2-integrin. (29) A variety of mechanisms have been advocated, including H_2O_2
stimulation of Src, increased cap-dependent translation, or direct activation of STAT5 by Type IV collagen via Src-dependent pathways. (30)

Caveolins, primary structural components of caveolae (active vesicular invaginations of the cell membrane), are Src kinase substrates. Moreover, *Caveolin-1* and *-2* are two of the six-gene set expression of which correlates with *in vitro* response to dasatinib (Section 26.5.1). In general, caveolin acts as a tumor suppressor, but the relationship between caveolin activation and malignancy is complex. On one hand, mutation or inactivation of caveolin-1 leads to highly metastatic o r invasive phenotype and non-genomic nuclear signaling via ER is markedly stimulated by direct interaction with caveolin-1,even in the absence of ligand. (31) Most recently, direct stimulation of proliferation by progestin and full transcriptional activity of PgR were reported to be mediated by tyrosine phosphorylation of caveolin-1.(32) Consistent with its tumor-suppressor role, inactivating mutations of Cav-1 gene are found in approximately 35% of ER-positive breast cancer samples (more than half of which are the dominant-negative P132L mutation), but in none of the ER-negative samples.(33) On the other hand, an inverse relationship has been reported between caveolin-1 expression and ErbB-mediated signaling. (34)

Finally, Src-family kinases are known to mediate VEGF-induced angiogenesis and vascular permeability.(35) Both VEGF production and downstream VEGF action appear to require Src kinase. (36, 37) Normal endothelial cell Src-family kinase activity (specifically Src or Yes, but not Fyn) appears to be responsible for VEGF-stimulated disruption of the cadherin-catenin complex leading to tumor cell invasion and metastasis. (38)

Pharmacodynamic data with the biomarker phospho-SRC (pSRC) was recently presented, to evaluate the relationship between dasatinib exposure and inhibition of SRC kinase activity. (39) The level of pSRC in peripheral blood mononuclear cells was used in two trials (CA 180002 and CA 180003) as a surrogate biomarker of the level of kinase activity of the SRC family members. On the BID regimen, pSRC inhibition was approximately dose-dependent across the dosing range. SRC family kinase activity was substantially inhibited at the exposures of dasatinib achieved.

3.5.4 EphA2 Kinase

EPH kinases are the largest family of receptor tyrosine kinases, best characterized for their role in embryogenesis, cell migration and cytoskeletal organization, and more recently shown to be involved in malignancy.(40) Overexpression of EphA2 has been demonstrated in breast cancer specimens by several independent investigators.(41) Direct inhibition of EphA2 using monoclonal antibody provided selective reversal of malignant behavior of breast carcinoma cells *in vitro*. (42, 43)

3.5.5 Circulating Tumor Cells (CTCs)

Micrometastasis was originally a theoretical concept: CTC were presumed to exist because cancers spread hematogenously, but by definition CTC were not detectable by standard clinical methods. New and emerging technologies now make it possible to sensitively detect CTC in peripheral blood and bone marrow of patients with cancer. Yet, the biological and clinical meanings of CTC have not been fully determined.

In principle, CTC technologies can be applied to many aspects of cancer management, including detection, staging/prognosis, assessment of treatment response, surveillance during remission, validation of novel therapies, and isolation of tumor cells for molecular analysis. For example, CTC detection may be of particular value in the initial management of breast cancer by better identifying those patients at risk of metastasis, thus allowing more judicious use of chemotherapy. Indeed, multiple studies have now shown that detection of CTC in marrow by immunocytochemistry (ICC) is strongly prognostic of recurrence and death. (44-46)

A number of studies have suggested that the presence of CTCs in patients with metastatic disease is associated with poorer survival and disease burden. (47, 48) Recent studies have quantified CTCs in late stage breast cancer to evaluate prognosis and monitor response to therapy. (49) These studies have found high sensitivity in detecting CTC using immunomagnetic bead enrichment of peripheral blood samples. The threshold for detection is often only a few cells/ml of peripheral blood, however many metastatic patients have > 100 CTC/ml. (50) Using a newly developed technology for automated ICC (CellSearch System), the presence of at least 5 or more CTCs per 7.5 ml of blood at start of treatment and first follow-up in a study of 177 patients with metastatic disease, correlated with a shorter median progression free survival, and overall survival. In a multivariate analysis, CTCs were the most significant independent predictor of progression free and overall survival.

Studies of targeted agents in the treatment of breast cancer have been complicated by the lack of target identification to individualize therapy. The success of specific targeting has been demonstrated in the treatment of HER2 positive cancers with trastuzumab. (51) Evaluating potential targets is particularly difficult in metastatic disease, where novel therapeutics undergo initial testing and generally meet with success or failure - due in part to the difficulty in obtaining initial and serial tissue for study. CTCs provide a readily accessible source of tumor cells for comparison of markers pre- and post-therapy.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

This is an open-label, single arm, dose-escalation phase I-II study to determine the MTD of dasatinib in combination with weekly paclitaxel, and to determine the safety and efficacy of this combination when given to patients with advanced and metastatic breast carcinoma.

4.2 Intervention

A treatment cycle will consist of 28 days, according to the following schedule:

- Dasatinib PO once daily
- Weekly paclitaxel 80 mg/m^2 given intravenously over 1 hour on day 1, 8, and 15 of a 28 day cycle

The trial will initially test the combination of weekly paclitaxel and dasatinib given PO, once daily, continuously. In case of 2 dose-limiting toxicities (DLT) in the first cohort (0), the next cohort will test dasatinib given with a different schedule, 5 days on and 2 days off, omitting dasatinib the day prior and the day of administration of paclitaxel.

4.3 Phase I Design

A standard, three-patient per cohort, dose escalation schedule will be used. Between 6 and 54 patients will likely be necessary to determine the MTD of dasatinib in combination with weekly paclitaxel. There will be no intrapatient dose escalation. The starting dose of dasatinib is 70 mg once daily, continuously (Dose level 0). One dose reduction level have been included, if necessary.

The dose for each cohort in this phase I is presented below:

Cohort	Paclitaxel dose	Dasatinib dose
+ 3	80 mg/m^2 weekly 3/4	150 mg daily continuously
+2	80 mg/m^2 weekly 3/4	120 mg daily continuously
+1	80 mg/m^2 weekly 3/4	100 mg daily continuously
0	80 mg/m^2 weekly 3/4	70 mg daily continuously
-1	80 mg/m^2 weekly 3/4	70 mg daily 5 days out of 7

In the event that the MTD is with dasatinib $\leq$ 100 mg daily given continuously, then the dasatinib dosing of 5 days out of 7 will be explored ; if tolerated, escalation may resume on the intermittent schedule. If dasatinib $\geq$ 120 mg daily continuous is tolerated, there will be no need to investigate the intermittent dose-schedule.

Cohort	Paclitaxel dose	Dasatinib dose
+ 3b	80 mg/m^2 weekly 3/4	150 mg daily 5 days out of 7
+2b	80 mg/m^2 weekly 3/4	120 mg daily 5 days out of 7
+1b	80 mg/m^2 weekly 3/4	100 mg daily 5 days out of 7
-1	80 mg/m^2 weekly 3/4	70 mg daily 5 days out of 7
-2	80 mg/m^2 weekly 3/4	50 mg daily 5 days out of 7

Dose limiting toxicity is defined as:

- Clinically significant grade 3 or 4 non-hematologic toxicity according to the NCI CTC version 3. (for example, not alopecia or nausea and vomit controlled by anti-emetics)
- Grade 3 or 4 hematologic toxicity including neutropenia and thrombocytopenia lasting greater than 2 weeks. Grade 3 or 4 anemia lasting two weeks despite the use of red blood cell growth factor support. Usage of growth factor support will be noted for data analysis.
- Grade 2 non-hematologic toxicity that persists despite adequate medical management and requires withholding dasatinib for more than 4 days in cycle #1.

The following dose escalation scheme will be utilized:

- If none of the initial three patients in a cohort experience dose-limiting toxicity (DLT), then a new cohort of three patients will be treated at the next higher dose level.

- If one of the three patients in a cohort experiences DLT, then up to three additional patients will be treated at the same dose level. Escalation will continue if only one of the six patients experiences DLT.

- If two or more patients in a cohort experience DLT, then the maximum tolerated dose (MTD) will have been exceeded, and no further dose escalation will occur. The previous dose level will be considered as the MTD.

- If the MTD is *exceeded* at any given dose level ≤ 120 mg daily, on the continuous schedule, then *that dose* will be assessed on 5 days per week schedule, corresponding to a 28% reduction in total weekly dose. If tolerated, escalation may resume on the intermittent schedule. (if > 120 mg daily continuous is tolerated, there will be no need to investigate the intermittent dose-schedule).

  Therefore:

  - If two or more patients in cohort 0 (paclitaxel 80 mg/m^2 and dasatinib 70 mg daily continuously), experience DLT, then patients will be enrolled at dose level -1, with paclitaxel 80 mg/m^2 weekly and dasatinib 70 mg **daily 5 days out of 7, omitting dasatinib the day prior and the day of administration of paclitaxel.** If this dosing is tolerated, escalation may resume on the intermittent schedule.

  - Similarly, if two or more patients in cohort 1 (paclitaxel 80 mg/m^2 and dasatinib 100 mg daily continuously), experience DLT, then patients will be enrolled at dose level +1b, with paclitaxel 80 mg/m^2 weekly and dasatinib 100 mg **daily 5 days out of 7, omitting dasatinib the day prior and the day of administration of paclitaxel.** If this dosing is tolerated, escalation may resume on the intermittent schedule.

  - Similarly, if two or more patients in cohort 2 (paclitaxel 80 mg/m^2 and dasatinib 120 mg daily continuously), experience DLT, then patients will be enrolled at dose level +2b, with paclitaxel 80 mg/m^2 weekly and dasatinib 120 mg **daily 5 days out of 7, omitting dasatinib the day prior and the day of administration of paclitaxel.** If this dosing is tolerated, escalation may resume on the intermittent schedule.

- If only three patients were treated at a dose level under consideration as the MTD, then up to three additional patients will be accrued. If no more than one of the six patients at that dose level experiences a DLT, then that dose level will be confirmed as the MTD. If two or more patients in that cohort experience DLT, then the previous dose level will be studied in the same fashion.

- If the MTD is *exceeded* at any given dose level on the continuous schedule, and the previous dose-level had only 3 patients in the cohort, than that cohort will be expanded to 6 patients, to establish MTD on the continuous schedule.

- If greater than or equal to 2 DLTs are experienced by cohort -1 (with dasatinib 70 mg daily 5 days out of 7), then cohort –2 (with dasatinib 50 mg daily 5 days out of 7) will be under consideration for the MTD. Six patients will be accrued at this level to establish the MTD. If one or less DLTs are experienced by cohort –2, then this will be considered the MTD. If 2 or more DLTs occur within cohort –2, then the study will be terminated and the IRB will be notified.

- If the MTD were not to be exceeded, additional dose-escalation steps in the 30% range will be explored.

All patients within a cohort will be observed for toxicity for one cycle (28 days) prior to entering patients at the next dose level.

We do not expect peripheral neuropathy to be a side effect of dasatinib, but rather of paclitaxel. For patients experiencing peripheral neuropathy, the following dose-modification schema can be utilized:

Dose-modification schema for peripheral neuropathy caused by paclitaxel:

Level	0	-1	-2
Dose (mg/m2)	80	70	60

Neuropathy:

Grade	Dose reduction
0-1	No change
2	One level
3	Two levels
4	Two levels or stop therapy

4.4 Phase II design

The phase II portion of this trial has a Simon two-stage design to determine the efficacy of dasatinib when administered in combination with paclitaxel. The eligibility criteria for the phase II study are more restrictive than for the phase I study because patients must have had 0 to 2 prior regimens; these additional eligibility criteria may be found in Section 6.0. Patients treated at the MTD in the Phase I portion of the study will not be included in the Phase II portion, as eligibility criteria are different for the two portions.

Twenty-three patients will initially enter the study. As described further in Section 14 Biostatistics, if there are 3 or fewer responses observed, the study will be terminated early and declared to have a negative result. If 4 or more responses are observed, enrollment will be extended to 55 patients. If $\geq$ 12 responses are observed among the 55 patients, the study will be considered to have a positive result and this regimen would be considered worthy of further testing. patients will be entered into Stage 1 of the phase II trial. Overall, a minimum of 23 patients and a maximum of 55 patients will be entered on the phase II portion of this study.

The phase II study will be conducted at the maximum tolerated dose of dasatinib in combination with paclitaxel, determined upon completion of the phase I portion of the protocol. The treatment schedule will be the same as for the phase I portion of this trial. A treatment cycle will consist of 28 days, according to the following schedule:

Agent	Dose	Route	Treatment days	Interval	Notes
Dasatinib	120 mg	PO	Recommended phase II schedule	28 days	Daily dose once
Paclitaxel	80 mg/m^2	IV	Day 1, 8 and 15	28 days	Weekly 3/4

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1 Dasatinib

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.

5.1.1 Dasatinib Product Identification

Dasatinib will be supplied in two different strengths by BMS.

- 20 mg film-coated tablets, biconvex, round, white to off-white in appearance with"BMS" or "20" debossed on one side and "527" on the other side
- 50 mg film-coated tablets, biconvex, oval, white to off-white in appearance with"BMS" or "50" debossed on one side and "528" on the other side

5.1.2 Packaging and Labeling

Dasatinib will be packaged in bottles as follows:

- dasatinib 20 mg film-coated tablets, 30 tabs/bottle
- dasatinib 50 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.

5.2 Storage, Handling and Dispensing of Dasatinib

5.2.1 Storage

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

5.2.2 Handling and Disposal

Procedures for proper handling and disposal of anticancer drugs should be considered.

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. 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.

5.2.3 Dispensing

It is the responsibility of the Investigator to ensure that dasatinib is only dispensed to study subjects. Dasatinib must be dispensed only from official study sites by authorized personnel according to local regulations.

The Investigator (or assigned designee, i.e., study pharmacist) will dispense the proper number of each strength tablet to the subject to satisfy dosing requirements for the study. The containers provided to the subject should be labeled with proper instructions for use. The lot numbers, dosing start dates and the number of tablets for each dosage strength must be recorded on the drug accountability pages of record for the site. The subject must be instructed to return all unused dasatinib in the provided packaging at each subsequent visit.

5.3 Drug Ordering and Accountability

Drug Supply – Initial Order and Re-supply

Initial Orders of dasatinib are requested by completing the Dasatinib (Sprycel®) Drug Supply Form for Investigator Sponsored Studies and submitting the request form electronically via e-mail at least 5-7 business days before the expected delivery date. Deliveries will be made Tuesday through Friday.

Initial drug supply is provided for a 12-week treatment period per subject.

Send the drug supply form with the subject heading: "BMS CA180-xxx [insert assigned number] Dasatinib Supply Request" to bms.ist@acculogix-usa.com.

Please note: The BMS study ID (CA180-XXX) and "dasatinib" must be recorded in the subject line of the email.

Send re-supply requests to the appropriate email address. Check "Re-supply" on the drug supply form. Re-supply requests should be submitted at least 5-7 business days before the expected delivery date. Deliveries will be made Tuesday through Friday.

5.4 Dasatinib Accountability

It is the responsibility of the Investigator to ensure that a current record of dasatinib disposition is maintained at each study site where dasatinib is inventoried and disposed. Records or logs must comply with applicable regulations and guidelines, and should include:

- Amount received and placed in storage area.
- Amount currently in storage area.
- Label ID number or batch number and use date or expiry date.
- Dates and initials of person responsible for each dasatinib inventory entry/movement.
- Amount dispensed to and returned by each subject, including unique subject identifiers.
- Amount transferred to another area/site for dispensing or storage.
- Non-study disposition (e.g., lost, wasted, broken).
- Amount returned to BMS, if applicable.
- Amount destroyed at study site, if applicable.
- Retain samples sent to third party for bioavailability/bioequivalence, if applicable.

Dasatinib dispensing record/inventory logs and copies of signed packing lists must be maintained at the investigational site. Batch numbers for dasatinib must be recorded in the drug accountability records.

5.5 Destruction of Dasatinib

It is the Investigator's responsibility to ensure that arrangements have been made for disposal and that procedures for proper disposal have been established according to applicable regulations, guidelines, and institutional procedures. Appropriate records of the disposal must be maintained.

Consult with the PI for instructions on disposal of unused dasatinib tablets.

5.6 Paclitaxel

Paclitaxel is a taxane originally derived from the bark of the Pacific yew tree (*Taxus Brevifolia*) one of the most active and commonly used agents for the treatment of breast carcinoma. Paclitaxel binds to the dimeric tubulin preventing microtubule disassembly by stabilizing microtubules so that the spindle cannot be dismantled. This microtubule disruption halts mitosis, interferes with other critical interphase functions and subsequently leads to cell death.

Paclitaxel (as Taxol) is generically available and will not be supplied by Bristol-Myers Squibb Company .

Paclitaxel is a clear colorless to slightly yellow viscous solution. Paclitaxel is supplied as a non-aqueous solution intended for dilution with a suitable parenteral fluid prior to intravenous
infusion. Paclitaxel is available as 30 mg (5 ml) 100 mg (16.7 ml) and 300 mg (50 ml) multiuse vials.

Each mL of sterile nonpyrogenic solution contains 6 mg paclitaxel, 527 mg of purified Cremophor® EL and 49.7% of v/v dehydrated alcohol.

5.6.1 Packaging and Labeling - Paclitaxel

Paclitaxel will be supplied as 100 mg (16.7 ml) multi-use vials. Vials will be labeled according to the respective country regulations and may contain information regarding the product strength, quantity, storage conditions and direction of use. Sites will be responsible for recording the label batch number/lot number of the supplies dispensed on drug accountability forms. Commercial supply for paclitaxel will be utilized for the study.

5.6.2 Storage Requirements/Stability - Paclitaxel (Taxol)

The Investigator should ensure that the investigational product is stored in accordance with the environmental conditions (temperature, light and humidity) as determined by the Sponsor and defined in the SmPC/reference label.

Unopened vials of Taxol (paclitaxel) injection are stable until the date indicated on the package when stored between 20 - 25°C (68 - 77°F), in the original package. Neither freezing nor refrigeration adversely affects the stability of the product. Upon refrigeration components in the paclitaxel vial may precipitate, but will re-dissolve upon reaching room temperature with little or no agitation.

5.6.3 Preparation and Administration - Paclitaxel

Prior to infusion paclitaxel should be diluted in 0.9% Sodium Chloride injection or 5% Dextrose injection or 5% Dextrose and 0.9% Sodium Chloride or 5% Dextrose in ringers lactate to a final concentration of 0.3 to 1.2 mg/mL. Paclitaxel must be prepared in glass, polypropylene or polyolefin containers and non-PVC containing (nitroglycerin) infusion sets. Prepared solutions are stable at room temperature (20 - 25°C (68 - 77°F)) and protected from light, up to 72 hours. In-line filtration with micropore no greater than 0.22 micron filter is required. Chemo dispensing pin devices or similar devices with spikes should not be used with paclitaxel vials since they can cause the stopper to collapse resulting in loss of sterile integrity.

5.6.4 Incompatibilities - Paclitaxel

Data collected for the presence of the extractable plasticizer DEHP [di-(2-ethylhexyl) phthalate] show that levels increase with time and concentration when dilutions are prepared in PVC containers. Consequently, the use of plasticized PVC containers and administration sets is not recommended. Therefore, paclitaxel solutions should be prepared and stored in glass, polypropylene, or polyolefin containers. Non-PVC containing administration sets, such as polyethylene-lined, should be used.

5.6.5 Safety Precautions - Paclitaxel

Paclitaxel does not contain any antimicrobials therefore extreme care must be taken to ensure sterility of the prepared solution.
Subjects should be premedicated to reduce risk of severe hypersensitivity reaction.

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

6.1 Subject Inclusion Criteria

1. Female or male patients with diagnosis of invasive adenocarcinoma of the breast confirmed at MSKCC.

    - **For the phase I portion**, patients with any ER/PR/HER2 disease status, no longer eligible for hormonal therapy or HER2-targeted therapy, will be eligible.

    - **For the phase II portion**, there needs to be documentation of negative HER2 (IHC 0-1+ or FISH/CISH negative) status. Patients with any ER/PR disease status are eligible.

2. A paraffin-embedded tissue block or unstained slides from prior surgery must be available.

3. Evidence of recurrent or progressive locally advanced or metastatic breast cancer

4. Presence of

    - **For the phase I portion:** at least one evaluable or measurable metastatic lesion ,

    - **For the phase II portion**: at least one measurable metastatic lesion according to the RECIST criteria which has not been irradiated (i.e. newly arising lesions in previously irradiated areas are accepted).Ascites, pleural effusion, and bone metastases are not considered measurable. Minimum indicator lesion size: $\geq$ 10 mm measured by spiral CT or $\geq$ 20 mm measured by conventional techniques.

5. Prior therapies:

   - **For the phase I portion**: Any number of prior endocrine or biologic therapies is permitted. In addition, patients may be untreated in the metastatic setting or have received any number of prior cytotoxic regimens**.**
   - **For the phase II portion**: 0-2 prior chemotherapies for metastatic disease are allowed. Prior taxane therapy, either in the adjuvant or in the metastatic setting, either deliver weekly, q 2 weeks or q 3 weeks, will be permitted. Prior therapy with bevacizumab will be allowed. All previous chemotherapy, radiotherapy and intravenous bisphosphonates must have been discontinued at least 3 weeks prior to study entry, 3 weeks also for trastuzumab and bevacizumab. All acute toxic effects (excluding alopecia) of any prior therapy must have resolved to NCI CTC (Version 3) Grade ≤1.

6. Endocrine therapy with an aromatase-inhibitor, SERM (i.e., tamoxifen) or fulvestrant is permitted, however it must be discontinued before enrolling in the study.

7. ECOG performance status of 0 or 1.

8. Age ≥ 18 years old.

9. Adequate Organ Function
   - Total bilirubin ≤ 1.5 times the institutional Upper Limit of Normal (ULN)
   - Hepatic enzymes (AST, ALT) ≤ 2.5 times the institutional ULN
   - Serum Na, K^{+}, Mg^{2+}, Phosphate and Ca^{2+}ε Lower Limit of Normal (LLN)
   - Serum Creatinine ≤ 1.5 time the institutional ULN
   - Neutrophil count, Platelets, both Grade 0-1
   - PT (INR) and PTT Grade 0-1, except for patients on Coumadin or low molecular weight heparin

10. Ability to take oral medication (dasatinib must be swallowed whole)
11. Concomitant Medications:
    - Patient agrees to discontinue St. Johns Wort while receiving dasatinib therapy
    - Patient agrees that IV bisphophonates will be withheld for the first 8 weeks of dasatinib therapy due to risk of hypocalcemia. Concomitant Medications, any of the following should be considered for exclusion:
    - Patient agrees to discontinue QT-prolonging agents strongly associated with Torsades de Pointes (patients must discontinue drug ≥7 days prior to starting dasatinib) such as [see also section 6.2.1]:

- quinidine, procainamide, disopyramide
- amiodarone, sotalol, ibutilide, dofetilide
- erythromycin, clarithromycin
- chlorpromazine, haloperidol, mesoridazine, thioridazine, pimozide
- cisapride, bepridil, droperidol, methadone, arsenic, chloroquine, domperidone, halofantrine, levomethadyl, pentamidine, sparfloxacin, lidoflazine.

- The concomitant use of H2 blockers or proton pump inhibitors with dasatinib is not recommended. The use of antacids should be considered in place of H2 blockers or proton pump inhibitors in patients receiving dasatinib therapy. If antacid therapy is needed, the antacid dose should be administered at least 2 hours prior to or 2 hours after the dose of dasatinib.
- Patient may not be receiving any potent CYP3A4 inhibitors. These are prohibited (patients must discontinue drug ≥7 days prior to starting dasatinib) and include: [see also section 6.2.1]
  - itraconazole, ketoconazole, miconazole, coriconazole
  - amprenavir, atazanavir, fosamprenavir, indinavir, nelfinavir, ritonavir
  - ciprofloxacin, clarithromycin, diclofenac, doxycycline, enoxacin, imatinib, isoniazid
  - ketamine, nefazodone, nicardipine, propofol, quinidine, telithromycin

12. Women of childbearing potential (WOCBP) must have:
    - A negative serum or urine pregnancy test within 72 hours prior to the start of study drug administration
    - Persons of reproductive potential must agree to use an adequate method of contraception throughout treatment and for at least 4 weeks after study drug is stopped
    - Pregnant or nursing women may not participate. Patients of reproductive potential may not participate unless they have agreed to use an effective method of contraception and to continue contraception for 30 days from the date of the last study drug administration. Postmenopausal woman must be amenorrheic for at least 12 months to be considered of non-childbearing potential.
13. Signed written informed consent including a HIPAA form according to institutional guidelines.

6.2 Subject Exclusion Criteria

1. Life expectancy < 3 months.

2. Prior severe allergic reaction to paclitaxel therapy.

3. Presence of new or recurrent pleural effusion which is symptomatic and/or requiring medical intervention (NCI CTC Grade 2, 3, or 4)

4. Completion of previous chemotherapy regimen < 3 weeks prior to the start of study treatment.

5. Prior hormonal therapy must be discontinued prior to treatment start. Biologic therapy (eg, bevacizumab, trastuzumab) for the treatment of metastatic disease must be discontinued ≥3 weeks from the start of protocol treatment.

6. Concurrent medical condition which may increase the risk of toxicity.

Patients may not have any clinically significant cardiovascular disease including the following:

- myocardial infarction or ventricular tachyarrhythmia within 6 months
- prolonged QTc ≥480 msec (Fridericia correction)
- ejection fraction less than institutional normal
- major conduction abnormality (unless a cardiac pacemaker is present)

Patients with any cardiopulmonary symptoms of unknown cause (e.g. shortness of breath, chest pain, etc.) should be evaluated by a baseline echocardiogram with or without stress test as needed in addition to electrocardiogram (EKG) to rule out QTc prolongation. The patient may be referred to a cardiologist at the discretion of the principal investigator. Patients with underlying cardiopulmonary dysfunction should be excluded from the study.

- Subjects with hypokalemia or hypomagnesemia if it cannot be corrected prior to dasatinib administration
- History of significant bleeding disorder unrelated to cancer, including:
  - Diagnosed congenital bleeding disorders (e.g., von Willebrand's disease)
  - Diagnosed acquired bleeding disorder within one year (e.g., acquired anti-factor VIII antibodies)
  - Ongoing or recent (δ 3 months) significant gastrointestinal bleeding
- Other medical condition which in the opinion of the Investigator might confer an unacceptable increase in risk.

7. Patients with symptomatic CNS metastases that remain untreated by radiation therapy are excluded from this trial. The presence of asymptomatic brain metastases or brain metastases that have been previously irradiated are not grounds for trial exclusion.

8. History of uncontrolled seizures, central nervous system disorders or psychiatric disability judged by the investigator to be clinically significant, precluding informed consent, or interfering with compliance of oral drug intake.

9. Presence of uncontrolled gastrointestinal malabsorption syndrome.

10. Unwillingness to give written informed consent or unwillingness to participate or inability to comply with the protocol for the duration of the study. Willingness and ability to comply with scheduled visits, treatment plan, laboratory tests and other study procedures are necessary for participation in this clinical trial.

11. Concurrent radiotherapy is not permitted for disease progression on treatment on protocol, but might allowed for pre-existing non-target lesions with approval from the principal investigator of the trial.

12. Patients with > Grade 1 neuropathy will be excluded form this trial.

6.2.1 Prohibited and Restricted Therapies During Study

6.2.1.1 Prohibited Therapies

No other systemic therapy for treatment of breast cancer is permitted while subject is on study.

Subjects requiring any of the following prohibited therapies should not be enrolled. If enrolled, the prohibited agent(s) must be withdrawn prior to first dose of study drug:

Bisphosphonates

Intravenous bisphosphonates will be withheld for the first 8 weeks of treatment due to the risk of hypocalcemia. After the need for Ca^{2+} supplementation has been assessed and levels documented to be > LLN, subjects on prior bisphosphonate may be restarted with caution at investigator discretion.

QT-prolonging agents strongly associated with Torsades de Pointes

Patients must discontinue drug ≥7 days prior to starting dasatinib such as:

- quinidine, procainamide, disopyramide
- amiodarone, sotalol, ibutilide, dofetilide
- erythromycin, clarithromycin
- chlorpromazine, haloperidol, mesoridazine, thioridazine, pimozide
- cisapride, bepridil, droperidol, methadone, arsenic, chloroquine, domperidone, halofantrine, levomethadyl, pentamidine, sparfloxacin, lidoflazine

Potent CYP3A4 inhibitors

Dasatinib is primarily metabolized by the CYP3A4 enzyme. In drug-drug interaction studies, concomitant use of ketoconazole (a potent CYP3A4 inhibitor) produced an increase of >5-fold in

dasatinib exposure. Therefore, potent inhibitors of CYP3A4 are prohibited during study; for such medications, a wash-out period of ≥7 days is required prior to starting dasatinib (less potent inhibitors, inducers and substrates of CYP3A4 are restricted, see section 6.2.1.2). Subjects should be advised not to consume substantial quantities of grapefruit or pomegranate juice.

Most commonly-used potent CYP3A4 inhibitors are:

- itraconazole, ketoconazole, miconazole, voriconazole
- amprenavir, atazanavir, fosamprenavir, indinavir, nelfinavir, ritonavir
- ciprofloxacin, clarithromycin, diclofenac, doxycycline, enoxacin, imatinib, isoniazid
- ketamine, nefazodone, nicardipine, propofol, quinidine, telithromycin

St. John's wort (Hypericum perforatum) may decrease dasatinib plasma concentrations unpredictably. Patients receiving dasatinib should not take St. John's wort.

6.2.1.2 Restricted Therapies

Restricted treatments are allowed to be used during the study. However, they should be used with caution when medically indicated.

Medications that inhibit platelet function or anticoagulants:

Caution should be exercised if subjects are required to take medications that inhibit platelet function or anticoagulants. Antiplatelet agents or anticoagulants should be avoided in the setting of Grade 3 or 4 thrombocytopenia.

Medications that prolong QT Interval:

Ideally, subjects enrolled in this study should not begin taking other medications known to prolong the QT interval. Information on where to find a partial list of medications known to prolong the QT interval is found at http://www.azcert.org/medical-pros/drug-lists/bycategory.cfm.

However, should the investigator believe that beginning therapy with a potentially QT prolonging medication (other than the ones explicitly prohibited) is vital to an individual subject's care, then additional ECG(s) will be done at the investigator's discretion to ensure the subject's safety.

As stated above, subjects enrolled in this study should not take or begin to take concomitant medications **strongly** associated with Torsades de Pointes such as:

- quinidine, procainamide, disopyramide
- amiodarone, sotalol, ibutilide, dofetilide

- erythromycin, clarithromycin
- chlorpromazine, haloperidol, mesoridazine, thioridazine, pimozide
- cisapride, bepridil, droperidol, methadone, arsenic, chloroquine, domperidone, halofantrine, levomethadyl, pentamidine, sparfloxacin, lidoflazine

CYP3A4 Inducers, Inhibitors and Substrates

Caution is warranted when administering dasatinib to subjects taking drugs that are highly dependent on CYP3A4 for metabolism and have a narrow therapeutic index. (See Appendix C).

Dasatinib is a CYP3A4 substrate. Drugs that induce CYP3A4 activity may decrease dasatinib plasma concentrations. In patients in whom CYP3A4 inducers (eg, dexamethasone, phenytoin, carbamazepine, rifampicin, phenobarbital) are indicated, alternative agents with less enzyme induction potential should be preferred.

Concomitant use of dasatinib and drugs that inhibit CYP3A4 (e.g., ketoconazole, itraconazole, erythromycin, clarithromycin, ritonavir, atazanavir, indinavir, nefazodone, nelfinavir, saquinavir, telithromycin) may increase exposure to dasatinib.

CYP3A4 substrates known to have a narrow therapeutic index such as alfentanil, astemizole, terfenadine, cisapride, cyclosporine, fentanyl, pimozide, quinidine, sirolimus, tacrolimus, or ergot alkaloids (ergotamine, dihydroergotamine) should be administered with caution in patients receiving dasatinib.

Potent CYP3A4 inhibitors are prohibited (See Section 6.2.1.1). Less-potent inhibitors, inducers, and substrates of CYP3A4 are restricted (see also Appendix C).

In patients receiving treatment with dasatinib, close monitoring for toxicity and a dasatinib dose reduction should be considered if systemic administration of a potent CYP3A4 inhibitor cannot be avoided.

Subjects should be advised not to consume substantial quantities of grapefruit juice.

Antacids

Nonclinical data demonstrate that the solubility of dasatinib is pH dependent. Simultaneous administration of dasatinib with antacids should be avoided. If antacid therapy is needed, the antacid dose should be administered at least 2 hours prior to or 2 hours after the dose of dasatinib. Medicines that neutralize stomach acid, such as MAALOX® (aluminum hydroxide/magnesium hydroxide),

TUMS® (calcium carbonate), or ROLAIDS® (calcium carbonate and magnesia) may be taken up to 2 hours before or 2 hours after dasatinib. (1)

H2 Blockers/Proton Pump Inhibitors

Long-term suppression of gastric acid secretion by H_2 blockers or proton pump inhibitors (eg, famotidine and omeprazole) is likely to reduce dasatinib exposure. The concomitant use of H_2 blockers or proton pump inhibitors with dasatinib is not recommended. The use of antacids should be considered in place of H_2 blockers or proton pump inhibitors in patients receiving dasatinib therapy.

6.2.1.3 Other Restrictions and Precautions

Based on pre-clinical data, dasatinib might increase the likelihood of bleeding. Hence, subjects undergoing surgical procedures, including dental procedures, should be instructed to inform their doctors of this potential increased risk.

7.0 RECRUITMENT PLAN

Patients will be recruited through the outpatient clinics of the Breast Cancer Medicine Service of Memorial Sloan-Kettering Cancer Center. All patients will be under the care of attending medical oncologists of MSKCC. Both men and women and members of all ethnic groups are eligible for this trial.

Patients will be required to sign a statement of informed consent that meets the requirements of the code of Federal Regulations (Federal Register Vol. 46, No. 17, Jan. 27, 1981, part 50) and the IRB of this center. A consent form is appended. The medical record will include a statement that written informed consent was obtained (and documented in the record the date written consent was obtained before) and the patient is enrolled in the study.

The estimated number of study participants is between 29 and 109 patients. The target population estimates have been based upon 109 patients participating. As the incidence of breast cancer in men is rare, exact numbers for enrollment have not been provided, although men are not excluded from this trial. Efforts to maintain similar proportions to that of the female study participants will be made.

8.0 PRETREATMENT EVALUATION

- Diagnosis of invasive adenocarcinoma of the breast confirmed at MSKCC by histology.

- Baseline extent of disease evaluation up to 4 weeks prior to treatment on Cycle #1 including

- CT scans of the chest, abdomen and pelvis with oral and intravenous contrast (if not contraindicated)
- Bone scan. Target lesions according to RECIST criteria should be identified at this time.

- Baseline demographics and medical history will be collected within 4 weeks of initiating study treatment. These data will include but is not limited to confirmation of breast pathology review at MSKCC, menopausal status, estrogen receptor status, progesterone receptor status, Her2 status (by immunohistochemistry or FISH), prior treatment history (including: chemotherapy, hormone therapy, trastuzumab, bevacizumab and radiation).
- Baseline history and physical examination within 14 days of treatment initiation including ECOG performance status assessment, blood pressure, pulse, temperature, weight and height.
- Baseline laboratory/cardiac assessment may be performed up to 14 days prior to treatment including:
  - CBC
  - Comprehensive metabolic panel
  - Serum or urine pregnancy test, if applicable
  - EKG
  - PT/PTT
  - Magnesium
  - Phosphorous

9.0 TREATMENT/INTERVENTION PLAN

9.1 Phase I Study

A treatment cycle consists of 28 days, according to the following schedule:

Agent	Route	Treatment days	Interval	Notes
Dasatinib	PO	Per cohort schedule (either continuously or 5 days out of 7)	28 days	Daily dose once
Paclitaxel 80 mg/m^2	IV	1, 8, 15	28 days	Weekly 3/4

9.1.1 Dasatinib

Dasatinib will be administered orally and is expected to be taken as an outpatient. No premedications are required for the oral administration of dasatinib as hypersensitivity reactions are not anticipated.

Dasatinib will be administered in the Phase I dose escalation study at a starting total daily dose of 70 mg DAILY, on a continuous schedule. The dose of dasatinib will be fixed, not calculated on the basis of body surface area.

As explained in Section 4.3, if the MTD is with dasatinib $\leq$ 100 mg daily given continuously, then *that dose* will be assessed on 5 days per week schedule, corresponding to a 28% reduction in total weekly dose. In that case dasatinib tablets will be administered 5 days a week, starting the day after the administration of paclitaxel, and stopping two days before the following administration of paclitaxel. (i.e. Day 1 paclitaxel, Day 2-6 dasatinib, Day 7 off). During the week off (4th week of every treatment cycle), patients will continue with dasatinib daily, stopping two days prior the administration of the next dose of paclitaxel (Day 1 of following cycle).

The total daily dose of dasatinib should be taken once daily and may be taken with meals. Patients will be provided with a diary in which to record study drug intake. Dosing times may be adjusted if required. If doses are missed for toxicity, they should not be replaced. If a dose is not taken due to an error, it may be taken up to 12 hours later**.** If vomiting occurs within 30 minutes of intake, that dose may be repeated.

Both gastrointestinal tolerance and drug absorption may be improved if dasatinib is taken with a light meal; there is no requirement, however, to administer with food.
Based on pre-clinical data, dasatinib may inhibit platelet aggregation and uncommon instances of increased bleeding risk have been observed. Dasatinib should be interrupted for 1 day before and 1 - 2 days after any surgical (including dental) procedure or until adequate hemostasis is achieved.

Patients will keep a drug diary (Appendix D) to document degree of compliance with dasatinib therapy. For phase I patients within the first two cycles of treatment, the exact number of pills necessary for 2 weeks of treatment will be dispensed to patients at one time. For phase II patients, the exact number of pills necessary for one cycle (four weeks) of treatment will be dispensed to patients at one time.

Patients should be evaluated for signs and symptoms of underlying cardiopulmonary disease prior to initiating dasatinib and during treatment. Symptoms of pulmonary arterial hypertension (PAH) include dyspnea, fatigue, hypoxia, and edema. Since other medical conditions may also cause these symptoms, non-invasive procedures (including echocardiogram) should be done first to rule out more the common etiologies of these symptoms, such as pleural effusion, pulmonary edema, anemia, and lung infiltration. Right heart catherization can confirm the diagnosis of PAH. Hypertension is "pre-capillary" and not a consequence of left heart failure or chronic lung disease if there is normal pulmonary capillary wedge pressure (<15 mm Hg) but elevated pulmonary artery pressure (mean pulmonary artery pressure >25 mm Hg). Since PAH may be reversible upon discontinuation of dasatinib, a diagnostic approach of interruption of dasatinib treatment may be considered at the discreation of the treating physician; however, if PAH is confirmed, dasatinib should be permanently discontinued.

9.1.2 Paclitaxel

Body surface area (BSA) should be calculated at the start of therapy (Day 1 of Cycle 1). In calculating surface areas actual heights and weights should be used; that is, there should be no adjustment to "ideal" weight.

Paclitaxel will be administered as a 1 hour IV infusion on Days 1, 8 and 15 in a 28 day cycle, provided the subject meets the re-treatment criteria. The initial dose will be 80 mg/m^2.

Due to the potential for allergic reactions to paclitaxel and/or the Cremophor vehicle, all subjects should be premedicated prior to each paclitaxel administration to prevent or reduce the risk of severe hypersensitivity reactions.

• Pre-medicate subject 30 - 60 minutes prior to paclitaxel administration with Dexamethasone 10 mg IV, Diphenhydramine 50 mg IV and Cimetidine (300 mg IV) or other H2 receptor antagonist (e.g. Ranitidine 50 mg IV). It is also acceptable to utilize the investigative sites standard pre-medication regimen for paclitaxel dosing. If no allergic reaction occurs after the first 3 doses of paclitaxel, it is acceptable to modify the pre-medication regimen at the discretion of the investigator.

9.2 Phase II Study

A treatment cycle consists of 28 days, according to the following schedule:

Agent	Route	Treatment days	Interval	Notes
Dasatinib	PO	120 mg	28 days	Daily dose once
Paclitaxel 80 mg/m^2	IV	1, 8, 15	28 days	Weekly 3/4

The Phase II dasatinib dose will be the maximum tolerated dose and schedule determined in the Phase I study. Paclitaxel will be administered at 80 mg/m^2 weekly on day 1, 8 and 15 of a 28 day cycle.

Dasatinib and paclitaxel will be administered with the modalities described above.

Patients enrolled on this trial may be asked to undergo additional radiographic studies performed solely for the purpose of this research. In the event that a patient has a complete response (CR), confirmatory imaging (4 weeks after imaging which documented CR) is requested per RECIST criteria.

The duration of patient treatment will depend on individual response and tolerance. Patients with clearly documented progressive disease by RECIST criteria will be taken off treatment at time of progression. Patients who are responding (complete or partial), or whose disease is stable, will be

treated until progression of disease, intolerable toxicity, patient refusal to continue with study, or investigator decision to remove patient from study. (Definitions of response, progressive disease and stable disease are found in Section 12.0 Criteria for Therapeutic Response/Outcome Assessment).

All patients who receive at least one full cycle (4 weeks) of dasatinib and paclitaxel will be evaluable for response.

9.3 Correlative Studies

Correlative studies will be performed in II portion of the study and will include:

1. Analysis of mRNA expression on the tumor specimens, for a gene expression profiling of the responders versus non-responders, in order to identify potential predictors of response to dasatinib. If differentially interesting candidates emerge, immunohistochemistry assays including EphA2, IGFBP2, caveolin and phospho-caveolin and other potential predictive markers and SFK substrates as available will also be performed.

2. Exploratory tumor biomarker data: assays of p-SRC, VEGFR2, and Collagen Type IV (or N-Telopeptide NTX) in plasma, obtained at baseline and after 2 cycles of treatment (8 weeks), will be performed by enzyme-linked immunosorbent assay.

3. Circulating tumor cells (CTC) at baseline and after 2 cycles of treatment (8 weeks).

4. Exploratory somatic gene mutations detection in archived tumor samples.

9.3.1 Analysis of mRNA expression

Total RNA will be isolated using TRIzol (Invitrogen, Carlsbad, CA), passed over a Qiagen RNeasy column (Qiagen, Valencia, CA), and double-stranded cDNA will be synthesized by a chimeric oligonucleotide with an oligo-dT and a T7 RNA polymerase promoter. Reverse transcription will be then carried out, followed by biotin labeling and approximately 250-fold linear amplification by *in vitro* transcription (Enzo Biochem, New York, NY). From each sample, ~5 micrograms of labeled cRNA will be hybridized onto an Affymetrix U133 GeneChip.

Tissue will be analyzed for expression of mRNA. If differentially interesting candidates emerge, we will also perform immunohistochemistry assays including EphA2, IGFBP2, caveolin and phospho-caveolin and other potential predictive markers and SFK substrates as available. These data will be integrated with those from other studies to explore the relationship between tumor phenotype and response to dasatinib.

9.3.1.1 Methods

Analysis of mRNA expression will be performed at Memorial Sloan Kettering Cancer Center. A paraffin block of either a metastatic lesion or the primary breast lesion biopsy specimen will be

forwarded to Dr. Jorge Reis-Filho at the address below. If a paraffin block is unavailable, 10 or more unstained slides may be sent for analysis.

Jorge Reis-Filho, MD, PhD
Memorial Sloan Kettering Cancer Center
C/O Research Team
300 E 66th Street, 9th Floor
New York, NY 10065

9.3.2 Assays of p-SRC, VEGFR2, and Collagen Type IV

The level of p-SRC in peripheral blood mononuclear cells (PBMCs) will be determined by enzyme-linked immunosorbent assay, and will be used a surrogate biomarker of the level of kinase activity of the SRC family members.

Assays of VEGFR2 and Collagen Type IV in plasma, obtained at baseline and after 2 cycles of treatment, will be performed by enzyme-linked immunosorbent assay.

9.3.2.1 p-SRC

Specimens for determination of the level of p-SRC in peripheral blood mononuclear cells (PBMCs) will be shipped to Targeted Molecular Diagnostics lab.

9.3.2.1.1 Methods, p-SRC

Two 8-mL CPT blood collection tubes manufactured by BD (blue/black top) will be collected from patients. These tubes are provided by Targeted Molecular Diagnostics. MSKCC will

- Isolate the PBMCs
- Spin down to a pellet
- Snap freeze and ship on dry ice to:

Quintiles - Westmont
Attention: Janice Spohn
777 Oakmont Lane, Suite 100
Westmont, IL 60559
www.quintiles.com
Phone: 630-203-6224

9.3.2.2 VEGFR2

MSKCC will each perform VEGFR2 and collagen Type IV on the specimens of their respective patients.

9.3.2.2.1 Methods, VEGFR2

VEGFR2 is assayed in heparin plasma (EDTA tube); collagen Type IV (or N-telopeptide- NTX) is measured in serum (red and black top tube). Approximately 15 cc of whole blood will be drawn. Samples at MSKCC will be sent to Quest Labs for analysis at baseline and after 2 cycles of treatment.

9.3.3 Circulating tumor cells (CTC)

The measurement of CTC enumeration will be performed with a semiautomated system, CellSearch, which uses an EpCAM antibody-based immunomagnetic antibody-capture technology developed by Immunicon Corporation. Blood collected in CellSave tubes will be processed on the CellTracks AutoPrep System with the CellSearch Circulating Tumor Cell Kit which is intended for the enumeration of CTC of epithelial origin (CD45-, EpCAM+, and cytokeratins 8, 18+, and/or 19+) in whole blood. A magnetic field is used to collect cells of interest without centrifugation. After unbound supernatant is removed, the enriched samples are processed for nucleic acid staining with DAPI and for markers for epithelial cells with anti-cytokeratin CK-PE. Leukocytes are excluded with an anti-CD45 immunofluorescent antibody. Stained cells are analyzed on a fluorescence microscope using the Cell Track Analyzer II and CTC are defined as cytokeratin positive, DAPI nucleated cells lacking CD45 markers. Automatically selected images are reviewed by the operator for identification as tumor cells and quality control is maintained via standard procedures.

9.3.3.1 Methods, CTCs

MSKCC and will each perform these assays on the specimens of their respective patients. For the measurement of the circulating epithelial cells, 10 cc of whole blood will be drawn into Cell Save Tubes (purple and yellow tiger top tubes). The tubes are to be kept at room temperature and shipped to the MSKCC Clinical Chemistry Research Laboratory.

9.3.4 Somatic Gene Mutations Detection

Next generation sequencing analyses will be coordinated in Jorge Reis-Filho's laboratory (MSKCC, Pathology), involving Francois-Clement Bidard (Visiting investigator, SKI/Pathology), Kiu Yan Charlotte Ng (Post doctoral research fellow, SKI/Pathology) and Britta Weigelt (Assistant Member, Pathology).

9.3.4.1 Methods, Next Generation Sequencing

DNA will be extracted from the following materials, whenever available:

- archived tumor tissue (primary breast cancer and/or metastasis), after microdissection
- previously collected plasma samples

- previously collected peripheral blood mononuclear cells (PBMC)

No supplementary biopsy or blood sampling will be required for this correlative study, which will use previously collected samples from the above mentioned correlative studies. Next generation sequencing analyses will be performed in the Genomics Core Laboratory. Tumor specific point mutations, small insertions and deletions, and somatic rearrangements will be detected by comparing tumor DNA (from tumor tissue and/plasma) to its paired normal DNA (from PBMC) and using a series of algorithms to identify somatic events, as previously described (Shah *et al*, Nature 2012; Stephens *et al*, Nature 2012; Banerji *et al*, Nature 2012). Germline information obtained by means of massively parallel sequencing will only be employed in this study for the purpose of comparison to the tumor genome. Using computational algorithms, those variant calls in the tumor that are also present in the germline will be removed, or "masked" from output reports to investigators. VCF or other files of germline data will not be separately analyzed in the future without additional informed consent, anonymization, and/or human subjects review by the IRB.

Once the repertoire of somatic mutations and the mutation allelic frequency of each mutation in tumor tissue and/or plasma are defined, we will seek any somatic mutation that may be associated with a specific pattern of sensitivity to the treatments tested in this clinical trial.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

A number of measures will be taken to ensure the safety of patients participating in this trial. These measures will be addressed through exclusion criteria and routine monitoring.

Patients enrolled in this study will be evaluated clinically and with standard laboratory tests before and at regular intervals during their participation in this study, as detailed in the table below.

For the phase I portion of the study, safety evaluations will be performed every 2 weeks and will consist of:

- medical interviews (+/- 1 day)
- recording of adverse events (+/- 1 day)
- review of diary of study drug (+/- 1 day)
- physical examinations with vital signs (blood pressure, pulse, temperature), height and body weight and ECOG performance status. (+/- 1 day)
- laboratory measurements. (CBC will be performed weekly) (+/- 1 day)

For the phase II portion of the trial, patients will be evaluated every four weeks. Safety evaluations will consist of:

- medical interviews (+/- 1 day)

- recording of adverse events (+/- 1 day)
- review of diary of study drug (+/- 1 day)
- physical examinations with vital signs (blood pressure, pulse, temperature), height and body weight, and ECOG performance status. (+/- 1 day)
- laboratory measurements. (CBC will be performed weekly only on weeks that paclitaxel is administered +/- 1 day)

Patients will be evaluated for adverse events (all grades), serious adverse events, and adverse events requiring study drug interruption or discontinuation at each study visit for the duration of their participation in the study.

Patients will be provided with a diary in which to record their intake of study drug.

Patients will be assessed radiographically for tumor response after the first two cycles of treatment (Cycle #2, Week #4), and every three cycles thereafter (eg, Cycle #5, Week 4; Cycle #8, Week 4; etc.). Follow-up imaging should be performed according to the same protocol as the baseline assessment of the target lesions. However, after the pre-study evaluation, the bone scan is only required at the treating physician's discretion.

- In responding patients, the response should be confirmed a minimum of 4 weeks after the first response has been recorded, if possible.

- Tumor assessments should be made on each patient within 2 weeks of study completion, withdrawal or discontinuation, as appropriate, even if the patient demonstrates physical evidence of progressive disease and withdraws from the study before the first scheduled tumor assessment.
- End-of-Study Evaluation: At the time patients are removed from study, laboratory (eg, CBC, CMP) and clinical evaluations to assess adverse events (AEs) will be performed. Radiologic studies for antitumor response will be repeated if they have not been done within the previous 28 days.

10.1 Patients discontinued from the treatment phase of the study for any reason will be evaluated with a clinical visit ~30days (28–42 days) after the decision to discontinue treatment.

Time and Events Schedule: Phase II

Study Parameter	Prestudy (Day –28 to 1)	Prestudy (Day -14 to 1)	CYCLE 1-CYCLE 2				≥ CYCLE 3 [g]				Study Termination
			Day 1 (+/- 1 day)	Day 8 (+/- 1 day)	Day 15 (+/- 1 day)	Day 22 (+/- 1 day)	Day 1 (+/- 1 day)	Day 8 (+/- 1 day)	Day 15 (+/- 1 day)	Day 22-28 (+/- 1 day)	
Informed consent	X										
Pathology review	X										
History	X		X				X				X
Pill count and diary review							X				
Physical examination			X				X				X
Vital signs[b]			X				X				X
Height and body weight			X				X				X
ECOG performance status			X				X				X
Tumor assessment (clinical)			X				X				X
Tumor assessment (radiological)	X[a]									X[d,e]	X
Toxicity assessment by NCI CTC v.3			X				X				X
CBC with differential, platelet count		X		X	X		X	X	X		X
Serum Chemistry[h]		X	X[i]				X				X
PT/PTT		X									
EKG		X	X[i]				X				
Pregnancy test[c]		X									
Correlative blood samples		X								X[f]	
Analysis of mRNA expression on the tumor specimens											X
Protocol Therapy			T +D*	T+D	T+D	D	T+D	T+D	T+D	D	

* T= paclitaxel

D= dasatinib

Amended: 06/15/15

IRB PB

a Radiographic tumor assessment should include CT scan chest, abdomen and pelvis and bone scan to determine extent of disease and identify target lesions to be used for judging response per RECIST criteria. After pre-study evaluation, a bone scan is only needed per the physician's discretion. If target lesion best visualized using MRI, then subsequent tumor assessment must utilize this same imaging modality (see d). A chest x-ray can be performed as the discretion of the investigator.

b Vital signs include blood pressure, pulse and temperature.

c Pregnancy test may be from serum or urine.

d Tumor assessments performed after the first 2 cycles, and then every 3 cycles: Cycle 2, Cycle 5 , etc. All assessments obtained during the study should be made using the same types of study, e.g., CT scan, as was used at baseline. If possible, the same equipment should be used.

e Only the indicator lesions need to be assessed every 3 cycles, unless a complete response is claimed, in which case the patient must undergo a complete assessment of all sites of disease.

f Blood samples for correlative studies will be drawn at baseline and after 2 cycles of therapy (day 1 of cycle 3) on the phase II portion of the study only.

g During the phase I trial, patients will be evaluated every two weeks; during the phase II trial, patients will be evaluated every 4 weeks.

h Serum Chemistry includes Na, K, Cl, CO2, BUN, Creat, Ca, Glu, T. Protein, Alb, Alk Phos, T. Bili, AST, ALT, Magnesium, and Phosphorous.

i The serum chemistry and EKG are not required for cycle 1, Day 1 as they were assessed in pre-study workup.

11.0 TOXICITIES/SIDE EFFECTS

This study will use the NCI Common Toxicity Criteria (CTC) version 3.0 for toxicity.

11.1 Dasatinib Toxicity

Myelosuppression

Treatment with dasatinib 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. In a Phase 3 dose-optimization study in patients with chronic phase CML, Grade 3 or 4 myelosuppression was reported less frequently in patients treated with 100 mg once daily than in patients treated with 70 mg twice daily. (1)

Bleeding Related Events

In addition to causing thrombocytopenia in human subjects, dasatinib caused platelet dysfunction *in vitro*. In all clinical studies, severe central nervous system (CNS) hemorrhages, including fatalities, occurred in <1% of patients receiving dasatinib. Severe gastrointestinal hemorrhage occurred in 4% of patients and generally required treatment interruptions and transfusions. Other cases of severe hemorrhage occurred in 2% 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. In some trials, the use of anticoagulants, aspirin, and non-steroidal anti-inflammatory drugs (NSAIDs) was allowed concurrently with dasatinib if the platelet count was >50,000. Caution should be exercised if patients are required to take medications that inhibit platelet function or anticoagulants. (1)

Fluid Retention

Dasatinib is associated with fluid retention. In all clinical studies, severe fluid retention was reported in 8% 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% of patients. 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.

In the Phase 3 dose-optimization study in patients with chronic phase CML, fluid retention events were reported less frequently in patients treated with 100 mg once daily than in patients treated with 70 mg twice daily. (1)

The following guidelines may be helpful:

- Chest x-rays are recommended if the subject presents with symptoms suggestive of pleural effusion
- Early institution of diuresis – eg, furosemide 20-40 mg po daily and/or spironolactione 25-50 mg po daily, titrate to symptoms
- Management of symptomatic pleural effusion with thoracentesis
- For symptomatic pleural effusion, study drug interruption and administration of corticosteroids (e.g. prednisone, 40 mg/day for 3– 5 days, with taper) has been effective in some patients.
- Dose reduction may be necessary if fluid retention or pleural effusion is severe or recurrent
- Complaints of chest discomfort - echocardiogram should be performed to investigate possible pericardial effusion
- Severe or recurrent pleural effusions – interrupt dosing, permit recovery to Grade 0-1, and restart dosing at appropriate dose level

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. (1)

Other side effects might include:

- Fatigue
- Gastrointestinal side effects**,** including nausea, vomiting or diarrhea, loss of appetite, abdominal pain, constipation, or bloating
- Muscle and joint aches
- Headaches
- Skin rashes
- Fever
- Dizziness, confusion, depression, tiredness, sleepiness, trouble sleeping, tingling or numbness in the arms or legs, changes in taste, and ringing in ears have been seen in patients taking dasatinib**.**

11.2 Paclitaxel Toxicity

Side-effects include alopecia, myelosuppression, fatigue, neuropathy, arthralgia, myalgia,
onycholysis, taste changes, amenorrhea, teratogenesis, hypersensitivity (hypotension, flushing, chest pain, abdominal or extremity pain, skin reactions, pruritus, dyspnea, bronchospasm, and tachycardia), sinus bradycardia, complete heart block, sinus tachycardia,
premature ventricular beats, ventricular tachycardia, bigeminy, syncope, myocardial infarction, hypotension, hypertension, dizziness, visual changes, radiation recall, abdominal
pain, diarrhea, typhlitis, ischemic colitis, abnormal liver function, pancreatitis, abnormal triglyceride, and seizures.

11.3 Chemotherapy Delay

Treatment may be delayed no more than 3 weeks to allow recovery from acute toxicity. If toxicity requires a dosing delay/interruption of more than three weeks, the patient will be withdrawn from the study for toxicity reasons. If treatment is delayed for 2 consecutive weeks and is ready to resume treatment on day 22 of a cycle, the patient should advance to the next cycle and start a new cycle. Patients that require discontinuation of dasatinib, but who have not progressed, should continue paclitaxel until unacceptable toxicity/progression. These patients should be counted (intent to treat) towards endpoints such as progression-free survival.

11.4 Dose modification for paclitaxel

This study will use the NCI Common Toxicity Criteria (CTC) version 3.0 for toxicity. If a patient develops multiple toxicities, delay treatment or modify dose based on the greatest indicated dose reduction or delay. Dose re-escalations are not allowed.

- If on the day that paclitaxel is due, platelet counts are <100, 000/μL and/or ANC <1,000/μL and/or non-hematologic toxicities (excluding alopecia, fatigue, peripheral neuropathy, nausea and arthralgias, and hepatic toxicities, described below) have not recovered to δGrade 1, treatment should be delayed by up to 1 week and CBC and toxicity grading repeated weekly. For hepatic toxicities (including ALT, AST, and total bilirubin), if the patient develops any hepatic grade 3 or 4 toxicity thought to be related to study therapy, paclitaxel may be held until the toxicity resolves, at the physician's discretion. If hematologic and non-hematologic toxicity have not recovered, a further delay of up to one week is allowed. <u>If a chemotherapy treatment delay of >3 consecutive weeks is required, the patient will be removed from the study.</u>

<u>Other non-hematologic toxicities:</u> For patients who experience peripheral neuropathy, paclitaxel may be dose-reduced following the schema presented in Section 4.3.

- <u>Hypersensitivity Reactions</u>: If a hypersensitivity reaction occurs symptoms such as flushing, skin reactions, dyspnea, hypotension, or tachycardia may require temporary

interruption of the infusion. However, severe reactions, such as hypotension requiring treatment, dyspnea requiring bronchodilators, angioedema or generalized urticaria require immediate discontinuation of paclitaxel and aggressive symptomatic therapy. Patients who develop severe hypersensitivity reactions may be re-challenged following premedication that the institution would typically use. (Appendix B) There are no dose reductions for hypersensitivity reactions. Consider discontinuation of therapy if patient experiences 2 episodes of Grade 3 hypersensitivity reaction to paclitaxel.

11.5 Dose modification for dasatinib

11.5.1 Dose modification for dasatinib in the phase I trial:

See also Section 4.3. Dasatinib dose may be withheld for any adverse event if the investigator feels it is due to dasatinib, and/or not well-controlled by medications, and/or still present at the time of visit.

If the dasatinib was held for a DLT at Cycle#1, the patient will be removed from study. If, in subsequent cycles, a patient develops a Grade 2 or 3 adverse event felt to be related to dasatinib, and the investigator deems the patient is benefiting from study therapy, dasatinib might be held and then resumed either at full dose, or at the reduced dosing of the previous cohort.

11.5.2 Dose modification for dasatinib in the phase II trial:

Dasatinib dosage may be adjusted so that any drug-related toxicity is adequately managed with outpatient therapy or deemed by the physician as clinically acceptable.

The dose adjustment of dasatinib in case of toxicity will follow the dosing schedule schema presented for the phase I portion of the study. For example, if the phase II dosing of dasatinib is 120 mg daily continuously, and a patient develops a toxicity related to dasatinib, the dosing may be modified as follows:

Grade	
0-1	No change
2	Not adequately managed with outpatient therapy and/or deemed to be clinically-unacceptable: decrease one dosing level (to 100 mg daily continuously) at the physician's discretion
3	Not adequately managed with outpatient therapy and/or deemed to be clinically unacceptable: decrease one dosing level (to 100 mg daily continuously) or two dosing levels (to 70 mg daily continuously) at physician's discretion
4	Decrease two levels or stop therapy at the physician's discretion

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

All patients who receive at least one dose of dasatinib or paclitaxel will be evaluable for toxicity. All patients who receive at least one full cycle of dasatinib and paclitaxel (4 weeks) will be evaluable for MTD assessment and response.

12.1 Primary Endpoint

Phase I Portion

The primary endpoint of the phase I portion of the trial is the maximum tolerated dose (MTD) of dasatinib when given in conjunction with paclitaxel. The MTD is defined as the highest dose studied for which the incidence of DLT is less than 33%. The MTD will be determined as described in Section 4.3 Phase I Design.

Phase II Portion

The primary endpoint of the phase II trial is the overall response rate of dasatinib plus paclitaxel, for patients with HER2 negative metastatic breast carcinoma. This is defined as the percentage of patients who achieve either an objective complete or partial target lesion response that is confirmed based on the RECIST criteria as described in Appendix E (Response Evaluation Criteria in Solid Tumors (RECIST). Only measurable lesions which have not been irradiated will be used as indicator lesions. These must have a minimum size of at least one diameter of 10 mm for liver, soft tissue lesions, lung, skin and lymph node lesions.

12.2 Secondary Endpoints

Phase I Portion

- To obtain preliminary data on the therapeutic activity of dasatinib when administered in combination with weekly paclitaxel.

Phase II Portion:

- To obtain safety and tolerability of dasatinib when administered in combination with weekly paclitaxel.
- To estimate secondary efficacy endpoints of this combination including clinical benefit (CR+PR+SD > 6 months), time to tumor progression (TTP), and progression free survival (TTP) and duration of response.
- To obtain exploratory tumor biomarker data: assays of p-SRC, VEGFR2 and Collagen Type IV (NTX) in plasma, obtained at baseline and after 2 cycles of treatment (8 weeks), will be performed by enzyme-linked immunosorbent assay.
- To perform analysis of mRNA expression on the tumor specimens, for a gene expression profiling of the responders versus non-responders, in order to identify potential predictors of response to dasatinib. If differentially interesting

candidates emerge, immunohistochemistry assays including EphA2, IGFBP2, caveolin and phospho-caveolin and other potential predictive markers and SFK substrates as available will also be performed.

- To collect circulating tumor cells (CTC) at baseline and after 2 cycles of treatment (8 weeks).
- To correlate somatic genetic alterations with tumor response.

Definitions:

- Clinical benefit
  Clinical benefit is defined as the sum of the percentage of patients achieving complete response, partial response and stable disease for greater than 6 months (as defined by RECIST criteria)
- Time to tumor progression
  Time to disease progression (TTP) will be measured from the start of treatment to the time the patient is first recorded as having disease progression. If a patient never progresses while being followed, the patient will be censored at the time he or she terminates the study or by the date of death. Patients who die without progression of disease will be censored at time of death.
- Duration of response
  Duration of response is measured from the time measurement criteria are met for CR/PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

A secondary endpoint of the phase II trial is to assess the overall safety profile of this regimen. The toxicity rate will be reported. The type, frequency, severity, timing and relationship of each toxicity will be determined as per the NCI Common Toxicity Criteria, version 3.

Safety & tolerability measures include but are not limited to:

- the incidence of treatment-emergent adverse events (AEs) and serious adverse events (SAEs)
- hematologic toxicity including neutropenia, anemia and thrombocytopenia
- non-hematological toxicity changes from baseline in hematology and clinical chemistry values
- physical examination
- the incidence of patients experiencing dose modifications, dose interruptions, and/or premature discontinuation of study drug.

13.0 CRITERIA FOR REMOVAL FROM STUDY

Patients have a right to withdraw from the study at any time for any reason. The investigator also has the right to withdraw patients from the study in the event of intercurrent illness, adverse events, and treatment failure or protocol violations. As an excessive rate of withdrawals can

render the study un-interpretable, unnecessary withdrawals should be avoided. When a patient discontinues treatment early, the investigator will make every effort to contact the patient and to perform a final evaluation. The reason for withdrawal will be recorded. Criteria for terminating participation in the study are as follows:

1. Intercurrent illness which would in the judgment of the investigator effect assessments of clinical status to a significant degree or require discontinuation of drug.

2. Severe or unacceptable toxicity.

3. Progressive disease

4. Request by the patient to withdraw.

5. General or specific changes in the patient's condition that would render the patient ineligible for further treatment according to the inclusion/exclusion criteria.

6. Administrative reasons (including but not limited to): non-compliance with medication, failure to return for follow up visits, termination of the clinical trial by the investigator.

7. Chemotherapy treatment delay of >3 consecutive weeks.

8. If at any time the patient is found to be ineligible for the protocol as designated in the section on Criteria for Patient/Subject Eligibility (i.e., a change in diagnosis), the patient will be removed from the study.

Patients who receive additional therapy or deviate from the treatment program by adding another breast cancer directed agent, undergoing surgery or receiving radiation for an identifiable lesion or substantially modify the dosage or schedule as prescribed will be considered a major protocol violation.

14.0 BIOSTATISTICS

This is a multi-center (two centers), open-label phase I/II trial with MSKCC serving as the lead center. The objective of the phase I portion of the trial is to determine the MTD of dasatinib when given in combination with a fixed dose of weekly paclitaxel. Dasatinib will be dose escalated to determine the MTD using a standard, three-patient per cohort dose escalation schedule. The starting dose of dasatinib is 70 mg daily, given continuously (Dose level 0). Three dose escalations with 2 schedules (continuous and intermittent) from dose level 0 are possible. If the MTD is with dasatinib < 100 mg daily given continuously, then *that dose* will be assessed on 5 days per week schedule, corresponding to a 28% reduction in total weekly dose. If tolerated, escalation may resume on the intermittent schedule. One dose reduction level has been included, if necessary. Between 6 and 54 patients will be required to determine the MTD of dasatinib. There will be no intrapatient dose escalation. Details of the dose escalation strategy are provided in section 4.3.

It is expected that the maximum of 54 patients for the Phase I portion of the study will be enrolled in less than one year, between the two centers. This portion of the study will be open to all patients with metastatic breast carcinoma, regardless of ER/PR/HER2 status and number of prior regimens. Summary statistics such as mean, median, counts and proportions will be calculated for all secondary analyses.

Once the MTD for dasatinib has been identified, additional patients will be enrolled into the Phase II portion of the study to determine the efficacy and of dasatinib at the MTD when administered in combination with weekly paclitaxel. Efficacy will be measured in terms of response rate as assessed after two cycles of therapy. The target population for the Phase II portion of the study will be metastatic breast cancer patients who have had 0 to 2 prior regimens and have HER2 negative disease. Early studies on breast cancer therapy have shown that paclitaxel as monotherapy induces response rates ranging from 20 - 50 %. (16, 17) However, more recent randomized trials have reported more modest response rates, in the range of 14-16%, likely due to the fact that more patients are receiving overall more chemotherapy in the adjuvant setting. (18, 19) Based on these data, we have chosen a 15% as the response rate under which the therapy of dasatinib with paclitaxel will not be considered for further investigation. We have chosen a response rate of 30% where the dasatinib with paclitaxel regimen will be considered for further study.

Simon's two-stage optimal design will be used to test the null hypothesis of a 15% response rate against the alternative of a 30% response rate. Setting both the Type I and Type II errors at 10%, 23 patients will initially enter the study. If there are 3 or fewer responses observed, the study will be terminated early and declared to have a negative result. If 4 or more responses are observed, enrollment will be extended to 55 patients. If 12 or more responses are observed among the 55 patients, the study will be considered to have a positive result and this regimen will be considered worthy of further testing. For this design, the probability of early termination is 54% if the true response probability is $\leq$ 15%. Upon completion of the study, the true response rate will be estimated via the observed response rate and an exact confidence interval will be constructed.

There are several secondary analyses planned for the Phase II portion of this study. Selected non-hematologic and hematologic toxicities, as measured by the NCI CTCAE Version 3.0, will be described by frequency and grade, by cycle and over all cycles, with the maximum grade over all cycles used as the summary measure per patient. Clinical benefit will be estimated via the observed rate and exact 95% confidence interval while time to tumor progression and progression free survival will be estimated using Kaplan-Meier methodology.

For all other secondary endpoints, such as of p-SRC, VEGFR2, Collagen Type IV, CTC and mRNA expression analysis on tumor specimens, summary statistics such as mean, median, counts and proportions will be calculated. If required, summary statistics will be calculated and graphed by cycle to uncover any temporal trends. No formal statistical analysis on the secondary endpoints will be done.

For the Phase II portion of the study, accrual of a maximum of 55 patients will take close to one year with anticipated enrollment of 4 patients per month over the two centers. In total, both Phase I and Phase II portions of this study will take a maximum of 3 years to complete.

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. Registrations must be submitted via the PPR Electronic Registration System (http://ppr/). The completed signature page of the written consent/RA or verbal script/RA, a completed Eligibility Checklist and other relevant documents must be uploaded via the PPR Electronic Registration System.

16.0 DATA MANAGEMENT

16.1 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled "Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials" which can be found at: http://cancertrials.nci.nih.gov/researchers/dsm/index.html. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at: *http://mskweb2.mskcc.org/irb/index.htm*

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, Plus, there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Center's Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) will be addressed and the monitoring procedures will be established at the time of protocol activation.

16.2 Regulatory Documentation

Prior to implementing this protocol at MSKCC, the protocol, informed consent form, HIPAA authorization and any other information pertaining to participants must be approved by the MSKCC Institutional Review Board/Privacy Board (IRB/PB).

17.0 PROTECTION OF HUMAN SUBJECTS

This study will be conducted in compliance with the protocol, Good Clinical Practice (GCP), Guidelines of the International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, and in full compliance with the World Medical Association Declaration of Helsinki and its most recent amendments.

Participation in this study is entirely voluntary on the part of the patient. During the process of informed consent, the potential risks and benefits of trial participation will be discussed with the patient. Alternative treatment options will be reviewed as well. Patients who have enrolled on study have a right to withdraw from the protocol at any time for any reason as described in Section 13.0 Criteria for Removal from Study.

Regarding the inclusion of children in research, this protocol/project does not include children because the occurrance of metastatic breast cancer in children is rare and because the majority of these affected children are already accessed by a nationwide pediatric cancer research network. This statement is based on exclusion 4b of the NIH Policy and Guidelines on the Inclusion of Children as Participants in Research Involving Human Subjects.

17.1 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board.

17.2 Serious Adverse Event (SAE) Reporting

Any SAE must be reported to the IRB as soon as possible but no later than 5 calendar days. The IRB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at sae@mskcc.org. The report should contain the following information:

Fields populated from the CRDB:

- Subject's name (generate the report with only initials if it will be sent outside of MSKCC)
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following information:
  - A explanation of how the AE was handled
  - A description of the subject's condition
  - Indication if the subject remains on the study
  - If an amendment will need to be made to the protocol and/or consent form

The PI's signature and the date it was signed are required on the completed report.

17.2.1 Adverse Events

An Adverse Event (AE) is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of dasatinib whether or not considered related to dasatinib.

During clinical trials, adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. (In order to prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more adverse events.)

A ***serious AE*** is any untoward medical occurrence that at any dose:

- results in death,
- is life-threatening (defined as 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 causes prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect,

- results in the development of drug dependency or drug abuse,
- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the patient or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.) For reporting purposes, also consider the occurrences of pregnancy or overdose (regardless of adverse outcome) as events which must be reported as important medical events.

NOTE:
• Hospitalizations (exceptions): Criteria for hospitalizations not reported as SAEs include admissions for:

– Planned as per protocol medical/surgical procedure
– Routine health assessment requiring admission for baseline/trending of health status documentation (e.g., routine colonoscopy)
– Medical/surgical admission for purpose other than remedying ill health state (planned prior to entry into study trial; appropriate documentation required)
– Admission encountered for other life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g. lack of housing, economic inadequacy, care-giver respite, family circumstances, administrative)

17.2.3 Reporting of SAEs

Following the subject's written consent to participate in the study, all SAEs should be collected and reported, including those thought to be associated with clinical trial procedures. Following study completion, any SAE thought to be related to study drug or clinical trial procedures should also be reported to BMS.

SAE terminology and severity grading will be based on (*i.e. CTCAEv3*).

The following categories and definitions of causal relationship to study drug should be considered for use for all clinical studies supported by BMS:

- **Certain:** There is a known causal relationship between the study drug and the SAE. The event responds to withdrawal of study drug (dechallenge), and recurs with rechallenge when clinically feasible. (>95% certainty)
- **Probable:** There is reasonable causal relationship between the study drug and the SAE. The event responds to dechallenge. Rechallenge is not required. (65%-95% probability)
- **Possible:** There is reasonable causal relationship between the study drug and the SAE. Dechallenge information is lacking or unclear. (35%-65% probability of relatedness)

- **Not likely:** There is temporal relationship to study drug administration, but there is not a reasonable causal relationship between the study drug and the SAE. (5-35% probability of relatedness)
- **Not related:** There is not a temporal relationship to study drug administration (too early, or late, or study drug not taken), or there is known causal relationship between the SAE and another drug, concurrent disease, or other circumstance. (<5% chance of relatedness)

- Adverse events classified as "serious" require expeditious handling and reporting to BMS to comply with regulatory requirements.
- All SAEs whether related or unrelated to dasatinib, must be immediately reported to BMS (by the investigator or designee) within 24 hours of becoming aware of the event. If only limited information is initially available, follow-up reports are required. The original SAE form must be kept on file at the study site.

All SAEs should be faxed or emailed to BMS at:

Global Pharmacovigilance & Epidemiology
Bristol-Myers Squibb Company
Fax Number: 609-818-3804
Email: Worldwide.safety@bms.com

- Collection of complete information concerning SAEs is extremely important. Full descriptions of each event will be followed by BMS. Thus, follow-up information which becomes available as the SAE evolves, as well as supporting documentation (e.g., hospital discharge summaries and autopsy reports), should be collected subsequently, if not available at the time of the initial report, and immediately sent using the same procedure as the initial SAE report.
- An overdose is defined as the accidental or intentional ingestion of any dose of a product that is considered both excessive and medically important. For reporting purposes, BMS considers an overdose, regardless of adverse outcome, as an important medical event.
- AEs should be followed to resolution or stabilization, and reported as SAEs if they become serious. This also applies to subjects experiencing AEs that cause interruption or discontinuation of dasatinib, or those experiencing AEs that are present at the end of their participation in the study; such subjects should receive post-treatment follow-up as appropriate.
- In BMS supported trials, all SAEs must be collected which occur within 30 days of discontinuation of dosing or completion of the patient's participation in the study if the last scheduled visit occurs at a later time. In addition, the Investigator should notify BMS of any SAE that may occur after this time period which they believe to be certainly, probably, or possibly related to dasatinib.

17.3 Safety Reports

- MSKCC must submit safety reports to the MSKCC IRB/PB according to institutional guidelines.

18.0 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.
Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

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20.0 APPENDICES

Appendix A ECOG Performance Status
Appendix B Management of Acute Hypersensitivity For Paclitaxel
Appendix C Prohibited and Restricted Concomitant Medications with Dasatinib
Appendix D RECIST Criteria

APPENDIX A

ECOG PERFORMANCE STATUS SCALE

Grade	Description
0	Fully active, able to carry out all normal activity without restriction (Karnofsky 100)
1	Restricted in physically strenuous activity but ambulatory and able to carry out light work (Karnofsky 90-80)
2	Ambulatory and capable of all self-care but unable to carry out any work. Up and about more than 50% of waking hours (Karnofsky 70-60)
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours (Karnofsky 50-40)
4	Completely disabled. Cannot carry out any self-care. Totally confined to bed or chair (Karnofsky 30-20)
5	Death

APPENDIX B

MANAGEMENT OF ACUTE HYPERSENSITIVITY

Severity of Symptoms	Treatment Guidelines
Mild symptoms: localized cutaneous reactions such as mild pruritus, flushing, rash	· consider decreasing the rate of infusion until recovery from symptoms, stay at bedside and monitor patient · then, complete taxane infusion at the initial planned rate
Moderate symptoms: any symptom that is not listed above (mild symptoms) or below (severe symptoms) such as generalized pruritus, flushing, rash, dyspnea, hypotension with systolic BP > 80 mm Hg	· interrupt taxane infusion · give diphenhydramine 50 mg IV with or without dexamethasone 10 mg IV; monitor patient until resolution of symptoms · resume taxane infusion after recovery of symptoms; depending on the physician's assessment of the patient, taxane infusion should be resumed at a slower rate, then increased incrementally to the initial planned rate, *(eg. infuse at an 8 hour rate for 5 minutes, then at a 4-h rate for 5 minutes, and then finally, resume at the 3-h infusion rate)* · depending on the intensity of the reaction observed, additional oral or IV premedication with an antihistamine should also be given for the next cycle of treatment, and the rate of infusion should be decreased initially and then increased back to the initial planned rate, *(eg. infuse at an 8 hour rate for 5 minutes, then at a 4-h rate for 5 minutes, and finally, administer at the 3-h infusion rate)*
Severe symptoms: any reaction such as bronchospasm, generalized urticaria, systolic BP ≤ 80mm Hg, angioedema	· immediately discontinue taxane infusion · give diphenhydramine 50 mg IV with or without dexamethasone 10 mg IV and/or epinephrine as needed; monitor patient until resolution of symptoms · the same treatment guidelines outlined under moderate symptoms (i.e. the third and fourth bullets) should be followed.
Anaphylaxis (NCI grade 4 reaction)	· **NO FURTHER STUDY DRUG THERAPY**

APPENDIX C

KNOWN INDUCERS & INHIBITORS OF ISOENZYME CYP3A4

Inducers	
Carbamazepine Dexamethasone Ethosuximide **Glucocorticoids** Griseofulvin Nafcillin Nelfinavir Nevirapine Oxcarbazepine Phenobarbital Phenylbutazone	Phenytoin Primidone Progesterone Rifabutin Rifampin Rofecoxib (mild) St John's wort Sulfadimidine Sulfinpyrazone
Inhibitors	

Amiodarone	Ketoconazole
Anastrozole	Metronidazole
Azithromycin	Mibefradil
Cannabinoids	Miconazole (moderate)
Cimetidine	Nefazodone
Clarithromycin	Nelfinavir
Clotrimazole	Nevirapine
Cyclosporine	Norfloxacin
Danazol	Norfluoxetine
Delavirdine	Omeprazole (weak)
Dexamethasone	Oxiconazole
Diethyldithiocarbamate	Paroxetine (weak)
Diltiazem	Propoxyphene
Dirithromycin	Quinidine
Disulfiram	Quinine
Entacapone (high dose)	Quinupristin and dalfopristin
Erythromycin	Ranitidine
Ethinyl estradiol	Ritonavir
Fluconazole (weak)	Saquinavir
Fluoxetine	Sertindole
Fluvoxamine	Sertraline
Gestodene	Troglitazone
Grapefruit juice	Troleandomycin
Indinavir	Valproic acid (weak)
Isoniazid	Verapamil
Itraconazole	Zafirlukast
	Zileuton

Concomitant use of H2 blockers or Proton Pump inhibitors:
The concomitant use of H2 blockers or proton pump inhibitors with dasatinib is not recommended. The use of antacids should be considered in place of H2 blockers or proton pump inhibitors in patients receiving dasatinib therapy. If antacid therapy is needed, the antacid dose should be administered at least 2 hours prior to or 2 hours after the dose of dasatinib.

APPENDIX D

RECIST CRITERIA

Tumor measurement

- Measurable disease: the presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.
- Measurable lesions: lesions that can be accurately measured in at least one dimension with the longest diameter ε2.0 cm. With a spiral CT scan, the lesion must be ε1.0 cm in at least one dimension.
- Non-measurable lesions: all other lesions, including small lesions (longest diameter <2.0 cm with conventional techniques or <1.0 cm with spiral CT scans) and other non-measurable

lesions. These include: bone lesions; leptomeningeal disease; ascites; pleural/pericardial effusion; inflammatory breast disease; lymphangitis cutis/pulmonis; abdominal masses that are not confirmed and followed by imaging techniques; and cystic lesions.

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

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules, palpable lymph nodes). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesions is recommended.

Baseline documentation of target and non-target lesions

- All measurable lesions up to a maximum of 10 lesions representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline.
- Target lesions should be selected on the basis of their size (lesions with the longer diameter) and their suitability for accurate repetitive measurements (either by imaging techniques or clinically).
- A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.
- All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as "present" or "absent."

Response criteria

Evaluation of target lesions

- Complete response (CR) disappearance of all target lesions.
- Partial response (PR) at least a 30% decrease in the sum of the LD of the target lesions taking as reference the baseline sum LD.
- Progression (PD) at least a 20% increase in the sum of the LD of the target lesions taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.
- Stable disease (SD) neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD since the treatment started.

Evaluation of non-target lesions

- Complete response (CR) disappearance of all non-target lesions and normalization of tumor marker level.
- Non-complete response (non-CR)/non-progression (non-PD) persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal limits.

- Progressive disease (PD) appearance of one or more new lesions. Unequivocal progression of existing non-target lesions. Although a clear progression of non-target lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed later on by a review panel (or study chair/primary investigator).

Evaluation of best overall response

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

Table 6: Evaluation of best overall response

Target lesions	Non-target lesions	New lesions	Overall response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

- Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "symptomatic deterioration." Every effort should be made to document the objective progression even after discontinuation of treatment.
- In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends upon this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

Confirmation

- To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat studies that should be performed no less than 4 weeks after the criteria for response are first met.
- In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of 6-8 weeks.