Phase I/II Study of Dasatinib in Combination with Zoledronic Acid for the Treatment of Breast Cancer Bone Metastasis

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Stacy Moulder, M.D., M.S.C.I.
Breast Medical Oncology, Unit 1354
The University of Texas M.D. Anderson Cancer Center
P.O. Box 301438
Houston, TX 77230-1439

Physical address:
1155 Pressler Street
Houston, TX 77030

Telephone:
(713) 792-2817

Fax:
(713) 794-4385

email:
smoulder@mdanderson.org

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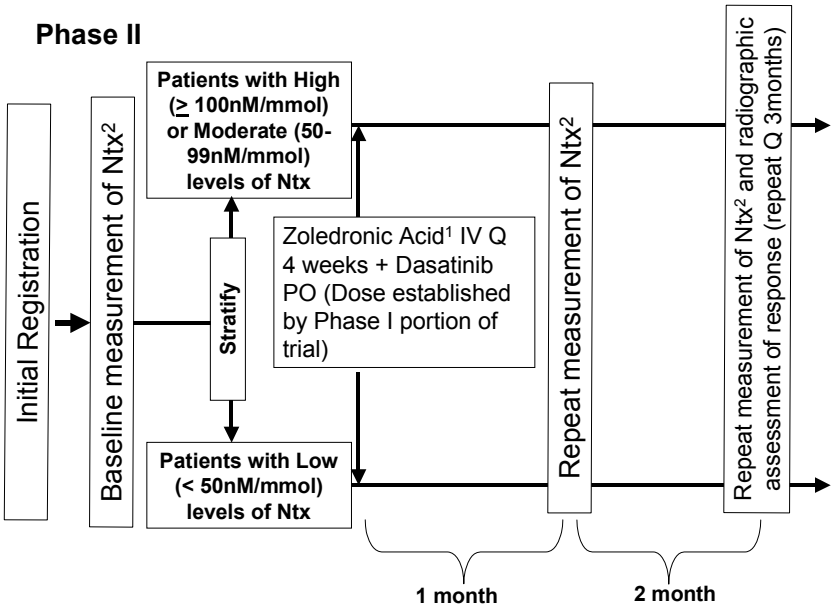
Co-principal Investigator:	**Marjorie Green, M.D.**
Co-investigators:	**Gabriel Hortobagyi, M.D.** **Lajos Pusztai, M.D**.
Statistician:	**Kristine Broglio/Marcy Johnson, M.S.**
Imaging Collaborator:	**Juri Gelovani, M.D., PhD**
Laboratory Collaborator:	**Bryant G. Darnay, PhD**

TABLE OF CONTENTS

PROTOCOL SYNOPSIS

<table>
<tr><td>Protocol Title:</td><td>Phase I/II Study of Dasatinib in Combination with Zoledronic Acid for the Treatment of Breast Cancer Bone Metastasis</td></tr>
<tr><td># Sites and Names:</td><td>Multicenter: M.D. Anderson Cancer Center, University of Chicago, and Duke University</td></tr>
<tr><td>Phase I Study Schema:</td><td>
Phase I Dose Escalation Schema
<table>
<tr><td><u>Dosing cohort 1:</u>
•Three patients will be initially treated in cohort 1.
•If ≤ 1 out of 3 patients in cohort 1 develop DLT, 3 additional patients will be accrued to cohort 2.
•If If ≤ 1 out of 3 patients in cohort 2 develop DLT, 3 additional patients will be accrued.
•If ≥ 2 out of 3 or ≥ 2 out of 6 patients develop DLT, further accrual will continue until 6 patients are treated at the next lower dosing cohort.
•If ≤ 1 out of 6 patients develop DLT, this dose level will be deemed the recommended phase 2 dose (RP2D) and the phase II portion of the trial will open to accrual.</td></tr>
<tr><td>Dosing Cohort –1 :
•Three patients will be initially treated
•If ≤ 1 out of 3 patients develop DLT, 3 additional patients will be accrued.
•If ≤ 1 out of 6 patients develop DLT, this dose level will be deemed RP2D and the phase II portion of the trial will open to accrual.
•If ≥ 2 out of 3 or 2 out of 6 patients develop DLT, the study will be closed due to intolerability of the combination</td></tr>
</table>
Phase I Drug Administration
<table>
<tr><th>Dosing Cohort</th><th>Dasatinib</th><th>Zoledronic Acid</th></tr>
<tr><td>-1</td><td>70 mg po daily</td><td>Standard dosing (see section 7.3.2)</td></tr>
<tr><td>1</td><td>100 mg po daily</td><td>Standard dosing (see section 7.3.2)</td></tr>
</table>
</td></tr>
</table>

Phase II Study Schema	 1. Zoledronic acid to be administered at standard doses as defined in section 7.3.2. 2. Ntx= N-telopeptide of Type I Collagen
Study Design:	This is an open label, Phase I/II, dose escalation study to evaluate the safety and tolerabilit of dasatanib in combination with a standard 28-day cycle of zoledronic acid in patients wit metastatic breast cancer to bone. Zoledronic acid will be administered as a 15-minute infusion on day 1(+/- 48 hours) of 28-day cycle. The dose of zoledronic acid administered will be calculated using th standard dosing guidelines outlined in section 7.3.2. Dasatinib will be taken by mouth using a daily dosing schedule on days 1-28 of each cyc (i.e. continuous dosing). The primary objective of the phase I portion of this trial will be to determine the RP2D fo dasatinib in combination with zoledronic acid. The first 3 patients will be treated with 100 mg of dasatinib daily in combination with fu dose zoledronic acid. If ≤ 1 patient develop(s) DLT within cycle one of therapy, then additional patients will be accrued. If ≤ 1 of 6 enrolled patients experiences DLT, then this will be deemed the RP2D an accrual will begin for the phase 2 portion of the trial. If ≥ 2 patients develop DLT during initial accrual to any cohort (n=3) or during the dos expansion (n=6), further accrual will continue at the next lower dosing cohort until a total o six patients are treated at the lower dosing cohort. If ≥ 2 patients develop DLT during accrual to cohort –1, the study will be closed due t

intolerability.

Definition of Dose Limiting Toxicity

Toxicity will be assessed using the NCI Common Toxicity Criteria (CTC) Scale, versio 3.0.

By definition a DLT must occur during cycle 1, be suspected as being drug-related, an includes any of the following:

- Any grade 4 non-hematological toxicity
- Grade 4 neutropenia lasting > 7 days or any febrile neutropenia
- Grade 3 non-hematological toxicity that persists >72 hours despite adequa supportive care
- Grade 2 or greater neutropenia (ANC<$1.5x10^9$/L) or thrombocytopen (PLT<$75x10^9$/L) which fails to revert to ≤grade 1 (level at which treatment permitted) by the time of the scheduled start of cycle 2
- 50% decrease in calculated creatinine clearance
- Diagnosis of osteonecrosis of the jaw

Phase II Study Design

The dose identified as the RP2D in the phase I portion of this study will be evaluated fo efficacy in the phase II portion of this trial. The primary objective of this phase II study is t assess the efficacy (as defined by disease response) of dasatinib in combination wit zoledronic acid. Patients will be divided into two cohorts dependant upon Ntx leve obtained prior to the initiation of therapy. All patients will receive same therapy, lab draw and radiographic assessment for response.

Patients treated at the RP2D in the phase I portion of this study will be included in the efficacy analysis for the phase II portion of the trial.

Up to 25 patients will be enrolled in this phase II study to assess the efficacy of dasatinib in combination with zometa (zoledronic acid). The primary endpoint is the response rate. Th trial will be conducted by the Simon's two-stage design using the minimax criterion and th response rate will be estimated accordingly [1].

It is assumed that dasatinib in combination with zometa will have a target response rate of 25%. A response rate of 5% or lower is considered a failure. When the probability of accepting a "bad" regimen (i.e. response rate ≤ 5%) is 0.05 and the probability of rejecting "good" regimen (i.e. response rate ≥ 25%) is 0.10, Simon's design to minimize the maximum sample size requires 15 patients in the first stage. If no patients respond to the treatment, the trial will be stopped and the regimen will be declared as ineffective. If at least one of the first 15 patients respond to the treatment, 10 additional patients will be entered in the study to reach a total of 25 patients. By the end of the study, the new regime will be rejected if response rate is less than or equal to 3 out of 25 patients and will be

	accepted otherwise. The operating characteristics of the trial are given as follows. When the true response rate is 0.05, the probability of stopping the trial early is 46.3%. On the other hand, if the true response rate is 0.25, the probability to stop the trial early is 1.3%. The expected sample sizes are 20.37 and 24.97 when the true response rates are 0.05 and 0.25, respectively. At the end of the study, the response rate and the 95% confidence interval will be reported and the toxicity profile of the regimen will be summarized. If the trial continues to the second stage (i.e., accrual = 25) and the 5 of 25 patients response for a point estimate of 20.0%, the exact binomial 95% confidence interval would be (6.8%, 40.7%).
Accrual Goal: (Total number of patients)	6-28 patients 6-18 patients may be enrolled in the phase I portion of the trial. In the phase II portion of the trial, 25 patients will be needed to establish efficacy. We will accrue an additional 10% of patients, a maximum of 28, to account for inevaluable patients
Accrual Rate: (Number of patients expected per month)	2-3 patients/month
FPFV: **LPLV:** **Follow Up:** **(dd-mm-yy)**	11/06 11/10 Follow up will be completed on all patients by 11/30/11
Trial Objectives: **(Primary and Secondary)**	**Primary Objectives:** Phase I: To determine the maximum tolerated dose (MTD) and recommended phase II dose (RP2D) for dasatinib in combination with zoledronic acid. Phase II: Determine the efficacy of dasatinib in combination with zoledronic acid usin disease response. **Secondary Objectives:** Phase I: To determine toxicity of combination therapy with dasatinib and zoledronic acid in patients with breast cancer and bone metastasis. Phase II: • Changes in Ntx levels will be correlated with traditional measurements of tumo response in bone (using WHO criteria), progression free survival and overa survival at 2 years • Biologic characterization of response using ^{18}F-FDG PET • Determination of molecular correlates of response in paraffin embedded or froze tissue • Determine time to tumor progression, response, time to tumor respons

	progression free survival (PFS) up to 2 years • Determine overall survival (OS) at 2 years • Banking of blood for future studies of novel markers of bone health • Determine changes in urinary Ntx with protocol therapy • Correlate response with tumor estrogen receptor (ER), progesterone receptor (PR and HER2 status
Correlative Studies:	**Biologic Characterization of Response Using ^{18}F-FDG PET:** Characterization of response using PET will be performed through collaboration with D Juri Gelovani, chair of the Department of Experimental Diagnostic Imaging in the Divisio of Diagnostic Imaging at MDACC. Patients who consent to participate will underg optional ^{18}F-FDG PET imaging at baseline, between days 12-16 of therapy (2 week value and at disease progression. The fold change from baseline will be calculated for the 2 wee value and progression of disease value. These values will then be graphed using scatterplo against urine Ntx to assess the relationship of response to this biologic endpoint. I addition, it is known that variations in ^{18}F-FDG PET avidity can occur within different fo of disease in individual patients. These variations may represent biologic difference between responding and non-responding cells; therefore, core needle biopsies will b performed on a subset of patients who consent to biopsy of ≥2 sites of metastatic tumo that differ in ^{18}F-FDG PET uptake (see Molecular Correlates below). **Determination of Molecular Correlates in Paraffin Embedded or Frozen Tissue:** Tumor biopsies will be obtained by imaging (CT or Ultrasound) guided core needle biops from patients who sign informed consent for tissue biopsy. Biopsies will be obtained at pr treatment and at day 28 of cycle 1 (+/- 48 hours). Tumor samples will be analyzed fo biomarkers of SRC, PDGFR, c-kit, FAK, and EPHA2 total kinase and phosphorylate activity, including Caveolin, Phospho-Caeolin, IGFBP2 and pSTAT3 using standar immunohistochemistry techniques. Furthermore, markers of angiogenesis VEGF and IL- will also be analyzed using the same techniques. Fresh tissue obtained by core needle biopsy of metastatic sites differing in PET avidity i individual patients (see above) will be snap frozen and used for microarray analysis t determine potential pathways of drug resistance. When possible, these results will b confirmed using IHC, western blotting or PCR. **Banking of Blood Samples:** Patients will be asked to donate 10cc of blood pretreatment and on days 28 (+/- 48 hours) c cycles 1 and 3. Blood will also be collected at the time of disease progression. Serum wi be banked for future treatment efficacy analyses including potential markers of bon turnover.
Inclusion Criteria:	*1)* Patients must have a pathologically confirmed diagnosis of invasive carcinoma o the breast. *2)* Patients must carry a diagnosis of metastatic breast cancer with predominant bon involvement. For the purposes of this study, predominant bone involvement will b

	defined as radiographically detected bone metastasis in the presence or absence of oth sites of metastatic breast cancer (i.e. visceral involvement). If visceral involvement present, patients must be asymptomatic and have no tumors in visceral organs th measure >3cm in size. *3)* Patients must agree to serial urine collections for measurement of Ntx according t the schedule defined in sections 8 and 9. *4)* Age ≥18 years. *5)* Patients must be able to swallow oral medications. Dasatinib must be taken whol and cannot be crushed. *6)* Patients must have evaluable disease using WHO Criteria for Assessment of Diseas Response in Bone or the MDACC modified response criteria in bone (Post te supplement 4). *7)* Patients must not have >1 chemotherapy regimen for metastatic disease. Patien with metastasis diagnosed ≤6 months after completion of adjuvant chemotherapy a considered to have had chemotherapy for metastatic breast cancer. *8)* Patients with ER positive disease must have had disease progression on at least on prior hormonal therapy for metastatic disease. Patients must also have developed diseas progression on their most recent hormonal therapy regimen and be agreeable to continu this regimen in combination with protocol therapy. For the purposes of this study diseas progression while receiving hormonal therapy will be defined as: • Radiographic evidence of progressive disease according to RECIST or WH criteria • Progression of disease by physical exam in patients with skin involvement. • 25% increase in tumor marker as measured on two evaluations no less than 72 hou apart. *9)* Patients must have and ECOG performance status of ≤2. *10)* Patients must not require concurrent radiation, or chemotherapy therapy whi receiving protocol therapy. *11)* Patients must not have an active infection requiring the use of intravenou antibiotics. The use of oral antibiotics as prophylaxis is allowed. *12)* Patients must have a baseline ECG with QTc within the normal range within 2 days prior to registration. *13)* Patients must be informed of the investigational nature of the study and must sig and give written informed consent. *14)* Patients may have received previous radiation but must have completed radiation least 2 weeks (8 weeks for radiation to the brain) prior to registration. Patients wit irradiated tumor as the only site of evaluable disease will not be eligible for protoc therapy unless there is documented disease progression within the previously radiated sit *15)* Patients must have recovered to grade ≤1 from all acute toxicity of previou radiation or hormonal therapy. *16)* Adequate hematologic and hepatic function: • Granulocyte count ≥ 1,500/mcL • Platelet count ≥ 100,000/mcL • Bilirubin ≤ 1.5 x ULN

	• AST and/or ALT ≤ 2 x ULN • Alkaline phosphatase (liver component, if fractionated) ≤ 2 x ULN • Serum Na, K^{+}, Mg^{2+}, Phosphate and Ca^{2+} ≥ Lower Limit of Normal (LLN) [subjec with low electrolyte levels must be repleted to normal for protocol entry] 17) Patients must not receive any concurrent bisphosphonate therapy other than th prescribed by the study. 18) Sexually active patients with reproductive potential must agree to use an effectiv method of birth control during the course of the study and for no less than 4 weeks aft discontinuing study drug. Contraceptives must be used in a manner such that risk failure is minimized. Oral contraceptives should be avoided in women with estrogen progesterone receptor positive breast cancer. 19) Prior to study enrollment, women of childbearing potential (WOCBP) must b advised of the importance of avoiding pregnancy during trial participation and th potential risk factors for an unintentional pregnancy. In addition, men enrolled on th study should understand the risks to any sexual partner of childbearing potential ar should practice an effective method of birth control. 20) All WOCBP MUST have a negative serum or urine pregnancy test within 72 hou prior to the start of study drug administration. If the pregnancy test is positive, the patie must not receive investigational product and must not be enrolled in the study. 21) Patients with disease progression while receiving previous therapy in combinatio with bisphosphonates (including zoledronic acid) will be considered eligible for protoc participation.
Exclusion Criteria:	1) No malignancy (other than breast cancer) which required radiotherapy or system treatment within the past 5 years. 2) Concurrent medical condition which may increase the risk of toxicity, including: • Pleural or pericardial effusion of any grade • Clinically-significant coagulation or platelet function disorder (e.g. known vo Willebrand's disease) 3) Cardiac Symptoms, including the following: • Uncontrolled angina, congestive heart failure or MI within (6 months) • Diagnosed congenital long QT syndrome • Any history of clinically significant ventricular arrhythmias (such as ventricul tachycardia, ventricular fibrillation, or Torsades de pointes) • Prolonged QTc interval on pre-entry electrocardiogram (> normal range) • Subjects with hypokalemia or hypomagnesemia if it cannot be corrected 4) 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-fact VIII antibodies)

	• Ongoing or recent (≤ 3 months) significant gastrointestinal bleeding 5) Concomitant Medications, consider the following prohibitions (Drugs must b discontinued for 7 days prior to starting protocol therapy): • Drugs that are generally accepted to have a risk of causing Torsades de Pointe including: quinidine, procainamide, disopyramide, amiodarone, sotalol, ibutilide, dofetilide, erythromycins, 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 n recommended. The use of antacids should be considered in place of H2 blockers o proton pump inhibitors in patients receiving dasatinib therapy. • Patient agrees to discontinue St. Johns Wort while receiving dasatinib therapy • Patient may not be receiving any prohibited CYP3A4 inhibitors [see section 7.6] 6) Women and men of child bearing potential: • Who are unwilling or unable to use an acceptable method to avoid pregnancy for th entire study period and for at least 4 weeks after cessation of study drug, or • Women of childbearing potential (CBP) who have a positive pregnancy test baseline, or • Women who are pregnant or breastfeeding • Sexually active men and women capable of reproduction must use an effectiv method of birth control during the course of the study, in a manner such that risk o failure is minimized • Prior to study enrollment, men and women capable of reproduction must be advise of the importance of avoiding pregnancy during trial participation and the potenti risk factors for an unintentional pregnancy *7)* Prisoners or subjects who are compulsorily detained (involuntarily incarcerated) fo treatment of either a psychiatric or physical (e.g., infectious) illness *8)* Untreated or uncontrolled brain metastasis *9)* Patient inability to take or absorb oral medications 10) Current active dental problems including: ongoing infection of the teeth or jawbon (maxilla or mandibula); current exposed bone in the mouth; and current or prio diagnosis of osteonecrosis of the jaw 11) Recent (within 8 weeks) or planned dental or jaw surgery (e.g., extraction, implants *12)* Diagnosis of metabolic bone disease other than osteoporosis (e.g., Paget's disease o bone) *13)* Known hypersensitivity to zoledronic acid or aspirin 14) Corrected serum calcium < 8.0 mg/dL (2.0 mmol/L) or ≥ 12.0 mg/dL (3.0 mmol/L at Visit 1. The formula to be used is: Corrected serum calcium (mg/dL) = Patient serum calcium (mg/dL) + [0.8 x Midrange Albumin (g/dL) – Patient's Albumin (g/dL)

	4.0g/dL to be used for the Midrange Albumin 15) Serum creatinine ≥ 1.5 times the institutional upper limits of normal or a creatinir clearance of <40 ml/min when calculated by the Cockroft and Gault formula: Estimated creatinine clearance= (140-age in years) x (weight in kg) (x 0.85, if female) 72 x creatinine (mg/dL)
Criteria for Evaluation: (Efficacy, safety, stopping rules, etc.)	For the phase 2 portion of the trial, a minimum of 15 and a maximum of 28 patients will b accrued. Simon's two stage design will be used to minimize the maximum sample size requires 15 patients in the first stage. If no patients respond to the treatment, the trial will be stopped and the regimen will be declared as ineffective. If at least one of the first 15 patients respond to the treatment, 10 additional patients will be entered in the study to reacl a total of 25 patients.

1 INTRODUCTION

1.1 Hypothesis

Dasatinib in combination with zoledronic acid will disrupt both the autocrine and paracrine loops that sustain breast cancer metastasis within the bone. This therapeutic effect will result in a significant reduction of N-telopeptide of Type I Collagen (Ntx), a marker of bone resorption, which will correlate with clinical outcomes of tumor response, time to progression and overall survival.

1.2 Background

1.2.1 Bone Metastasis in Patients with Breast Cancer

Up to 70% of patients with advanced breast cancer develop metastasis to the bone and, although bone metastasis is associated with a favorable prognosis, a median survival of 9 months has been published in patients with progressive bony metastasis or hormone refractory breast cancer (1, 2). Many of these patients are also ineligible for most clinical trials because of the complexities involved in measuring the therapeutic response of lesions within the bone(3). Skeletal related events (SREs) resulting from bone metastasis are also the source of a significant amount of morbidity, which may necessitate additional medical or surgical intervention.

Metastasis to the bone initiates a vicious cycle where the metastatic cells stimulate osteoclast mediated bone resorption and growth factors released from resorbed bone promote tumor growth (1). Src has been implicated in the autocrine and paracrine interactions involved in promoting bone metastasis and is thus a potential target for the treatment of breast cancer that has metastasized to the bone.

1.2.2 The Role of Src in Breast Cancer

Src kinase modulates oncogenic signal transduction through multiple pathways such as EGFR, HER2, PDGFR, FGFR and VEGFR. Approximately 50-65% of human breast carcinomas overexpress c-Src and up to 85% of human breast carcinomas have detectable levels of activated Src (Src-pY^{416})(4-6). Src signaling is significantly activated in breast tumors compared with normal breast tissue and has been correlated with chemotherapy resistance and antiapoptotic signaling in both cell lines and clinical trials involving breast cancer patients (7, 8). Elevated Src tyrosine kinase activity has also been associated with poor metastasis-free survival in breast cancer patients and with tumor colonization in bone and lung in animal models of human breast cancer metastasis(9). In these

models, activation of Src was associated with increased tumor cell production of PTH-rP, a powerful stimulator of osteoclast bone resorption that has been shown to play an important role in osteolytic bone metastasis.

1.2.3 The Role of Src in the Regulation of Osteoclast Function

Osteoclasts are multinucleated giant cells which contain ruffled borders or polarized portions of cell membrane where bone resorption occurs. Osteoclasts express high levels of Src which is involved with osteoclastogenesis, cytoskeletal organization and formation of ruffled boarders [reviewed in (10)]. Expression of c-Src in osteoclasts is controlled by NF-κB and AP-1 and disruption of this pathway using antisense *src* or c-*src* knock-out mice results in perturbations in the formation of multinucleated cells and morphologically abnormal cells with aberrant cytoskeletal organization and absence of ruffled borders [reviewed in (10)]. Disruption of Src with small molecular weight tyrosine kinase inhibitors (TKIs) has also been demonstrated to dose-dependently reduce interleukin-ıβ induced hypercalcemia in mice and partially prevented bone loss in young ovariectomized rats(11).

1.2.4 The Role of PDGF in Bone Metastasis

Breast cancer specimens evaluated by immunohistochemistry (IHC) show expression of PDGF in the cancer cells and associated expression of PDGFRs predominately within the stroma, including vascular endothelial cells in the periepithelial stroma [reviewed in (12)]. In addition, inhibition of PDGF by neutralizing antibody, transfection with antisense vector or treatment with tyrosine kinase inhibitors (TKIs) has been shown to block cancer induced osteoblastic bone formation, reduce bone metastasis and inhibit growth of established metastasis in breast cancer xenograft models(12, 13).

2 OVERVIEW OF STUDY DRUGS

2.1 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(14).

Drug discovery and nonclinical pharmacology studies showed that Dasatinib(15):

- 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

2.1.1 Preclinical Anti-tumor Activity

2.1.1.1 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, PDGFRβ, and SRC kinases, respectively(15).

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(14).

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(15).

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.

2.1.1.2 In Vivo Studies

The activity of dasatinib against CML cells *in vitro* was reproduced *in vivo* against several human CML xenograft models grown subcutaneously in SCID mice. Against the K562/imatinib mesylate/R CML model, Dasatinib was curative in 100% of the treated animals. In contrast, at its optimal dose and schedule, imatinib mesylate was inactive.

2.1.2 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(15).

2.1.3 Clinical Pharmacokinetics

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

2.1.3.1 Absorption

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

Data from a study of 54 healthy subjects administered a single, 100-mg dose of Dasatinib 30 minutes following consumption of a high-fat meal resulted in a 14% increase in the mean AUC of dasatinib. The observed food effects were not clinically relevant.

2.1.3.2 Distribution

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

2.1.3.3 Metabolism

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

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

2.1.3.4 Elimination

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

2.1.4 Clinical Experience with Dasatinib

Four single-arm multicenter studies were conducted to determine the efficacy and safety of dasatinib in patients with CML or Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) resistant to or intolerant of treatment with imatinib. Resistance to imatinib included failure to achieve a complete hematologic response (within 3–6 months) or major cytogenetic response (by month 12) or progression of disease after a previous cytogenetic or hematologic response. Imatinib intolerance included inability to tolerate 400 mg or more of imatinib per day or discontinuation of imatinib because of toxicity. The studies are ongoing. The results are based on a minimum of 6 months follow-up after the start of DASATINIB therapy. Most patients had long disease histories with extensive prior treatment, including imatinib, cytotoxic chemotherapy, interferon, and stem cell transplant. The maximum imatinib dose had been

400–600 mg/day in about one-half of the patients and >600 mg/day in the other half(14).

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(14).

The primary efficacy endpoint in chronic phase CML was major cytogenetic response (MCyR), defined as elimination (complete cytogenetic response, CCyR) or substantial diminution (by at least 65%, partial cytogenetic response) of Ph+ hematopoietic cells. The primary endpoint in accelerated phase, myeloid blast phase, and lymphoid blast phase CML, and Ph+ ALL was major hematologic response (MaHR), defined as either a complete hematologic response or no evidence of leukemia as defined in Table 3.

Most cytogenetic responses occurred after 12 weeks of treatment, when the first cytogenetic analyses were performed. Hematologic and cytogenetic responses were stable during the 6-month follow-up of patients with chronic phase, accelerated phase, and myeloid blast phase CML. The median durations of major hematologic response were 3.7 months in lymphoid blast CML and 4.8 months in Ph+ ALL.

There were no age- or gender-related response differences(14).

Table 3: Efficacy in Dasatinib Clinical Studies (All Treated Populations)[a]

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

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

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

2.1.5 Safety of Dasatinib in Clinical Studies

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

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

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

All treatment-emergent adverse events (excluding laboratory abnormalities), regardless of relationship to study drug, that were reported in at least 20% of the patients in dasatinib clinical studies are shown in Table 4.

Table 4: Adverse Events Reported ≥20% in Clinical Studies

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

2.1.6 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 CML or Ph+ ALL than in chronic phase CML. Myelosuppression was reported in patients with normal baseline laboratory values as well as in patients with pre-existing laboratory abnormalities. (Table 5)

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

Grade 3 or 4 elevations of transaminases or bilirubin and Grade 3 or 4 hypocalcemia and hypophosphatemia were reported in patients with all phases of CML but were reported with an increased frequency in patients with myeloid or lymphoid blast CML and Ph+ ALL. Elevations in transaminases or bilirubin were usually managed with dose reduction or interruption. Patients developing Grade 3 or 4 hypocalcemia during the course of dasatinib therapy often had recovery with oral calcium supplementation. (Table 5).

Table 5: CTC Grades 3/4 Laboratory Abnormalities in Clinical Studies

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

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

2.1.7 Anticipated Adverse Events

Myelosuppression

Treatment with dasatinib B is associated with severe (NCI CTC Grade 3 or 4) thrombocytopenia, neutropenia, and anemia. Their occurrence is more frequent in patients with advanced CML or Ph+ ALL than in chronic phase CML. Complete blood counts should be performed weekly for the first 2 months and then monthly thereafter, or as clinically indicated. Myelosuppression was generally reversible and usually managed by withholding dasatinib temporarily or dose reduction(14).

Bleeding Related Events

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

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

Fluid Retention

Dasatinib is associated with fluid retention, which was severe in 9% of patients, including pleural and pericardial effusion reported in 5% and 1% of patients, respectively. Severe ascites and generalized edema were each reported in 1%. Severe pulmonary edema was reported in 1% of patients. Patients who develop symptoms suggestive of pleural effusion such as dyspnea or dry cough should be evaluated by chest X-ray. Severe pleural effusion may require thoracentesis and oxygen therapy. Fluid retention events were typically managed by supportive care measures that include diuretics or short courses of steroids(14).

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(14).

2.1.8 Phase I experience in Solid Tumors

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

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

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

To date, the safety profile in solid tumor subjects has been similar to that in CP CML subjects with the exception of severe myelosuppression,

which has not been observed in solid tumor subjects and is considered related to efficacy against the leukemia as noted above, and severe bleeding which is secondary to thrombocytopenia in most instances.

2.2 Zoledronic Acid

The principle pharmacologic action of zoledronic acid is inhibition of bone resorption. Although the antiresorptive mechanism is not completely understood, several factors are thought to contribute to this action. *In vitro*, Zoledronic acid inhibits osteoclastic activity and induces osteoclast apoptosis. Zoledronic acid also blocks the osteoclastic resorption of mineralized bone and cartilage through its binding to bone. Zoledronic acid inhibits the increased osteoclastic activity and skeletal calcium release induced by various stimulatory factors released by tumors.

Zoledronic acid has demonstrated efficacy and safety in phase II and phase III clinical trials(16). The phase II study was a randomized, double-blind study of 280 patients with malignant bone lesions associated with multiple myeloma or breast cancer(16). Patients were randomized to a 5-minute infusion of 0.4, 2.0, or 4.0 mg of zoledronic acid, or a 2-hour infusion of 90 mg of pamidronate. Adverse events with zoledronic acid and pamidronate were similar, and the 5-minute 4.0-mg zoledronic acid infusion was equally efficacious when compared to a 2-hour 90-mg pamidronate infusion.

Zoledronic acid has been evaluated in three prospective, randomized, controlled studies. [Study 0010] was a randomized, double-blind, double-dummy, 12-month study of 1,122 patients with at least one osteolytic bone lesion secondary to multiple myeloma or at least one bone metastasis secondary to Stage IV breast carcinoma(17). Patients were randomized to 15-minute 4-mg infusions of zoledronic acid, or to 120-minute 90-mg infusions of pamidronate, while receiving standard antineoplastic therapy. Analysis of the primary endpoint revealed that zoledronic acid was at least as effective as pamidronate at reducing skeletal related events. Tolerability was similar for the two drugs, with bone pain, nausea, and fatigue the most common adverse events. There was no significant difference between the renal safety profiles for zoledronic acid and pamidronate after renal safety amendments for zoledronic acid were implemented.

Two additional phase III studies demonstrated the safety and efficacy of zoledronic acid in patients with bone metastases associated with prostate cancer, and non-small cell lung cancer or other solid tumors. Saad, et al. reported a phase III, double-blind, placebo-controlled study that enrolled 422 patients with prostate cancer receiving antineoplastic therapy, and who had metastatic bone lesions and three consecutive rises in serum PSA level(18). In this study, zoledronic acid reduced the proportion of patients with SREs during the study by 25%, when compared with placebo.

Rosen, et al. published a phase III double-blind, placebo-controlled study in 507 patients with malignancies other than breast, prostate, or multiple myeloma, and documented bone metastases(19). This study demonstrated that treatment with zoledronic acid induced a significant delay to the first SRE and a significant reduction in the risk of developing SRE, compared to placebo. Both studies confirmed the safety profile of zoledronic acid.

For details regarding drug preparation mechanism of action and prescribing information please see section 10.7 and Post-text supplement 2 [Package Insert for Zometa (Zoledronic acid)].

3 STUDY RATIONALE

3.1 Rationale for the Use of Dasatinib in Combination with Zoledronic Acid for the Treatment of Hormone Receptor Negative Metastatic Breast Cancer and in Combination with Antiestrogen Therapy in Patients with Hormone Receptor Positive Breast Cancer

Dasatinib is a novel, broad spectrum ATP competitive inhibitor of tyrosine kinases such as BCR-ABL, Src, c-KIT, PDGFR and ephrin (EPH). Src has been implicated as a target in both hormone receptor positive and triple negative breast cancer cell lines. In estrogen receptor (ER) positive cell lines, inhibition of ER/Src interaction leads to inactivation of the Src/Erk pathway, reduction in cyclin D1 expression and DNA synthesis induced by estradiol.(20) Disruption of the ER/Src association also leads to inhibited tumor growth in MCF-7 xenografts.(20) Additionally, PGC-1 beta (a strong enhancer of ER activity) and SRC-1 have been demonstrated to functionally interact and promote the agonist activity of tamoxifen. Inhibition of the ER/Src association through peptide mimetics or Src inhibitors results in diminished growth in breast cancer xenograft models and restores sensitivity to anti-estrogen therapy in these same models.(20, 21) Preclinical data have demonstrated cytotoxic activity against the breast cancer cell line MDA-MB-231 and complete inhibition of PTH-stimulated bone resorption in thyro-parathyroidectomized rats(15).

Zoledronic acid has been evaluated in three randomized double blind trials which demonstrated reductions in the risk of skeletal events of 27-36% in patients with prostate cancer, non-small cell lung cancer and other solid tumors(22-24) . In breast cancer, zoledronic acid was compared to pamidronate and demonstrated an additional 20% reduction in skeletal events (25). Zoledronic acid is a nitrogen containing bisphosphonate that inhibits osteoclast mediated bone resorption by blocking FFP synthase and

subsequent prenylation of GTPases that regulate osteoclast cytoskeletal organization(26). As mentioned in section 1.2.3, Src is also important in regulating the cytoskeletal organization necessary for formation of the ruffled boarder where bone resorption occurs. As such, both agents may offer an additive effect in combination for the prevention of bone resorption and improvement in skeletal related events.

In addition, preclinical data indicate that zoledronic acid sensitizes endothelial cells to tumor necrosis factor (TNF) programmed cell death by inhibiting focal adhesion kinase (FAK) and protein kinase B (PKB/Akt)(27). These effects were independent of c-Src activation and suggest that treatment with zoledronic acid may augment the anti-angiogenic effects previously described with dasatinib.

Taken together, these data support a potential therapeutic role for the combined use of dasatinib with zoledronic acid to inhibit osteolytic bone destruction and the subsequent release of growth factors that support cancer cell proliferation and survival.

3.2 Rationale for Evaluating Therapeutic Response in Bone and Correlating N-telopeptide of Type I Collagen (Ntx) with Therapeutic Response

Traditional assessment of bone tumor response using imaging studies remains challenging and often limits patients with bone only metastasis from participating in clinical trials. In addition, traditional imaging studies such as bone scan or plain films used by the World Health Organization (WHO) criteria for response in bone were published in 1977 and show only moderate sensitivity when assessing for response, may lead to false diagnoses of tumor progression (flare phenomenon), or take months to show response because they measure skeletal architecture changes that occur slowly over time. These guidelines also were not adopted by RECIST or RECIST 1.1, creating a void regarding the evaluation of bone metastases. This void lead to the development of bone-specific response criteria at the M. D. Anderson Cancer Center in 2004.(45) The MDA criteria revised the evaluation of bone metastases previously adopted by UICC and WHO by modifying radiographic assessment and updating the technology to include both CT and MRI.

The MDA criteria divide response into 4 standard categories (CR, PR, PD and SD, see post-text supplement 4) and include quantitative and qualitative assessments of the behavior of bone metastases. Quantitatively, the MDA criteria adopted the method of physical measurement put forth by the UICC and WHO criteria which define PR as a decrease of 50% or more in the sum of the perpendicular measurements of a lesion and PD as a reduction of 25% or more in this sum.

Using the MDA criteria, complete response is defined as complete sclerotic fill-in of lytic lesions on radiographs (XR) or CT; the restoration of normal bone density at the site of a blastic lesion on XR or CT; the disappearance of abnormal tracer uptake on skeletal scintigraphy (SS); and normalization of signal intensity on MRI. The PR category includes the development of a sclerotic rim or partial (rather than complete) sclerotic fill-in of lytic metastases on XR or CT, $\geq$ 50% decrease in measurable lesions on XR, CT or MRI (Fig. 7), subjective unequivocal decrease in the size of blastic lesions on XR or CT that cannot be accounted for by changes in obliquity or slice placement and unequivocal decrease in tracer uptake on SS. A caveat to the PR designation involves osteosclerotic "flare." Interval visualization of sclerotic lesions or lytic lesions with sclerotic rims, in the setting of other signs of PR, does not indicate disease progression but the healing of previously inconspicuous lesions. The caveat does not apply if any pre-existing lesions show signs of progression (e.g. enlargement of lytic lesions, development of new lytic lesions). The PD category includes a $\geq$ 25% increase in the size of any measurable lesion on XR, CT or MRI; unequivocal increase in the size of unmeasurable (ill-defined) lytic or blastic lesions that cannot be accounted for by obliquity or slice placement, unequivocal increase in tracer uptake on SS; or the development of new metastases. Flare occurs when healing sclerosis results in more tracer uptake than was caused by the untreated lesion. Correlation of SS with another imaging modality is also suggested for the rapid resolution of tracer uptake in pre-existing lesions. Rarely, this is due to the rapid progression of lytic metastases. When lytic lesions progress quickly, the bone is unable to produce reparative sclerosis, rendering tracers such as methylene diphosphate (MDP) ineffective because they normally bind to the crystalline phase of hydroxyapetite laid down by the bone as it attempts to heal the damage caused by the lesion. In this unusual situation, the rapidly progressing metastases destroy pre-existing skeletal tissue that demonstrated tracer uptake. SD is defined as < 25% increase, < 50 % increase in measurable lesions or no change and no new lesions.

Upon comparison of the MDA, UICC and WHO criteria in a study of 41 breast cancer patients with bone-only metastases, MDA was shown to better differentiate responders from non-responders and was the only set of criteria to correspond to progression-free survival. Using the MDA criteria, time to disease progression was found to be 5.5 months for non-responders and 23.3 months for responders ($p = 0.025$) while it was 10.4 months and 12.4 months respectively, using the WHO criteria ($p = 0.55$). MDA identified non-responders earlier and was also found to better correlate with clinical response in the first 2-6 months of therapy in comparison to the WHO criteria. Early signs of disease progression are valuable, allowing ineffective therapy to be halted in a timely fashion and

the possible substitution of effective therapy. In addition to clinical utility, the MDA bone response criteria closely reflect the behavior of bone metastases on radiographs and CT and can be used as guidelines for the interpretation of these studies whether or not a patient is enrolled in a therapeutic trial. The MDA criteria can be considered for use in conjunction with other cancer response criteria or in patients with bone-only metastases and no measurable disease.

Though the MDACC bone response criteria have been helpful in determining response to therapy in bone metastasis, no imaging study exists to measure the benefits of bisphosphonates in preventing skeletal related events in patients with metastatic breast cancer. Such limitations, combined with the expense of traditional imaging studies, suggest a need for novel measures of tumor response and bone health. Ntx, a marker of bone resorption, has been found to significantly correlate with risk of negative clinical outcomes(28), response to systemic therapy(29) and response to bisphosphonate therapy(25).

Ntx has shown significant correlation with clinically relevant skeletal related events (SREs) ($r=0.62$, $p<0.001$) in patients with bone metastasis and in a multivariate logistic regression model was highly predictive for adverse event or death (30). In randomized bisphosphonate studies, reductions in Ntx and decreased SREs were similar in patients treated with zoledronic acid compared to pamidronate (23).

Several studies have correlated reductions in Ntx with response to therapy. Costa et al. demonstrated that increases in Ntx were strongly associated with progression in bone and that the increase in Ntx occurred independent of bisphosphonate administration(31). In this study, urinary Ntx was associated with the highest positive predictive value for disease progression (71%) when compared to other markers of bone metabolism. A second study conducted by Lipton, et al randomized patients to receive either pamidronate or placebo and correlated urinary Ntx with response to therapy(32). Of the 25 patients who received pamidronate, 21 had elevated Ntx prior to therapy administration and 12 of these patients normalized Ntx values. When compared to the patients who did not normalize Ntx, the proportion of patients with progression in bone was significantly higher ($p=0.03$) in those who did not normalize. Although not directly correlated with tumor response, several bisphosphonate dosing studies have also demonstrated a dose dependant effect on Ntx levels [summarized in (33)], suggesting that variations in bone resorption for individual patients can be detected using this technique. Finally, the existing clinical data for the use of Ntx to monitor response to bisphosphonate therapy has led to development and implementation of the Bisphosphonate Therapy Directed by Bone Resorption Markers (BISMARK) trial. This study is a large

randomized phase III trial currently accruing patients to prove non-inferiority between standard and marker-directed bisphosphonate therapy.

Because this proposal hypothesizes that combined therapy with dasatinib and zoledronic acid will inhibit bone resorption through combined effects on osteoclasts and may also exert anti-tumor effects through disruption of the tumor cell/osteoclast paracrine loop, biomarkers of bone resorption would be expected to accurately predict improvement in bone health as well as tumor response and time to disease progression in this special cohort of patients.

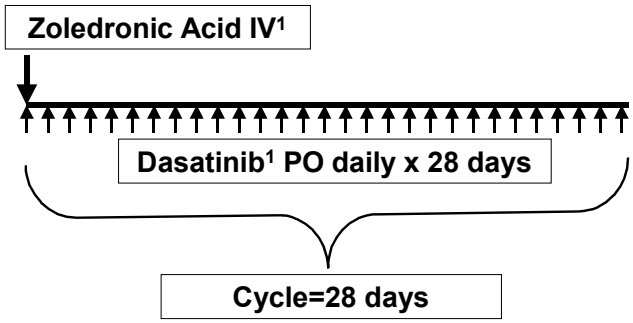
Urinary measures of Ntx will be performed using standard techniques at baseline, 1 month, 3 months and then every 3rd month until disease progression or protocol withdrawal. Patients will be divided into three categories: high (Ntx≥ 100nm/mmol creatinine), moderate (Ntx=50-99nm/mmol creatinine) and low (Ntx <50nm/mmol creatinine) as previously described by Coleman et al.(25). This study demonstrated that treatment with various systemic therapies in combination with zoledronic acid resulted in a 67% decrease in the proportion of patients with moderate to high levels of Ntx after only one month of therapy; an effect which was sustained for > 6 months. Zoledronic acid was also more effective than pamidronate in reducing the levels of Ntx from a moderate or high state to a low state within the breast cancer subset, a subset in which zoledronic acid significantly improved clinical benefit compared to pamidronate.

4 OVERVIEW OF STUDY DESIGN AND EVALUATION

4.1 Dose-finding and Cohort Definitions

This is an open label, Phase I/II, dose escalation study to evaluate the safety and tolerability of dasatanib in combination with a standard 28-day cycle of zoledronic acid (Figure 4.1) in patients with metastatic breast cancer to bone.

Figure 4.1: Dosing Schedule for Phase I and Phase II



1. Dosing as per protocol section 7.3.

Zoledronic acid will be administered as a 15-minute infusion on day 1(+/- 48 hours) of a 28-day cycle. The dose of zoledronic acid administered will be calculated using the standard dosing guidelines outlined in section 7.3.2.

Dasatinib will be taken by mouth using a daily dosing schedule on days 1-28 of each cycle (i.e. continuous dosing). Dasatinib will be given continuously unless patients develop toxicity requiring dose adjustment as outlined in section 7.4.

4.2 Phase I Study Design

The primary objective of the phase I portion of this trial will be to determine the RP2D for dasatinib in combination with zoledronic acid.

The first 3 patients (dosing cohort 1) will be treated with 100 mg of dasatinib daily in combination with full dose zoledronic acid using the dosing guidelines outlined in section 7.3.2. If $\leq$ 1 of 3 patients develops DLT within cycle one of therapy, then 3 additional patients will be accrued to this dose level .

If $\leq$ 1 of 3 patients treated in dosing cohort 1 develops DLT within cycle one of therapy, then 3 additional patients will be accrued to this cohort (n=6 total). If $\leq$ 1 of 6 enrolled patients experiences DLT, then this will be deemed the RP2D and accrual will begin for the phase 2 portion of the trial.

If $\geq$ 2 patients develop DLT during initial accrual (n=3) or during the dose expansion (n=6 total) of any cohort, further accrual will begin at the lower dosing cohort until a total of six patients are treated at the lower dosing cohort or accrual is halted due to DLT. The RP2D will be defined as the dose at which $\leq$ 1 out of 6 patients develops DLT.

If $\geq$ 2 patients develop DLT during accrual to cohort – 1 (100 mg of dasatinib daily), the study will be closed due to intolerability.

4.2.1 Definition of Dose Limiting Toxicity

Toxicity will be assessed using the NCI Common Toxicity Criteria (CTC) Scale, version 3.0.

By definition a DLT must occur during cycle 1, be suspected as being drug-related, and includes any of the following:

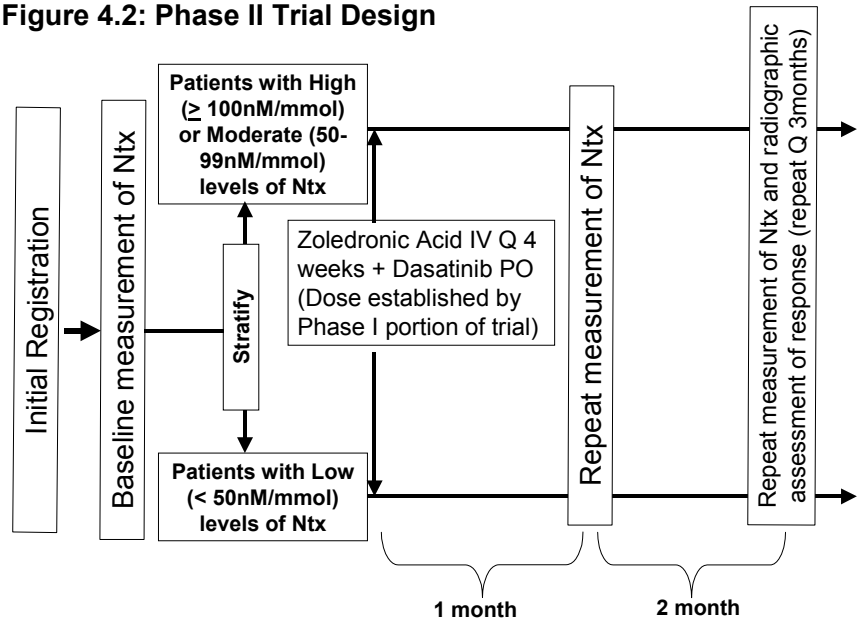
- Any grade 4 non-hematological toxicity
- Grade 4 neutropenia lasting > 7 days or any febrile neutropenia

- Grade 3 non-hematological toxicity that persists >72 hours despite adequate supportive care
- Grade 2 or greater neutropenia (ANC<$1.5x10^9$/L) or thrombocytopenia (PLT<$75x10^9$/L) which fails to revert to ≤grade 1 (level at which treatment is permitted) by the time of the scheduled start of cycle 2
- 50% decrease in calculated creatinine clearance
- Diagnosis of osteonecrosis of the jaw

4.3 Phase II Study Design

The dose identified as the RP2D in the phase I portion of this study will be evaluated for efficacy in the phase II portion of this trial. The primary objective of this phase II study is to assess the efficacy of dasatinib in combination with zoledronic acid as measured by disease response. . Patients will be stratified into two cohorts dependent upon Ntx levels obtained prior to the initiation of therapy (Figure 4.2). All patients will receive same therapy, lab draws and radiographic assessment for response.

Figure 4.2: Phase II Trial Design



The primary efficacy analysis will not be dependant upon urinary Ntx levels, but this the effects of therapy on urinary Ntx will be evaluated as a secondary endpoint.

Patients treated at the RP2D (n=6) in the phase I portion of this study will be included in the efficacy analysis for the phase II portion of the trial.

Up to 25 patients will be enrolled in the phase II study to assess the efficacy of dasatinib in combination with zometa (zoledronic acid). The primary endpoint is the response rate. The trial will be conducted by the Simon's two-stage design using the minimax criterion and the response rate will be estimated accordingly.

It is assumed that dasatinib in combination with zometa will have a target response rate of 25%. A response rate of 5% or lower is considered a failure. When the probability of accepting a "bad" regimen (i.e. response rate ≤ 5%) is 0.05 and the probability of rejecting a "good" regimen (i.e. response rate ≥ 25%) is 0.10, Simon's design to minimize the maximum sample size requires 15 patients in the first stage. If no patients respond to the treatment, the trial will be stopped and the regimen will be declared as ineffective. If at least one of the first 15 patients respond to the treatment, 10 additional patients will be entered in the study to reach a total of 25 patients. By the end of the study, the new regimen will be rejected if response rate is less than or equal to 3 out of 25 patients and will be accepted otherwise. The operating characteristics of the trial are given as follows. When the true response rate is 0.05, the probability of stopping the trial early is 46.3%. On the other hand, if the true response rate is 0.25, the probability to stop the trial early is 1.3%. The expected sample sizes are 20.37 and 24.97 when the true response rates are 0.05 and 0.25, respectively. At the end of the study, the response rate and the 95% confidence interval will be reported and the toxicity profile of the regimen will be summarized. If the trial continues to the second stage (i.e., accrual = 25) and the 5 of 25 patients response for a point estimate of 20.0%, the exact binomial 95% confidence interval would be (6.8%, 40.7%). Assuming that 10% of patients may not be evaluable for response, a total of 28 patients will be accrued to the study.

5 STUDY OBJECTIVES

5.1 Primary Objective

5.1.1 Phase I:

To determine the maximum tolerated dose (MTD) and recommended phase II dose (RP2D) for dasatinib in combination with zoledronic acid.

5.1.2 Phase II:

Determine the efficacy of dasatinib in combination with zoledronic acid as measured by disease response.

5.2 Secondary Objective(s)

5.2.1 Phase I:

To determine toxicity of combination therapy with dasatinib and zoledronic acid in patients with breast cancer and bone metastasis.

5.2.2 Phase II:

- Determine the effects of therapy on urinary Ntx levels.
- Changes in Ntx levels will be correlated with traditional measurements of tumor response in bone (using WHO criteria), progression free survival and overall survival at 2years
- Biologic characterization of response using ^{18}F-FDG PET
- Determination of molecular correlates of response in paraffin embedded or frozen tissue
- Determine time to tumor progression, response (as defined in section 9.1.2), time to tumor response, progression free survival (PFS) up to 2 years
- Determine overall survival (OS) at 2 years
- Banking of blood for future studies of novel markers of bone health
- Correlate response with tumor estrogen receptor (ER), progesterone receptor (PR) and HER2 status

6 SELECTION OF PATIENTS

6.1 Inclusion Criteria

For entry into the study, the following criteria MUST be met:

1) Patients must have a pathologically confirmed diagnosis of invasive carcinoma of the breast.
2) Patients must carry a diagnosis of metastatic breast cancer with predominant bone involvement. For the purposes of this study, predominant bone involvement will be defined as radiographically detected bone metastasis in the presence or absence of other sites of metastatic breast cancer (i.e. visceral involvement). If visceral involvement is present, patients must be asymptomatic and have no tumors in visceral organs that measure >3cm in size.
3) Patients must agree to serial urine collections for measurement of Ntx according to the schedule defined in sections 8 and 9.
4) Age $\geq$18 years.
5) Patients must be able to swallow oral medications. Dasatinib must be taken whole and cannot be crushed.

6) Patients must have evaluable disease using WHO Criteria for Assessment of Disease Response in Bone or MDACC Modified Response Criteria for Assessment of Disease Response in Bone (Post text supplement 4).

7) Patients must not have had >1 chemotherapy regimens for metastatic disease. Patients with metastasis diagnosed ≤6 months after completion of adjuvant chemotherapy are considered to have had chemotherapy for metastatic breast cancer.

8) Patients with ER positive disease must have had disease progression on at least one prior hormonal therapy for metastatic disease. Patients must also have developed disease progression on their most recent hormonal therapy regimen and be agreeable to continue this regimen in combination with protocol therapy. For the purposes of this study disease progression while receiving hormonal therapy will be defined as:

- Radiographic evidence of progressive disease according to RECIST or WHO criteria for measurement of response in bone
- Progression of disease by physical exam in patients with skin involvement
- 25% increase in tumor marker as measured on two evaluations no less than 72 hours apart

9) Patients must have and ECOG performance status of ≤2.

10) Patients must not require concurrent radiation or chemotherapy while receiving protocol therapy.

11) Patients must not have an active infection requiring the use of intravenous antibiotics. The use of oral antibiotics as prophylaxis is allowed.

12) Patients must have a baseline ECG with QTc within the normal range within 28 days prior to registration.

13) Patients must be informed of the investigational nature of the study and must sign and give written informed consent.

14) Patients may have received previous radiation but must have completed radiation at least 2 weeks (8 weeks for radiation to the brain) prior to registration. Patients with irradiated tumor as the only site of evaluable disease will not be eligible for protocol therapy unless there is documented disease progression within the previously radiated site.

15) Patients must have recovered to grade ≤1 from all acute toxicity of previous radiation or hormonal therapy.

- Adequate hematologic and hepatic function:
- Granulocyte count ≥ 1,500/mcL
- Platelet count ≥ 100,000/mcL
- Bilirubin ≤ 1.5 x ULN
- AST and/or ALT ≤ 2 x ULN
- Alkaline phosphatase (liver component, if fractionated) ≤ 2 x ULN
- Serum Na, K^{+}, Mg^{2+}, Phosphate and Ca^{2+}≥ Lower Limit of Normal (LLN) [subjects with low electrolyte levels must be repleted to normal for protocol entry]

16) Patients must not receive any concurrent bisphosphonate therapy other than that prescribed by the study.

17) Sexually active patients with reproductive potential must agree to use an effective method of birth control during the course of the study and for no less than 4 weeks after discontinuing study drug. Contraceptives must be used in a manner such that risk of failure is minimized. Oral contraceptives should be avoided in women with estrogen or progesterone receptor positive breast cancer.

18) Prior to study enrollment, women of childbearing potential (WOCBP) must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy. In addition, men enrolled on this study should understand the risks to any sexual partner of childbearing potential and should practice an effective method of birth control.

19) All WOCBP MUST have a negative serum or urine pregnancy test within 72 hours prior to the start of study drug administration. If the pregnancy test is positive, the patient must not receive investigational product and must not be enrolled in the study.

20) Patients with disease progression while receiving previous therapy in combination with bisphosphonates (including zoledronic acid) will be considered eligible for protocol participation.

6.2 Exclusion Criteria

Subjects may NOT have any of the following:

1) Any malignancy (other than breast cancer) that required radiotherapy or systemic treatment within the past 5 years.

2) Concurrent medical condition which may increase the risk of toxicity, including:

- Pleural or pericardial effusion of any grade
- Clinically-significant coagulation or platelet function disorder (e.g. known von Willebrand's disease)

3) Cardiac Symptoms, including the following:

- Uncontrolled angina, congestive heart failure or MI within (6 months)
- Diagnosed congenital long QT syndrome
- Any history of clinically significant ventricular arrhythmias (such as ventricular tachycardia, ventricular fibrillation, or Torsades de pointes)
- Prolonged QTc interval on pre-entry electrocardiogram (> normal range)
- Subjects with hypokalemia or hypomagnesemia if it cannot be corrected

4) 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 (≤ 3 months) significant gastrointestinal bleeding

5) Concomitant Medications, consider the following prohibitions (Drugs must be discontinued for 7 days prior to starting protocol therapy):

- Drugs that are generally accepted to have a risk of causing Torsades de Pointes including:

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

  erythromycins, 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.
- Patient agrees to discontinue St. Johns Wort while receiving dasatinib therapy
- Patient may not be receiving any prohibited CYP3A4 inhibitors [see section 7.6]

6) Women and men of child bearing potential:

- Who are unwilling or unable to use an acceptable method to avoid pregnancy for the entire study period and for at least 4 weeks after cessation of study drug, or
- Women of childbearing potential (CBP) who have a positive pregnancy test at baseline, or
- Women who are pregnant or breastfeeding
- Sexually active men and women capable of reproduction must use an effective method of birth control during the course of the study, in a manner such that risk of failure is minimized
- Prior to study enrollment, men and women capable of reproduction must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy

7) Prisoners or subjects who are compulsorily detained (involuntarily incarcerated) for treatment of either a psychiatric or physical (e.g., infectious) illness

8) Untreated or uncontrolled brain metastasis

9) Patient inability to take or absorb oral medications

10) Current active dental problems including: ongoing infection of the teeth or jawbone (maxilla or mandibula); current exposed bone in the mouth; and current or prior diagnosis of osteonecrosis of the jaw

11) Recent (within 8 weeks) or planned dental or jaw surgery (e.g., extraction, implants)

12) Diagnosis of metabolic bone disease other than osteoporosis (e.g., Paget's disease of bone)

13) Known hypersensitivity to zoledronic acid or aspirin

14) Corrected serum calcium < 8.0 mg/dL (2.0 mmol/L) or ≥ 12.0 mg/dL (3.0 mmol/L) at Visit 1. The formula to be used is: Corrected serum calcium (mg/dL) = Patient's serum calcium (mg/dL) + [0.8 x Midrange Albumin (g/dL) – Patient's Albumin (g/dL)]. 4.0g/dL to be used for the Midrange Albumin

15) Serum creatinine ≥ 1.5 times the institutional upper limits of normal or a creatinine clearance of <40 ml/min when calculated by the Cockroft and Gault formula:

Estimated creatinine clearance=

$$\frac{(140\text{-age in years}) \times (\text{weight in kg})}{72 \times \text{creatinine (mg/dL)}} \quad (\times 0.85, \text{if female})$$

7 TREATMENT OF SUBJECTS

This is an open label, Phase I/II, dose escalation study to evaluate the safety and tolerability of dasatanib in combination with a standard 28-day cycle of zoledronic acid in patients with metastatic breast cancer to bone. Zoledronic acid will be administered as a 15-minute infusion on day 1(+/- 48 hours) of a 28-day cycle (see section 7.3.2). Dasatinib will be taken by mouth using a daily dosing schedule on days 1-28 of each cycle (i.e. continuous administration). Dasatinib will be given continuously unless patients develop toxicity requiring dose adjustment as outlined in section 7.4.

Patients with ER positive disease who have progressed on their most recent hormonal therapy will continue this therapy (at the previously prescribed dose) in combination with protocol treatment.

7.1 Study Registration

Patients will be registered after the Principal Investigator or her designee has verified that they are eligible per criteria in Section 6.1 and Section 6.2. Patients withdrawn from the study prior to completing the first course of therapy due to non drug-related reasons will be replaced.

The Principal Investigator or her designee will provide the dosing assignment form. No subject may start therapy prior to dosing assignment.

7.2 Study Treatment

7.2.1 Phase I

On cycle 1, day 1, patients will receive a single IV infusion of zoledronic acid using dosing guidelines outlined in section 6.4.2. Dasatinib will be taken daily by mouth beginning on cycle 1 day 1 and continued daily for the duration of the cycle (Table 7.1 and section 4.2).

Table 7.1. Dosing of Dasatinib for Phase I Dosing Cohorts

Dose Level	Dose of Dasatinib (+ Full Dose Zoledronic Acid)
-1	70 mg po daily
1	100 mg po daily

Patients will be monitored for toxicity every week during the first cycle of therapy and on day 1 (+/- 48 hours) prior to therapy for subsequent cycles. ECG and laboratory assessments will be performed weekly (see section 7.0) during the first cycle. ECG will be performed on day 1 (+/- 48 hours) of cycle 2 only. Laboratory assessment will be performed on day 1 (+/- 48 hours) prior to therapy during cycle 2 and all subsequent cycles.

7.2.2 Phase II

Zoledronic acid will be administered as a single 15-minute infusion on day 1(+/- 48 hours) of a 28-day cycle. The dose of zoledronic acid administered will be calculated using the standard dosing guidelines outlined in section 7.3.2.

Dasatinib will be taken by mouth using a daily dosing schedule on days 1-28 of each cycle (i.e. continuous dosing). Dasatinib will be given continuously unless patients develop toxicity requiring dose adjustment as outlined in section 7.4.

Both drugs will be given at the RP2D as determined by the phase I portion of the trial.

Patients will be monitored for toxicity on day 1 (+/- 48 hours) prior to therapy during subsequent cycles. Physical exams and laboratory

assessments will be performed monthly (see section 8.0) on day 1 (+/- 48 hours) prior to therapy. Measurement of Ntx will occur at baseline (up to 7 days prior to therapy), at day 28 (+/- 48 hours) of cycle 1 and at day 28 (+/- 48 hours) of every 3rd cycle of therapy, (see figure 4.2 and section 8.0). Patients will undergo radiographic evaluation of response every 3 cycles.

7.3 Drug Dosing and Routes of Administration

NOTE: Although the dose of dasatinib may differ between the phase I and phase II portions of the trial, the schedule and route of administration of both dasatinib and zoledronic acid will be the same for the phase I and phase II portions of the trial.

7.3.1 Dasatinib

Dasatinib will be supplied as 20 and 50 mg tablets for oral administration. Patients treated at the 70 mg dose level will receive a 50 mg tablet and a 20 mg tablet daily. Patients treated at the 100 mg dose level will receive two 50 mg tablets daily.

If a dose is missed by more than 12 hours, the dose should be omitted. Missed doses should not be made up.

Tablets should not be crushed or cut; they should be swallowed whole. Dasatinib can be taken with or without a meal.

Patients will be provided with a diary to record drug dosing (see Appendix 3).

7.3.2 Zoledronic Acid

Zoledronic acid will be administered as a 15-minute IV infusion on day 1(+/- 48 hours) of each 28-day cycle. The dose of zoledronic acid administered will be calculated using the schema in Figure 7.1.

Figure 7.1: Dosing of zoledronic acid

Dosing will be based upon serum creatinine obtained within 2 weeks prior to zoledronate dose.

$$\text{Creatinine Clearance} = \frac{(140 - \text{age in years}) \times (\text{weight in kg})}{72 \times \text{serum Creatinine}} \quad (\times 0.85, \text{ if female})$$

Creatinine Clearance (mL/min)	Zoledronic acid Dose
>60	4 mg
50-60	3.5 mg
40-49	3.3 mg
30-39	3 mg

<30	**Hold**

Doses of zoledronic acid may not be given earlier than 48 hours prior to day 1 of each cycle of therapy; however doses of zoledronic acid may be delayed for up to 4 weeks if the delay in therapy is felt to be in the best interest of the patient. The duration and reason for the delay should be documented on the appropriate treatment forms. Patients should continue with laboratory and toxicity assessments as outlined in section 8.0. Patients requiring a delay in therapy administration for $\geq$ 4 weeks will discontinue protocol therapy. Dasatinib may be administered as a single agent while zoledronic acid is held.

Patients should receive supplemental oral calcium (1000-2000 mg daily) and vitamin D (400-800 IU daily) unless felt to be medically contraindicated by the treating physician.

7.4 Dose Modifications

7.4.1 Dasatinib

NOTE: Toxicity will be assessed using the NCI Common Toxicity Criteria (CTC) Scale, version 3.0. Dasatinib should be held for treatment related toxicities $\geq$ grade 2.

Treatment modifications will proceed as outlined in Table 7.2.

Table 7.2: Dose Modifications for Dasatinib.

Dose Level	Dose (if RP2D=100 mg daily)	Dose (if RP2D=70 mg daily)	
A	70 mg daily	50 mg daily	
B	50 mg daily	Discontinue protocol therapy	
C	Discontinue protocol therapy		
Toxicity	Treatment Modification		
Grade 1	Proceed without dose modification. Consider appropriate supportive measures.		
Grade 2	If 1st episode, hold therapy until recovery to Grade $\leq$1, then continue treatment at dose level A. If 2nd episode, hold therapy until recovery to Grade $\leq$1, then continue treatment at dose level B.		

	If 3rd episode, discontinue protocol therapy
Grade 3	If 1st episode, hold therapy until recovery to Grade ≤1, then continue treatment at dose level B. If 2nd episode, discontinue protocol therapy
Grade 4	May discontinue treatment permanently or, at the discretion of the treating physician, hold therapy until recovery to Grade ≤1, then continue treatment at dose level B. If any grade > 1 toxicity recurs, patient must discontinue protocol therapy.

Decisions for dose reductions will be made according to grade of toxicity and will be independent of the type of toxicity. For example, if a patient develops grade 2 hypokalemia that resolves and, upon re-treatment, develops grade 2 elevation of AST; the second event (i.e. elevated AST) will require a dose reduction because it is the second episode of grade 2 toxicity.

Zoledronic acid may be continued if dasatinib is held or discontinued.

The dosing time may be adjusted as 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.

7.4.2 Dose Modifications for Zoledronic Acid

Dosing of zoledronic acid will be performed as outlined in section 7.3.2. Zoledronic acid will be permanently held in patients who develop evidence of osteonecrosis, acute renal failure attributed to therapy with zoledronic acid, or hypersensitivity to zoledronic acid that recurs despite appropriate prophylaxis. Patients may continue single agent dasatinib if zoledronic acid is discontinued.

7.5 Discontinuation of Therapy

Study therapy must be immediately discontinued for the following reasons:

- Progressive disease at any time
- Any clinical adverse event, laboratory abnormality or intercurrent illness which, in the opinion of the Investigator, indicates that continued treatment with study therapy is not in the best interest of the subject
- Excessive toxicity despite dose reduction
- Withdrawal of informed consent (subject's decision to withdraw for any reason)

- Pregnancy
  - All WOCBP should be instructed to contact the Investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation. Institutional policy and local regulations should determine the frequency of on study pregnancy tests for WOCBP enrolled in the study.
  - The Investigator must immediately notify BMS in the event of a confirmed pregnancy in a patient participating in the study.

7.5.1 Follow up After Discontinuation of Protocol Therapy:

All patients will be followed for 2 years after discontinuation of protocol therapy or until death, whichever occurs first. Patients will follow up 1 month after completing therapy for toxicity assessment. After this, patients will be followed every 3-6 months for survival outcome. This follow up can include contacting an outside treating physician if the patient does not wish to return to the treating institution.

7.6 Prohibited and Restricted Therapies During Study

7.6.1 Prohibited Therapies

Subjects requiring any of the following prohibited therapies should not be enrolled.

CYP3A4

Dasatinib is primarily metabolized by the CYP3A4 enzyme. 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. Subjects should be advised not to consume substantial quantities of grapefruit juice.

Drugs that may increase dasatinib plasma concentrations

CYP3A4 Inhibitors: Dasatinib is a CYP3A4 substrate. Concomitant use of Dasatinib and drugs that inhibit CYP3A4 (See Appendix 2) may increase exposure to dasatinib and should be avoided. 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.

Medications that prolong QT Interval

Subjects enrolled in this study should not take or begin to take concomitant medications known to prolong the QT interval. For such medications, a wash-out period of ≥7days is required prior to starting

dasatinib. (Agents which may possibly prolong the QT interval are restricted). Medications known to prolong the QT interval (Class I; see http://www.qtdrugs.org/medical-pros/drug-lists/drug-lists.htm) are: Drugs that are generally accepted to have a risk of causing Torsades de Pointes include:

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

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, the Investigator must check that the subject's prior on-therapy ECG has not shown a QTcF ≥ 450 msec or an increase in QTc ≥ 60 msec over the baseline value.

7.6.2 Restricted Therapies

Drugs that may decrease dasatinib plasma concentrations

CYP3A4 Inducers: Drugs that induce CYP3A4 activity may decrease dasatinib plasma concentrations. In patients in whom CYP3A4 inducers (See Appendix 2) are indicated, alternative agents with less enzyme induction potential should be used. If dasatinib must be administered with a CYP3A4 inducer, a dose increase in dasatinib should be considered.

Drugs that may have their plasma concentration altered by dasatinib

CYP3A4 Substrates: 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.

Less-potent inhibitors, inducers, and substrates of CYP3A4 are restricted. (Appendix 2)

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

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.

H_2 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.

Medications that inhibit Platelet Function and Anticoagulants

Caution should be exercised if patients are required to take medications that inhibit platelet function or anticoagulants.

Subjects enrolled in this study should not take concomitant medications which durably inhibit platelet function. For such medications, a wash-out period of ≥7days is required prior to starting dasatinib. (Agents which inhibit platelet function transiently or inhibit coagulation by other mechanisms are restricted.)

Medications which directly and durably inhibit platelet function include:

- aspirin or aspirin-containing combinations, clopidogrel, dipyridamole
- tirofiban, dipyridamole, epoprostenol, eptifibatide, cilostazol, abciximab, ticlopidine, cilostazol

  Medications which directly and durably inhibit anticoagulation include:

- warfarin, heparin/low molecular weight heparin [e.g., danaparoid, dalteparin, tinzaparin, enoxaparin]
- Exceptions: low-dose warfarin for prophylaxis to prevent catheter thrombosis, and heparin for flushes of IV lines.

7.7 Concomitant Therapy

NOTE: For the phase I portion of the trial, concomitant medications may be used to treat symptoms that develop during therapy; however, the routine use of concomitant medications (except antibiotics) as prophylaxis for toxicity is not allowed during cycle 1 of therapy. Concomitant medications can be used as prophylaxis for toxicity during cycles > 1.

In general, the use of any concurrent medications deemed necessary for the care of the patient are allowed. For patients receiving cycles > 1 in the phase I portion of the trial and for all patients enrolled in the phase II portion of the trial, drugs may be given prophylactically to reduce the side effects of therapy. The following exceptions apply:

- No other investigational therapy will be given to patients.

- The use of anticancer agents (other than hormonal therapy) or radiation therapy is not allowed (an exception would be the use of megestrol acetate for the treatment of anorexia).
- Patients enrolled in the Phase I portion of the trial may not receive GCSF or GMCSF during cycle 1 of therapy. The use of hematologic growth factors for the treatment of anemia is allowed at any time during protocol therapy. Patients enrolled in the Phase II portion of the trial or those who have completed >1 cycle of therapy in the phase I portion of the trial may receive growth factor support as per ASCO guidelines.
- Where possible, drugs known to inhibit CYP3A4 should be avoided as systemic therapy as a pharmacokinetic interaction may occur during the concomitant use of dasatinib (see section 7.5).

8 STUDY PROCEDURES

8.1 Evaluation and visit schedule

Table 8.1 Evaluation and visit schedule in Phase I

Evaluations:	Screening	Dasatinib + Zoledronic Acid										Final visit
		Cycle 1					Continuing Cycles					
	D-14 to D- 0	Day 1	Day 8	Day 15	Day 21	Day 28	Day 1	Day 8	Day 15	Day 21	Every 3rd Cycle	
Selection (inclusion/exclusion criteria), medical history, demography, signed informed consent	X											
Physical exam[3], ECOG performance status	X	X		X			X				X	X
Blood pressure, pulse, temperature, weight	X	X		X			X					X
Laboratory[1]: hematology, serum biochemistry, urinalysis	X	X[1]	X	X	X		X					X
Pregnancy Test	X[8]											
Calculate Creatinine Clearance	X	X	X	X	X		X					X
Concomitant medications	X	Continuous ----------→										
Adverse events, toxicity		Continuous ----------→										
ECG[5]	X	X	X	X	X		X[5]					
Tumor evaluation/staging[2] (Radiographic imaging and/or tumor markers)	X										X	
Treatment: Dasatinib[3]		Continuous ----------→										
Zoledronic Acid[3]		X					X					
Response Assessment: Ntx[4]	X					X					X	
Imaging Studies[2]	X										X	
Correlative Studies (optional):												
PET scan	X			X								X[6]
Biopsy	X					X						
Blood Samples[7]	X					X[7]					X	X[6]

1. May be drawn on day 1 (+/- 48 hours) however, results must be reviewed prior to patient receiving day 1 of therapy. Hematology: hemoglobin, hematocrit, platelets, total white blood cell count (WBC) and differential. Serum biochemistry: sodium, potassium, chloride, bicarbonate, creatinine, blood urea nitrogen, albumin, total protein, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, uric acid, calcium, Glucose, magnesium. Urinalysis will only be performed during cycle 1 of therapy. Screening lab may be used for day 1 cycle 1 of therapy.
2. Baseline scans may be performed 1 month prior to initiating protocol therapy. All known sites of metastasis must be imaged prior to initiating protocol therapy. For response assessment, only imaging studies with detectable tumor-related abnormalities will be required to follow response. Bone scan will be performed pretreatment and used as a baseline for comparison, if needed, to verify disease progression or response as assessed using plain films.
3. Physical exam must be performed on day 1 (+/- 48 hours) of cycles 1 and 2 and occur prior to administration of protocol therapy. After cycle 2, physical exams can be performed every 2 cycles of therapy.
4. Urine for N-telopeptide. Must be collected as scheduled on day 28 (+/- 48 hours) of cycle 1 and every 3rd cycle of therapy(after 8 weks of treatment are completed and restagning takes place). Must be collected before receiving subsequent cycles of therapy.
5. ECG will only be performed during cycles 1 and 2 of therapy. Screening ECG may be performed up to 28 days prior to protocol therapy.
6. PET scan will be done at time of disease progression to restage patients. Blood samples will be drawn at the time of documented disease progression (+/- 14 days).
7. Blood will be collected for MUC-1 (CA27-29) levels and serum will be banked. Blood will be drawn during weeks 4, 8, 16 and 24. May be drawn +/- 72 hours of time points indicated but must be drawn prior to administration of therapy.
8. Must be performed in women of childbearing potential within 72 hours of dasatinib administration.

Table 8.2 Evaluation and visit schedule in Phase II

Evaluations:	Screening	Dasatinib + Zoledronic Acid										Final visit
		Cycle 1					Continuing Cycles					
	D-14 to D- 0	Day 1	Day 8	Day 15	Day 21	Day 28	Day 1	Day 8	Day 15	Day 21	Every 3rd Cycle	
Selection (inclusion/exclusion criteria), medical history, demography, signed informed consent	X											
Physical exam[3], ECOG performance status	X	X					X					X
Blood pressure, pulse, temperature, weight	X	X					X					X
Laboratory[1]: hematology, serum biochemistry,	X	X[1]					X					X
Pregnancy test	X[8]											
Calculate Creatinine Clearance	X	X					X					X
Concomitant medications	X	Continuous ----------→										
Adverse events, toxicity		Continuous ----------→										
ECG[6]	X					X						
Tumor evaluation/staging[2] (Radiographic imaging and/or tumor markers)	X						X				X	
Treatment: Dasatinib[3]		Continuous ----------→										
Zoledronic Acid[3]		X					X					
Response Assessment: Ntx[4]	X[4]					X					X	
Imaging Studies[2]	X										X	
Correlative Studies (optional):												
PET scan	X			X								X[6]
Biopsy	X					X						
Blood Samples[7]	X						X[7]					X[6]

1. May be drawn on day 1 (+/- 48 hours) however, results must be reviewed prior to patient receiving day 1 of therapy. Hematology: hemoglobin, hematocrit, platelets, total white blood cell count (WBC) and differential. Serum biochemistry: sodium, potassium, chloride, bicarbonate, creatinine, blood urea nitrogen, albumin, total protein, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, uric acid, calcium, Glucose, magnesium. Screening lab may be used for day 1 cycle 1 of therapy.

2. Baseline scans may be performed 1 month prior to initiating treatment. All known sites of metastasis must be imaged prior to initiating protocol therapy. For response assessment, only imaging studies with detectable tumor-related abnormalities will be required to follow response. Bone scan will be performed pretreatment and used as a baseline for comparison if needed to verify disease progression or response as assessed using plain films.

3. Physical exam must be performed on day 1 (+/- 48 hours) of cycles one and two and occur prior to administration of protocol therapy. After cycle 2 of therapy, physical exam may be performed every 2 cycles.

4. Urine for N-telopeptide. Must be collected as scheduled on day 28 (+/- 48 hours) of cycle 1 and every 3rd cycle of therapy(after 8 weeks of treatment are completed and restaging takes place). Must be collected before receiving subsequent cycles of therapy. Accrual to the low Ntx cohort will be capped after 10 patients. If > 10 patients with low levels of Ntx are accrued prior to study completion, patients will undergo screening Ntx prior to study entry.

5. Screening ECG may be performed up to 28 days prior to protocol therapy.

6. PET scan will be done at time of disease progression to restage patients. Blood samples will be drawn at the time of documented disease progression (+/- 14 days).

7. Blood will be collected for MUC-1 (CA27-29) levels and research serum will be banked. Research blood will be drawn during weeks 4, 8, 16 and 24. May be drawn +/- 48 hours of time points indicated but must be drawn prior to administration of therapy. (Cycle =28 days/4 weeks)

8. Must be performed in women of childbearing potential within 72 hours of dasatinib administration.

9 EFFICACY AND SAFETY ASSESSMENTS

9.1 Efficacy Assessment

9.1.1 Measurement of Tumor Response

Tumor response will be assessed for individual patients and will be used to determine which patients should continue protocol therapy in the absence of toxicity. Patients with bone only involvement or disease considered non-measurable by RECIST will be followed for response using the WHO Criteria for Assessment of Disease Response in Bone or the MDACC Modified Response Criteria for Assessment of Disease Response in Bone (Post-text supplement 4). Patients with measurable disease outside of the bone will have response measured using a combination of RECIST criteria (Post-text supplement 3) and the WHO Criteria for Assessment of Disease Response in Bone (Post-text supplement 4).

Patients who meet criteria for disease progression within the bone (as measured by WHO criteria) or in non-bone disease (as measured by RECIST criteria) or both will be considered to have progression of disease and will be withdrawn from protocol therapy.

It is feasible that patients may have response in bone metastasis and disease progression in non-bone metastasis. Because these patients have met criteria for disease progression, they will discontinue protocol therapy; however, (for statistical purposes only) patients with disease progression by RECIST in areas outside of the bone but disease response in the bone will be counted as responders for the purpose of correlation of bone response with changes in Ntx. Such patients will be counted as non-responders for all other endpoint analyses.

Radiologic assessments must account for all lesions that were present at screening and must use the same imaging techniques that were used at screening. Any bone lesions that have been previously treated with radiation therapy or surgical resection should not be used for tumor assessment unless the lesions have shown definite progression since treatment.

9.1.2 N-telopeptide of Type I Collagen (Ntx)

Ntx, a marker of bone resorption, has been found to significantly correlate with risk of negative clinical outcomes(28), response to systemic therapy(29) and response to bisphosphonate therapy(25). Because this proposal hypothesizes that combined therapy with dasatinib and zoledronic acid will exert anti-tumor effects through disruption of the tumor cell/osteoclast paracrine loop, biomarkers of bone resorption would be expected to accurately predict tumor response and time to disease progression in this special cohort of patients. Urinary measures of Ntx will be performed using standard techniques at baseline, 1 month, 3 months and then every 3rd months until disease progression or protocol withdrawal.

Urine for Ntx analysis will be obtained on day 28 (+/- 48 hours) of cycle 1, cycle 3 and every subsequent 3rd cycle while the patient is receiving protocol therapy. Urine will be obtained during the second morning void prior to day 1 of specified cycles. Assessment of Ntx will be performed through an outside lab using a commercially available assay.

Although Ntx will be used as an endpoint for efficacy analysis and statistical design, changes in Ntx will not be used for decisions regarding continuation of therapy on an individual patient basis.

9.2 Safety Assessment

Safety assessments will consist of monitoring and recording all adverse events (AEs) and serious adverse events (SAEs), the regular monitoring of hematology, blood chemistry and urine values, regular measurement of vital signs, and performance of physical examinations. The AE and SAE reporting period will begin with the signing of informed consent and end 30 days after discontinuation of protocol therapy. Reporting of AEs and SAEs will be as designated in section 11.

10 STUDY DRUG(S)

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.

10.1 Dasatinib Product Identification

Dasatinib will be supplied in two different strengths by Bristol-Myers Squibb.

- 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

10.2 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.

10.3 Storage, Handling and Dispensing of Dasatinib

10.3.1 Storage

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

10.3.2 Handling and Disposal

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

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

10.3.3 Dispensing

It is the responsibility of the Investigator to ensure that dasatinib is only dispensed to study subjects. The 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.

10.4 Drug Ordering and Accountability

10.4.1 Initial Orders

Initial Orders of dasatinib will be requested by the assigned BMS protocol manager. Initial drug supply is provided for a 12-week treatment period per subject.

10.4.2 Re-supply

Re-supply requests can be obtained by completing the SRC re-supply request form and submitting the request form electronically via e-mail to **srcsupply@bms.com**. Re-supply requests should be submitted at least 5-7 business days before the expected delivery date. Deliveries will be made Tuesday through Friday.

10.5 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.

10.6 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.

10.7 Zoledronic Acid

10.7.1 Supply

Zoledronic acid will be purchased from Novartis in plastic vials containing 4mg zoledronic acid in a 5 mL concentrate as a solution for infusion.

10.7.2 Preparation

The zoledronic acid 4 mg/5 mL concentrate solution is not for direct infusion and has to be further diluted prior to the use. Prior to administration, the 5 mL of the concentrate solution must be diluted with 100 mL calcium-free infusion solution (0.9% sodium chloride solution or 5% glucose solution). The appropriate volume of the reconstituted zoledronic acid solution is 105 mL. The necessary infusion bags/bottles, containing either 100ml calcium free 0.9% sodium chloride or 5 % dextrose solution, that have to be used for the set up of the infusion will be provided by the study center. Glass bottles and infusion bags or tubing made from polyvinylchloride (PVC), polypropylene (PP) and polyethylene (PE) are appropriate for use with Zoledronic acid.

If not used immediately after dilution with infusion media, for microbiological integrity, the final solution must be placed in a refrigerator with a temperature between 2-8°C. The refrigerated solution should then be equilibrated to room temperature prior to administration. The total time between dilution, storage in a refrigerator, and end of administration of the infusion must not exceed 24 hours. Reconstituted zoledronic acid solutions must be administered in no less than a 15-minute intravenous infusion in a line separate from all other drugs.

A peripheral or central intravenous site is to be used for the study drug infusion. Study drug should be administered as a single intravenous solution in a line separate from all other drugs. The bore of the needle or angiocatheter used to insert the intravenous line will be 20 to 22 gauge. The i.v. infusion will be preceded by and followed by a 10 ml normal saline flush of the intravenous line. In order to allow constant flow a vented infusion line should be used. Prior to application of the drug, the infusion line via a y-connector or other similar set-up will be flushed with approximately 10 ml of normal saline. Thereafter, the solution will be infused over a period of no less than 15 minutes. After emptying, approximately 3 mL of drug product solution may stay in the infusion bottle. Use at least 10 ml of normal saline to flush the infusion line.

A pharmacist or other qualified person will be responsible for the preparation of the i.v. The qualified person who prepares the drug to be infused must enter the appropriate drug preparation information requested on the sign off log for drug preparation. Documentation of trial-drug administration and amount infused will be maintained for every patient

Preparation of reduced dose solution for patients with mild to moderate renal impairment (CrCl <60 mL/min) at baseline (baseline defined as the serum creatinine, just prior to their first ever Zoledronic acid dose, approximately one year prior to study entry):

Withdraw an appropriate volume of the 5 mL – study drug concentrate:

4.4 mL	for 3.5 mg dose
4.1 mL	for 3.3 mg dose
3.8 mL	for 3.0 mg dose

The withdrawn concentrate must be diluted in 100 mL of sterile 0.9% sodium chloride, USP, or 5% dextrose injection, USP. The dose must be given as a single intravenous infusion over no less than 15 minutes.

Medication vial labels will comply with the legal requirements of the United States. They will supply no information about the patient, only the medication number. The storage conditions for study drug will be described on the medication label.

10.7.3 ANTICIPATED TOXICITY

Anticipated toxicities are as described in the package insert for zoledronic acid (see Post-text supplement 2).

10.7.3.1 Osteonecrosis of the Jaw (ONJ)

After study entry, any patient report of the following symptoms at interim visits will require an examination by a dental professional (dental hygienist or dentist) to rule out ONJ prior to further study drug dosing:

- exposed bone in the oral cavity
- rough area on the jawbone
- "heavy jaw", a dull aching sensation
- numbness/tingling of the jaw
- loosening of teeth
- tooth pain
- sudden change in the health of periodontal or mucosal tissue
- failure of oral mucosa to heal
- undiagnosed oral pain,
- soft tissue swelling, drainage or infection

If exposed bone is observed on dental examination, the patient will be referred to an oral surgeon for a panoramic x-ray, diagnosis, and treatment. While a precise definition of "ONJ" does not exist, Novartis has developed a working definition of ONJ based on input from a panel of experts. Thus, an AE is designated as a possible case of ONJ if **all** the following criteria are met:

1. There is exposed bone in the maxillofacial area that occurred either
   - spontaneously; or
   - was induced by dental surgery that has no evidence of healing for more than 3 to 6 weeks after appropriate care
2. May or may not be associated with infection
3. May or may not be associated with pain
4. Appeared in the absence of prior radiation to the head or neck

If a diagnosis of ONJ is made, study medication will be stopped permanently, the patient will be discontinued from the study, and further treatment will be at the discretion of the patient's physician. The patient will be followed for outcome of ONJ.

All such diagnosed cases of ONJ should be considered as "medically significant," irrespective of whether the event meets the definition of "serious adverse event (SAE)" under current health authority guidelines. These cases are therefore to be reported to Bristol-Myers Squibb as well as local health authorities under the guideline of SAE reporting (see section 11).

Osteonecrosis at any skeletal site, and osteomyelitis at any skeletal site, should also be considered as medically significant and reported to Bristol-Myers Squibb as well as local health authorities under the guidance of SAE reporting (see Section 11)

If diagnosis of ONJ is **not** made, then the patient will be allowed to continue on the study at the discretion of the investigator.

10.7.3.2 Monitoring of Renal Function

Serum creatinine should be monitored in all patients treated with zoledronic acid infusion prior to each dose. A 48-hour window for checking creatinine is allowed prior to the each study infusion. Elevations in serum creatinine above baseline values may require a delay in treatment.

Each pre-infusion serum creatinine during the study must be compared with the baseline serum creatinine and should be managed as follows:

- If the patient's baseline serum creatinine (before the first ever zoledronic acid dose) was < 1.4 mg/dL, an increase of 0.5 mg/dL or more will require that the study drug be delayed until the patient's serum creatinine returns to no higher than 10% above the baseline value.
- If the patient's baseline (before the first ever zoledronic acid dose) serum creatinine was ≥ 1.4 mg/dL, then an increase in the serum creatinine of 1.0 mg/dL or more will require that the study drug be delayed until the patients serum creatinine returns to no higher than 10% above the baseline value.
- Any doubling of the baseline (before the first ever zoledronic acid dose) serum creatinine will require that the study drug be delayed until the patient's serum creatinine returns to no higher than 10% above the baseline value.

Zoledronic acid should be re-initiated at the same dose as that prior to treatment interruption.

11 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 <u>any dose</u>:

- 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.**

11.1 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 **NCI Common Toxicity Criteria (CTC) Scale, version 3.0.**

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 Bristol-Myers Squibb at:

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

- For studies conducted under an Investigator IND, any event that is both serious and unexpected must be reported to the FDA as soon as possible and, in no event, later than 7 days (death or life-threatening event) or 15 days (all other SAEs) after the investigator's or institution's initial receipt of the information. Bristol-Myers Squibb will be provided with a simultaneous copy of all adverse events filed with the FDA. SAEs should be reported on the MedWatch Form 3500A, which can be accessed at: http://www.accessdata.fda.gov/scripts/medwatch/

  MedWatch SAE forms should be sent to the FDA at:

  MEDWATCH
  5600 Fishers Lane
  Rockville, MD 20852-9787
  Fax: 1-800-FDA-0178 (1-800-332-0178)
  http://www.accessdata.fda.gov/scripts/medwatch/

All SAEs should simultaneously be faxed or e-mailed to Bristol-Myers Squibb 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.

12 STATISTICAL METHODOLOGY

12.1 PHASE I

The primary objective of the phase I portion of this trial will be to determine the RP2D for dasatinib in combination with zoledronic acid.

The phase I portion of the trial will use a 3+3 dose escalation design. If at least 2 of 3 patients treated within a given dosing cohort develop DLT, it can be concluded with 90% confidence that the true probability of DLT at that dose is greater than 20%. If none of the 3 patients demonstrates DLT, it can be concluded with 90% confidence that the true probability of DLT is less than 55%. These criteria are considered a standard for dose selection using the proposed phase I clinical trial design.

When 1 of 3 patients develops DLT and the cohort is expanded to 6 patients, the proposed plan for dose escalation provides 91% probability that dose escalation will not be stopped at doses associated with DLT probability of < 10% and gives a 92% probability that escalation will not proceed beyond doses associated with DLT probability in excess of 60%. The stepwise dose escalation also protects against defining an RP2D associated with excessive toxicity. For a wide variety of dose-toxicity curves, the probability is approximately 85-90% that the defined RP2D will be associated with DLT probability of approximately 10-45%.

12.2 Phase II

The primary objective of this phase II study is to assess the efficacy of dasatinib in combination with zoledronic acid. Up to 25 patients will be enrolled in the phase II study to assess the efficacy of dasatinib in combination with zometa (zoledronic acid). The primary endpoint is the response rate. The trial will be conducted by the Simon's two-stage design using the minimax criterion and the response rate will be estimated accordingly.

It is assumed that dasatinib in combination with zometa will have a target response rate of 25%. A response rate of 5% or lower is considered a failure. When the probability of accepting a "bad" regimen (i.e. response rate ≤ 5%) is 0.05 and the probability of rejecting a "good" regimen (i.e. response rate ≥ 25%) is 0.10, Simon's design to minimize the maximum sample size requires 15 patients in the first stage. If no patients respond to the treatment, the trial will be stopped and the regimen will be declared as ineffective. If at least one of the first 15 patients respond to the treatment, 10 additional patients will be entered in the study to reach a total of 25 patients. By the end of the study, the new regimen will be rejected if response rate is less than or equal to 3 out of 25 patients and will be accepted otherwise. The operating characteristics of the trial are

given as follows. When the true response rate is 0.05, the probability of stopping the trial early is 46.3%. On the other hand, if the true response rate is 0.25, the probability to stop the trial early is 1.3%. The expected sample sizes are 20.37 and 24.97 when the true response rates are 0.05 and 0.25, respectively. At the end of the study, the response rate and the 95% confidence interval will be reported and the toxicity profile of the regimen will be summarized. If the trial continues to the second stage (i.e., accrual = 25) and the 5 of 25 patients response for a point estimate of 20.0%, the exact binomial 95% confidence interval would be (6.8%, 40.7%).

Patients will be divided into two cohorts dependent upon Ntx levels obtained prior to the initiation of therapy. All patients will receive same therapy, lab draws and radiographic assessment for response.

12.2.1 Cohort A: Patients with moderate or high baseline levels of Ntx:

We will determine the association between changes in Ntx levels and tumor response, progression-free survival (PFS), and overall survival (OS).

Changes in Ntx levels will be considered as both a continuous and a dichotomous measure. We will describe the percent change and the absolute change in Ntx levels from baseline to one month and from baseline to 3 months following the start of treatment with descriptive statistics such as the mean, median, range, and standard deviation. We will use the t-test or Wilcoxon's rank sum test to determine the association between changes in Ntx levels and tumor response. Considering the change in Ntx levels as dichotomous (maintained moderate or high level versus achieved low level) we will determine the association between achieving a low Ntx level and tumor response with a chi-square or Fisher's exact test as appropriate.

PFS will be measured from the date of first treatment to the date of disease progression or death from any cause. OS will be measured from the date of first treatment to the date of death from any cause. The percent change and the absolute change in Ntx levels will be included in Cox proportional hazards models to estimate the change in the hazard of death and the change in the hazard of progression associated with a change in Ntx levels. Considering the change in Ntx levels as dichotomous, the Kaplan-Meier product limit method will be used to estimate PFS and OS and the log-rank statistic will be used to test for differences between patients who do and do not achieve a low level Ntx level. Finally, we will consider the serial measurements of Ntx by including them as a time-dependent covariate in Cox proportional hazards models.

12.2.2 Cohort B: Patients with low levels of Ntx:

These patients have been reported to have a more favorable outcome compared to patients with moderate to high levels. However, it remains uncertain if further reduction of Ntx levels in this subset of patients would be predictive of response to therapy.

We will first describe the percent change and absolute change in Ntx levels from baseline to one month and from baseline to three months following the start of treatment with descriptive statistics such as the mean, median, range, and standard deviation. Percent change in Ntx will also be considered as a dichotomous measure using 30% as the threshold value. We will describe both the change in Ntx levels and the proportion of patients with at least a 30% decrease in Ntx levels separately by tumor response groups. We will plot the Kaplan-Meier curves of PFS and OS overall survival and visually assess the differences between patients who achieved at least a 30% decrease in Ntx and those who did not.

13 ADMINISTRATIVE SECTION

13.1 Compliance with the Protocol and Protocol Revisions

The study must be conducted as described in the final approved protocol. All revisions to the protocol must be provided to BMS along with IRB approval verification. The Investigator should not implement any deviation or change to the protocol without prior review and documented approval/favorable opinion from the IRB of an Amendment, except where necessary to eliminate an immediate hazard(s) to study patients.

Documentation of approval signed by the chairperson or designee of the IRB(s) must be sent to BMS.

13.2 Ethical Considerations and Informed Consent

The following must be observed in order to comply with Food and Drug Administration regulations for the conduct and monitoring of clinical investigations:

The principles of informed consent are described by Federal Regulatory Guidelines.

13.3 Records and Reports

An Investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the investigation on each individual treated with the investigational product.

13.4 Institutional Review Board (IRB)

Before study initiation, the Investigator must have written and dated approval/favorable opinion from the IRB for the protocol, consent form, patient recruitment materials/process (e.g., advertisements), and any other written information to be provided to patients. The Investigator should also provide the IRB with a copy of the product labeling, information to be provided to patients, and any updates.

The Investigator will provide the IRB with reports, updates, and other information (e.g., Safety Updates, Amendments, Administrative Letters) according to regulatory requirements and Institution procedures.

A detailed list of required regulatory documents also to be submitted to BMS will be sent upon final approval of the protocol.

13.5 Records Retention

The Investigator must retain investigational product disposition records, case report forms and source documents for the maximum period required by applicable regulations and guidelines, or Institution procedures.

If the Investigator withdraws from the study (e.g., relocation, retirement), the records shall be transferred to a mutually agreed upon designee (e.g., another Investigator, IRB). Notice of such transfer will be given in writing to BMS.

14 CORRELATIVE STUDIES

14.1 Biologic Characterization of Response Using ^{18}F-FDG PET:

Characterization of response using PET will be performed through collaboration with Dr. Juri Gelovani, chair of the Department of Experimental Diagnostic Imaging in the Division of Diagnostic Imaging at MDACC. ^{18}F-FDG PET imaging has demonstrated increased glucose consumption in breast cancer with substantial variation in uptake influenced by the number of viable tumor cells per volume, histological subtype (ductal > lobular), tumor grade, microvessel density and proliferative activity(35). In addition, changes in ^{18}F-FDG PET avidity have been correlated with breast cancer response to both hormonal therapy and chemotherapy and in patients with predominate bone metastasis in small non-randomized studies(36-38). Although FDG uptake has been studied in patients with solid tumors treated with the angiogenesis inhibitors(39), to date no published phase II data exists concerning changes in ^{18}F-FDG uptake in breast cancer patients treated with dasatinib. It is hypothesized that dasatinib's inhibition of the paracrine loop supporting bone metastasis will result in decreased cancer cell proliferation, decreased microvessel density and a decrease in the number of viable tumor cells, which will decrease tumor avidity for^{18}F-FDG. MDACC has had extensive experience with the use of PET to image intracellular receptors, such as ER, in breast cancer patients(40) and in the development of biologic markers of response in patients undergoing therapy with angiogenesis inhibitors(39).

Patients who consent to participate will undergo optional ^{18}F-FDG PET imaging at baseline, between days 12-16 of therapy (2 week value), and at disease progression. The fold change from baseline will be calculated for the 2 week value and progression of disease value. These values will then be graphed using scatterplots against urine Ntx to assess the relationship of response to this biologic endpoint. In addition, it is known that variations in ^{18}F-FDG PET avidity can occur within different foci of disease in individual patients. These variations may represent biologic differences between responding and non-responding cells; therefore, core needle biopsies will be performed on a subset of patients who consent to biopsy of $\geq$2 sites of metastatic tumors that differ in ^{18}F-FDG PET uptake (see Molecular Correlates below).

14.2 Determination of Molecular Correlates in Paraffin Embedded or Frozen Tissue:

Tumor biopsies will be obtained by imaging (CT or Ultrasound) guided core needle biopsy from patients who sign informed consent for tissue biopsy. Biopsies will be obtained at pre-treatment and at day 28 of cycle 1 (+/- 48 hours). Dasatinib inhibits a number of targets including SRC, PDGFR, c-kit, FAK and EPHA2. Expression of these markers may be correlated with tumor response and their expression in bone metastasis remains ill defined. Tumor samples will be analyzed for these targets using immunohitochemistry. In addition, Src regulates proteins such as cavelolin and STAT3, which are responsible for osteoblastic activity, tumor cell metastasis and migration, and response to chemotherapy (41) (42),(7). We will also use immunohistochemistry to measure phosphorylated activity of these proteins in pre- and post-treatment biopsies to determine the biologic effects of therapy. In addition, as stipulated in the biologic rationale for this study, Src has been linked to angiogenesis through downstream effects on STAT3(43, 44). We will therefore measure the effects of dasatinib on VEGF and IL-8 using immunohistochemistry in pre- and post-treatment samples.

Fresh tissue obtained by core needle biopsy of metastatic sites differing in PET avidity in individual patients (see above) will be snap frozen and used for microarray analysis to determine potential pathways of drug resistance. When possible, these results will be confirmed using IHC, western blotting or PCR.

14.3 Banking of Blood Samples

Patients will be asked to donate 10cc of research blood pretreatment and on day 28 (+/- 48 hours) of weeks 4, 8, 16, and 24. Research blood will also be collected at the time of disease progression. Serum will be banked for future treatment efficacy analyses including potential markers of bone turnover. (cycle = 28 days/4 weeks)

The following protocol will be followed:

1) Subject should be seated for at least five minutes prior to blood collection. The draw should be from the median cubital, cephalic, or basilic veins. If possible, the draws should be dedicated draws to collect these samples.

2) Using approved, standardized methods and supplies, trained phlebotomists will collect one 3cc priming discard blood sample. Then collect one 10 cc test sample from each donor into labeled blood collection tubes without anticoagulant (red top). Tubes must be completely filled.

3) Allow the blood to clot for 30 minutes to 1 hour at room temperature.

4) Pipette the serum off of the clot and place into a clean test tube. Clarify by centrifugation at 3000 rpm for 15 minutes. Carefully remove the stopper tops and

using a transfer pipette transfer the serum from the red top tube into 500 uL aliquot vials.

5) Enter the number of vials and the sample information into the Freezerworks database.

6) Store serum samples in -80 C freezer.

Patients confidentiality will be guaranteed. The information gathered from each individual patient will be used as part of a larger statistical analysis. Patients will not be harmed because data from individual patients will be coded. The samples will be coded using consecutive numbers (1, 2, 3, etc). A code breaker exists in the possession of the principal investigator. The code breaker can be used to match the coded specimens to the specimen source (i.e., pretreatment, post treatment). To minimize any potential breaches of confidentiality, specimens are stored in a secure freezer in the laboratory of Dr. Francisco Esteva at MDACC. Participants' data will be stored in the principal investigator's computer, which is password protected.

Banked samples will be available to future investigators at the discretion of the Principal Investigator and only for use through MDACC IRB approved protocols.

Samples will be shipped to:

Stacy Moulder, M.D., M.S.C.I.

The University of Texas M.D. Anderson Cancer Center

Department of Breast Medical Oncology, Unit 1354

1155 Herman P. Pressler, CPB5.3450

Houston, TX 77030-3721

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Post-Text Supplement 1 (See attached Dasatinib Investigator's Brochure)

Post-Text Supplement 2 (See attached Zometa (Zoledronic Acid) Package Insert)

Post-Text Supplement 3: RECIST Criteria

Solid Tumor Response Criteria (RECIST)

Malignant Disease Evaluation

To assess objective response, it is necessary to estimate the overall tumor burden at baseline to which subsequent measurements will be compared. Measurable disease is defined by the presence of at least one measurable lesion.

All measurements should be recorded in metric notation by use of a ruler or calipers. The same method of assessment and the same technique should be used to characterize each identified lesion at baseline and during follow-up. All baseline evaluations should be performed as closely as possible to the beginning of treatment and **never more than four weeks** before registration.

The term evaluable in reference to measurability will not be used because it does not provide additional meaning or accuracy.

At baseline, tumor lesions will be characterized as either measurable or non-measurable.

Measurable

Lesions that can be accurately measured in at least one dimension (longest diameter to be recorded) as > 20 mm (2.0 cm) with conventional techniques or as > 10 mm (1.0 cm) with spiral CT scan.

If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

Non-Measurable

All other lesions, including small lesions [longest diameter < 20 mm (2.0 cm) with conventional techniques or < 10 mm (1.0 cm) with spiral CT scan] and truly non-measurable lesions.

Lesions considered to be truly non-measurable include the following: 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.

Definitions of Response

Target Lesions

All measurable lesions up to a maximum of five lesions per organ and 10 lesions in total, representative of all involved organs. Target lesions should be selected on the basis of their size (those with the longest diameters) and their suitability for accurate repeated measurements.

The sum of the longest diameters of all target lesions will be calculated at baseline and reported as the bas*eline sum longest diameter*. The *sum longest diameter* will be used to characterize the objective tumor response. For lesions measurable in 2 or 3 dimensions, always report the longest diameter at the time of each assessment.

Complete Response (CR)

The disappearance of all target lesions. To be assigned a status of complete response, changes in tumor measurements must be confirmed by repeat assessments performed **no less than four weeks** after the criteria for response are first met.

Partial Response (PR)

At least a 30% decrease in the sum of the longest diameters of target lesions, taking as reference the *baseline sum longest diameter*. To be assigned a status of partial response, changes in tumor measurements must be confirmed by repeat assessments performed **no less than four weeks** after the criteria for response are first met.

Progressive Disease (PD)

At least a 20% increase in the sum of the longest diameters of target lesions, taking as reference the *smallest sum longest diameter* recorded since the baseline measurements, or the appearance of one or more new lesion(s).

Stable Disease (SD)

Neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease. To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval of eight weeks.

Nontarget Lesions

All other lesions or sites of disease. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

Complete Response (CR)

The disappearance of all nontarget lesions and normalization of tumor marker levels, if applicable. To be assigned a status of complete response, changes in tumor measurements must be confirmed by repeat assessments performed **no less than four weeks** after the criteria for response are first met.

Incomplete Response/Stable Disease (SD)

The persistence of one or more nontarget lesion(s) and/**or** the maintenance of tumor marker levels above the normal limits. To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval of eight weeks.

Progressive Disease (PD)

The appearance of one or more new lesion(s) and/or unequivocal progression of existing nontarget lesions.

Symptomatic Deterioration

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having symptomatic deterioration.

Evaluation of Patient's Best Overall Response

The best overall response is the best response recorded from registration until disease progression/recurrence, taking as reference for progressive disease the smallest measurements recorded since registration. The table below provides overall responses for all possible combinations of tumor responses in target and nontarget lesions, with or without new lesions.

To be assigned a status of complete or partial response, changes in tumor measurements must be confirmed by repeat assessments performed **no less than four weeks** after the criteria for response are first met.

To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval of eight weeks.

Overall Response for all Possible Combinations of Tumor Response

Target Lesions	Nontarget Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Incomplete response/SD	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

CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease

First Documentation of Response

The time between initiation of therapy and first documentation of PR or CR.

Confirmation of Response

To be assigned a status of complete or partial response, changes in tumor measurements must be confirmed by repeat assessments performed **no less than four weeks** after the criteria for response are first met.

Duration of Response

Duration of overall response – the period measured from the time that measurement criteria are met for complete or partial response (whichever status is recorded first) until the first date that recurrent or progressive disease is objectively documented, taking as reference the smallest measurements recorded since treatment started.

Duration of Overall Complete Response

The period measured from the time measurement criteria are met for complete response until the first date that recurrent disease is objectively documented.

Duration of Stable Disease

A measurement from registration until the criteria for disease progression is met, taking as reference the smallest measurements recorded since registration. To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval of eight weeks.

Post-text Supplement 4: WHO and MDACC Criteria for Response in Bone

Response Type	World Health Organization Definition[1,2]
Complete Response (CR)	Complete disappearance of all lesions on Xray or scan for at least 4 weeks
Partial Response (PR)	Partial decrease in size of lytic lesions, recalcification of lytic lesions or decreased density of blastic lesions for at least 4 weeks
No Change (NC) or Stable Disease (SD)	Because of the slow response of bone lesions, the classification of "no change" should not be applied until at least 8 weeks have passed since the start of therapy
Progressive Disease (PD)	Increase in size or extent of lesions or appearance of new lesions
Response Type	**MDACC Definition[3]**
Complete Response (CR)	Complete sclerotic fill-in of lytic lesions on XR or CT Normalization of blastic lesions on XR or CT Normalization of tracer uptake on SS Normalization of signal intensity on MRI
Partial Response (PR)	Development of a sclerotic rim or partial sclerotic fill-in of lytic lesions on XR or CT Osteosclerotic "flare" - Interval visualization of lesions with sclerotic rims or "new" sclerotic lesions in the setting of other signs of PR and absence of progressive bony disease ≥ 50% decrease in measurable lesions on XR, CT or MRI Unequivocal decrease in the size of blastic lesions on XR or CT Unequivocal decrease in tracer uptake on SS (verify

	rapid response)
No Change (NC) or Stable Disease (SD)	No change < 25% increase or < 50% decrease in measurable lesions No new lesions
Progressive Disease (PD)	≥ 25% increase in size of measurable lesions on XR, CT or MRI Unequivocal increase in the size of unmeasurable lytic or blastic lesions on XR, CT or MRI Unequivocal increase in tracer uptake on SS (verify flare) New bone metastases on CT or MRI or new symptomatic bone metastasis on bone scan

1. Occurrence of bone compression or fracture and its healing should not be used as the sole indicator for evaluation of therapy.

2. Tumor Measurements:
   - Lesions will be selected by size from the longest diameter and will be measured in centimeters.
   - The longest diameter will be used for measured lesions.
   - Size will be reported as the product of the diameters.

3. Measurements are based on the sum of a perpendicular, bidimensional measurement of the greatest diameters of each individual lesion

Post-Text Supplement 5: Eastern Cooperative Oncology Group (ECOG) Performance Status

Grade	Description
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead

APPENDIX 1 LIST OF ABBREVIATIONS

Abbreviation	Term
ANC	Absolute Neutrophil Count
BID	Twice a Day
CAT (or CT scan)	Computed Axial Tomography
CBC	Complete Blood Count
CR	Complete Response
DLT	Dose Limiting Toxicity
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
ECOG PS	Eastern Cooperative Oncology Group Performance Status
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
IRB	Institutional Review Board
MRI	Magnetic Resonance Imaging
PD	Progressive Disease
PFS	Progression Free Survival
PO	By Mouth
PR	Partial Response
QD	Once Twice daily
QoL	Quality Of Life
RECIST	Response Evaluation Criteria In Solid Tumors
SAE	Serious Adverse Event
SD	Stable Disease
TNM Staging	Tumor, Node and Metastasis Staging
ULN	Upper Limit of Normal
WBC	White Blood Count
WOCPB	Women of Child-Bearing Potential

APPENDIX 2 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 Troglitazone
Inhibitors	
Amiodarone Anastrozole Azithromycin Cannabinoids Cimetidine Clarithromycin Clotrimazole Cyclosporine Danazol Delavirdine Dexamethasone Diethyldithiocarbamate Diltiazem Dirithromycin Disulfiram Entacapone (high dose) Erythromycin Ethinyl estradiol Fluconazole (weak) Fluoxetine Fluvoxamine Gestodene Grapefruit juice Indinavir Isoniazid Itraconazole	Ketoconazole Metronidazole Mibefradil Miconazole (moderate) Nefazodone Nelfinavir Nevirapine Norfloxacin Norfluoxetine Omeprazole (weak) Oxiconazole Paroxetine (weak) Propoxyphene Quinidine Quinine Quinupristin and dalfopristin Ranitidine Ritonavir Saquinavir Sertindole Sertraline Troglitazone Troleandomycin Valproic acid (weak) Verapamil Zafirlukast Zileuton

APPENDIX 3 DASATINIB PATIENT DIARY FOR STUDY:CA180094

Your Study ID:	Your Doctor:	Your Nurse:
	Phone:	Phone:

Doctor's instructions for dasatinib:

Name of drug	Dosing		How Often	When
Dasatinib	___# of 20 mg tablets	___# of 50 mg tablets	____ day	___After Breakfast ___After supper

Cycle ____	**Week of _____ to _____**

Dasatinib dose (Check (✓) when you take it)

Insert Date	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
A.M.	☐ 20 mg ☐ 50 mg	☐ 20 mg ☐ 50 mg	☐ 20 mg ☐ 50 mg	☐ 20 mg ☐ 50 mg	☐ 20 mg ☐ 50 mg	☐ 20 mg ☐ 50 mg	☐ 20 mg ☐ 50 mg
P.M.	☐ 20 mg ☐ 50 mg	☐ 20 mg ☐ 50 mg	☐ 20 mg ☐ 50 mg	☐ 20 mg ☐ 50 mg	☐ 20 mg ☐ 50 mg	☐ 20 mg ☐ 50 mg	☐ 20 mg ☐ 50 mg

Notes (Anything you wish to tell your doctor/nurse?):

APPENDIX 4; Study Management Plan

1. **Introduction**

   The Study Management Plan outlines the procedures and requirements for institutions collaborating with UTMDACC in the conduct of an Investigator initiated trial sponsored by Bristol Meyer Squib. This plan is written in adherence to the overall BMO Medical Oncology Monitoring Plan.

2. **Purpose of a Protocol Specific Study Management Plan (SMP)**

   To establish standards that will ensure compliance with Federal Regulations, Good Clinical Practice Guidelines; and Health Insurance Portability and Accountability Act and the Institutions for both the lead and sub-investigator sites for MDACC Protocol 2006-0900.

3. **General Roles and Responsibilities of the *Lead Site***

Lead Principal Investigator's Role

Stacy Moulder MD and/or designated research staff is *responsible* for:

- **Protocol Development**: The coordination, development, submission, and approval of the protocol as well as subsequent amendments by the IRB and study sponsor.

- **Study Oversight**: PI and designee(s) will monitor the progress and overall conduct of the study at all participating institutions through the regular review of data. Data items to review are as follows: doctors examination for status of patients enrolled, eligibility, status of treatment (on/off treatment, any dose modifications, and dose delays), AE's and SAE's, and response evaluation. Also, included will be a review of regulatory and data management documents, including any amendments, memos regarding status of approvals, maintenance of regulatory documents, and queries.
- **Regulatory Management**: The lead site is responsible for timely distribution of approved protocols, amendments, revisions, or status changes to participating institutions. Collaborating sites will be corresponded with electronically, unless traditional mail methods are required. Main methods of correspondence will be email or fax. Outlook calendars will be created for each collaborating site. Required correspondence, expiration dates, or any dated or timed events will be noted on the calendars with a reminder to send necessary communication regarding those events to collaborating sites. Maintenance of regulatory binders with information from each site will be managed by a BMO designee with the help of the calendars. All regulatory documentation will be received by the Department of Breast Medical Oncology prior to initiation of any protocol related procedures. It is the participating institution's responsibility to notify its IRB of the above mentioned events.

- **Registration/Enrollment of Participants**: the lead site will review the signed informed consent, the registration form, completed eligibility CRF, Pathology Report, Relevant Scans and Labs. The registration form will include the sub-investigator's signature. The participant, if eligible,

will be registered in MDACC Clinical Research Oncology system (CORe). BMO will provide University of Chicago/Duke with confirmation of eligibility, registration and our assigned Core Number within 48 hours. If, however there is questionable data submitted to the department, source documents will be requested by Breast Medical Oncology (BMO) to verify and confirm participant's eligibility prior to enrollment. If the participant is deemed ineligible at the collaborating site, they will be notified via written correspondence (i.e. email, fax) as soon as possible, allowing for weekend/holidays by noon of the next business day.

- **Data Collection and Management**: the lead site will design and distribute case report forms (CRF's), monitor data quality, and issue queries via email if necessary. If questionable/conflicting data has been submitted to the department by a collaborating site, source documentation will be requested by BMO to verify the protocol specific data. Source documents (i.e. such as an on-study note will reflect the registration/consent/and eligibility process). Baseline and eligibility data should be received by the lead site within 7 days of registration. Protocol deviations will be reported to the lead site according to the collaborating site's IRB guidelines. Violations and serious adverse events will be reported within 24 hours of the research team being notified. SAE's should meet the guidelines of prompt reporting (causality; possible, probably, definitely related to study drug; serious and unexpected adverse events. The PI will notify the collaborating sites of these SAEs within 24 hours. Violations also need to be reported within 24 hours of the research team becoming aware. External Adverse Event will be emailed to collaborating sites received from the supporter. Response evaluations will be made available to the lead site every eight to 10 weeks. Serious Adverse Events will be reviewed by the Principal Investigator at the lead site (BMO), and shared with all collaborating sites through written correspondence (email).
- CRF's will be made available electronically to collaborating sites. The lead site will enter data into the Protocol Data Management System (PDMS) within a timely manner, 21 days from the date of submission).
- BMO will assure that all collaborating sites will provide MDACC with a copy of their IRB/IEC initial approval and continuing review approvals.

Miscellaneous Research Nurse Responsibilities

Coordinates and participates in the initiation meetings. Provides reports to the lead PI regarding the accrual, toxicities, and responses through the review of data. .

The lead site will request source documents or review documents already sent to BMO to monitor and audit participating institutions by the inspection of selected patient records. Audits will be conducted at the lead site. Documents and procedures that will be examined will be the registration process, informed consent process, eligibility, treatment, protocol compliance, outcome and response, toxicity, data quality and the documentation of each. Appropriate regulatory documents and the reporting of deviations, violations, and required adverse events will be included in the review.

Provide and maintain study contact list for all key personnel at each participating institution.

Ensure that a completed registration form and a copy of the signed Informed Consent are received at the lead site.

Ensure that eligible participants are registered on study at UTMDACC prior to the initiation of any study- related procedures, assessments or treatments.

Collect relevant de-identified radiology/nuclear medicine scans, lab, and pathology reports faxed or emailed to MDACC. These include CT Chest/Abdomen/Pelvis, Bone Scan and Survey, and MRI's performed, and ECG.

4. General Roles and Responsibilities of the *Collaborating Site*

Participating Institution ollaborating Investigator Role:

The investigator and/or designated research staff is ***responsible for compliance*** in the following areas: Protocol Management, Regulatory Management, Data Collection and Management.

Protocol Management:

- All regulatory documentation will be received by the Department of Breast Medical Oncology prior to initiation of any protocol related procedures.
- Source documentation will made available to the lead site to confirm protocol compliance (i.e. protocol eligibility, the informed consent process, protocol directed therapy compliance, adverse events, response evaluation, and other items as requested per the CRF) and in accordance with the Institution, the code of Federal Regulations, ICH, and Good clinical Practice. In addition the necessary regulatory items from each collaborating site will be shared in a timely manner with the lead site.
- Appropriate documentation regarding the investigational nature of the protocol and the alternative diagnostic or treatment approaches will be included.

Regulatory Management:

- A list of key study personnel and updates as needed on the delegation authority log.
- Submission of protocol and subsequent amendments to the local IRB prior to initiating any protocol changes or activity is the collaborating sites responsibility.
- Provide all required regulatory documents to the lead site: such as
    - Lab CLIA's and CAP
    - 1572
    - Training Log
    - Delegation of Authority Log
    - Screening Log
    - Subject Enrollment Log
    - IRB Acknowledgement of Investigational Drug Brochure/Package Insert
    - Lab Normal Ranges
    - IRB/Institutional Ethics Committee approval of the Informed Consent
    - Medical License
    - Curriculum vitae
    - Financial Disclosures
    - Initial IRB Approval

- Submission of all external and internal SAE's to local IRB with copies sent to the lead site.
- Participating sites will send the following required regulatory documents post-activation:
    - Annual Approval Documents (Continuing Reviews)
    - Informed Consent Revisions,
    - Serious Adverse Events
    - Evidence of the Participating Institution's IRB review of External Safety Reports
    - Protocol Violations and Deviations Submitted to the Participating Institution's IRB.
    - Any document that the study supporter may require.

Data Collection and Management

The Research Nurse and/or Clinical Coordinator and Data Manager will assure adherence to the protocol as noted in the source documentation and submitted as required:

- Ensure that all data is submitted into the database within 7 to 10 business days and in compliance with data entry schedule as outlined below:
    - Registration Form and required documents for enrollment at the time of registration.
    - All pre-study data should be submitted within 7 days of registration
    - Respond and resolve all data queries generated by the lead site within 3 weeks.
- Submit all subsequent protocol activity i.e. (doctor visits, treatments or exams, adverse events, protocol data/off treatment data) should be entered within 3 21 days of the event.
- Lead Site will notify participating sites of delinquent/incomplete data.
- Monitor / (QA) the completeness of data entered
- Obtain Required Regulatory Documents from Each Site Post-Activation.
    - annual approval documents (continuing reviews),
    - informed consent revisions
    - Serious Adverse Events
    - Evidence of Participating Institution's IRB review of External Safety Reports
    - Protocol Violations and Deviations submitted according to the participating institution's IRB policy.
    - Any document that the study supporter may require.

5. Patient Confidentiality and Authorization Statement:

All institutions involved in the trial will attempt to limit the use of PHI in its trials. However, because of the nature of these trials, certain PHI must be collected as per the sponsor requirements.

6. **Sub contract must be negotiated, signed and executed independently between BMS and University of Chicago and Duke University.**

7. Correlative Studies:

Supplies will be provided and paid for by the collaborating sites

Urine NTX levels will be performed according to collaborating sites policy.
All research lab samples (serum and tissue) will be processed by the participating institution. Samples will be shipped according to the participating sites shipping and handling policy to MDACC and will be stored in Dr. Francisco Esteva's lab at the expense of the collaborating sites contract when accrual is complete.

Sharell Cornet-Risher or Patricia Hutchinson CIT's will accept shipment of samples.
Address to be used is:

The University of Texas M.D. Anderson Cancer Center
Department of Breast Medical Oncology, Unit 1354
1155 Herman P. Pressler, CPB5.3450
Houston, Texas 77030-3721

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