Phase II Trial of Dasatinib in Patients with Metastatic Breast Cancer Using Real-Time Pharmacodynamic Tissue Biomarkers of Src Inhibition to Escalate Dosing

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ABSTRACT

Purpose: A phase II study of dasatinib, an inhibitor of multiple oncogenic tyrosine kinases including Src, was conducted to evaluate 16-week progression-free rate and tolerability in patients with previously treated metastatic breast cancer (MBC). Real-time assessment of potential tissue biomarkers of Src inhibition was used to optimize dosing.

Experimental Design: Eligibility criteria required that patients have measurable MBC, biopsiable tumor, and unlimited prior therapies. For the analysis of change in biomarkers of Src inhibition, p-FAK, p-pax, and p-Src, patients underwent metastatic biopsies at baseline and 4 weeks. Patients who tolerated the starting dose of dasatinib (50 or 70 mg orally twice daily) for the first 28-day cycle, and displayed suboptimal Src inhibition, were escalated to a higher dose (70 or 100 mg).

Results: The trial was closed early with 31 patients due to a statistical boundary that required at least 4 (13%) patients without disease progression to continue accrual. These 31 patients had a median of 2 prior lines of chemotherapy for MBC. The most notable toxicity was pleural effusions in 16 patients (52%). Twenty patients had evaluable metastatic biopsies. None of the tumors demonstrated the pre-defined optimal level of Src inhibition at week 4.

Conclusions: Single-agent dasatinib did not exhibit significant anti-tumor activity in patients with heavily pre-treated MBC. There were no clinically meaningful decreases before and after dasatinib exposure between exploratory tissue
biomarkers of Src inhibition which may be attributable to challenges in defining biomarker endpoints for multi-targeted tyrosine kinase inhibitors.

**Translational Relevance**

Src kinase dysregulation has been implicated in the pathogenesis of many cancers, including breast cancer. Src-mediated pathways are involved in multiple aspects of the malignant phenotype including cancer cell motility. Cancer cell motility involves FAK and paxillin, both of which are downstream targets of Src and activated by Src phosphorylation. Dasatinib is a potent inhibitor of multiple oncogenic tyrosine kinases including Src family kinases. To optimize drug dosing, efficacy, and patient selection for multi-targeted agents such as dasatinib there is a need for validated biomarker endpoints. Although this study did not meet the primary efficacy endpoint, it demonstrates that obtaining sequential tumor biopsies is a feasible research platform that should be useful in defining drug mechanisms and biomarkers of activity.
INTRODUCTION

Src kinase dysregulation is linked to the pathogenesis of many cancers, including breast cancer (1). Src-mediated pathways have been implicated in multiple aspects of the malignant phenotype including cell survival, angiogenesis, proliferation, and motility (2). Mediation of Src activity is complex including self-activation via autophosphorylation (3). Cancer cell motility, including cell migration and adhesion as well as tumor growth and invasion, involves focal adhesion kinase (FAK) and the adhesion protein paxillin (pax). Both FAK and pax are downstream targets of Src and are activated by Src phosphorylation (4-6).

In preclinical and early phase clinical studies, it appears that it is possible to down regulate Src activity. In a preclinical model using mouse xenografts and the Src inhibitor saracatinib (AZD0530), Src activity was measured by phosphorylation levels of the target proteins FAK (p-FAK) and pax (p-pax); and, as a corollary, Src inhibition induced by saracatinib was measured by decreased phosphorylation of these proteins (7). A recent phase I trial of saracatinib utilized this biomarker platform in 81 patients with advanced solid tumors, including 13 with metastatic breast cancer, and demonstrated a reduction in tumor Src activity (8). Specifically, paired tissue biopsies, before and after exposure to saracatinib, showed reduced p-FAK and p-pax levels by semiquantitative H-score analysis without a dose-response trend. Approximately 10% of patients demonstrated confirmed stable disease 6 weeks into protocol therapy (8).

Bone is a common site of metastasis in breast cancer with an estimated prevalence of 65-75% (9). Src is known to participate in normal osteoclast
function and physiologic bone resorption with deletion of the c-src gene resulting in osteopetrosis (10). In an animal model using human breast cancer cell lines, Src activity was associated with osteolytic bone metastases likely through regulation of both cell growth and production of parathyroid hormone-related protein (11). Given Src's role in lytic metastasis to bone, markers of bone resorption, such as urinary cross-linked N-telopeptide of type I collagen (NTX), may also serve as important biomarkers of Src activity.

Dasatinib, an FDA-approved therapy for chronic myelogenous leukemia and Philadelphia (Ph+) acute lymphoblastic leukemia, is a potent orally-available inhibitor of multiple oncogenic tyrosine kinases including Src family kinases (SFKs), the fusion protein Bcr-Abl, c-Kit, and platelet-derived growth factor receptor (PDGFR) (12-13). In the phase I setting investigating the use of dasatinib in advanced solid tumors, dasatinib demonstrated a generally tolerable safety profile, with a dose-limiting toxicity of pleural effusions, as well as preliminary evidence of efficacy (14-15).

In order to optimize drug efficacy and patient selection for targeted agents such as dasatinib there is a need for adaptive trial methodologies and validated biomarker endpoints. To further investigate dasatinib as a potential treatment for advanced breast cancer, we conducted a phase II study with paired metastatic tumor biopsies wherein real-time assessment of potential tissue biomarkers of Src inhibition, p-FAK and p-pax, were used to escalate dasatinib dosing.

**MATERIALS AND METHODS**
Study Design and Drug Administration

This was a 2-stage phase II single-arm multi-site study of dasatinib given as monotherapy for metastatic breast cancer. The total planned accrual was 60 patients with a planned interim analysis of progression in the first 31 patients (stage 1). Each treatment cycle of dasatinib was 28 days. Metastatic site biopsies were conducted at baseline and at the conclusion of cycle 1 (week 4). These tissue biopsies were analyzed using quantitative immunohistochemistry (IHC), measured in optical densitometry (OD) units, for the following tissue biomarkers: p-FAK, p-pax, and p-Src. These procedures are described in detail below under “Tissue Biomarker Analysis.”

For the purposes of these analyses we defined optimal evidence of Src inhibition by biomarker analysis was predefined as ≥80% inhibition of phosphorylation of both FAK and pax at the week 4 biopsy compared to the phosphorylation levels of these proteins from the baseline biopsy for the same patient. Accordingly, suboptimal evidence of Src inhibition by biomarker analysis was defined as <80% inhibition of phosphorylation of either FAK or pax compared to baseline phosphorylation levels.

Within the first 31 patients there were two pre-planned treatment groups: group A (the first 15 patients) and group B (the subsequent 16 patients). The starting dose of dasatinib for group A was 50 mg orally twice daily. In the study design, an escalation of the starting dose for group B to 70 mg orally twice daily was planned if less than 10 patients in group A had week 4 biopsies that showed ≥ 80% inhibition of both p-FAK and p-paxillin. The purpose of this planned
starting dose escalation was to increase the likelihood that the dose of dasatinib would be sufficiently high to induce changes in the tissue biomarkers of Src inhibition.

The dasatinib dosing strategy for each patient for cycle 2 and beyond was based upon individual tissue biomarker analysis as well as individual toxicity assessment. For patients who tolerated the starting dose of dasatinib (50 mg twice daily for group A and 70 mg twice daily for group B) and displayed suboptimal Src inhibition, the dose of dasatinib was escalated by one level for cycle 2: to 70 mg orally twice daily for group A and to 100 mg orally twice daily for group B as depicted in the study schematic, Figure 1. Patients who developed any toxicities ≥ grade 3 and/or developed any pleural or pericardial effusions of any grade during the first cycle of treatment with dasatinib were not eligible for dose-escalation for cycle 2 regardless of the results from tissue biomarker analysis. Dasatinib dose reduction by one level was also allowed by protocol for drug toxicity anytime during therapy. Treatment with dasatinib continued until disease progression, excessive toxicity, or patient withdrawal.

**Patients**

Women with histologically confirmed advanced breast cancer, defined as inoperable stage III or stage IV disease, were eligible. Measurable disease, defined as at least one measurable lesion according to Response Evaluation Criteria in Solid Tumors (RECIST), was required. In addition, patients were
required to have a site of metastatic disease that could be biopsied without posing excessive risk.

Patients were required to be at least 18 years of age, with Eastern Cooperative Group (ECOG) performance status of 0-1, and a life expectancy of at least 3 months. There was no limit to the number of prior therapies for MBC; however, women with hormone receptor (HR)-positive disease (estrogen receptor (ER)-positive and/or progesterone receptor (PR)-positive) were required to have progressed after at least one line of endocrine therapy while women with human epidermal growth factor receptor 2 (HER-2)-positive disease must have progressed after receiving trastuzumab in either the adjuvant or metastatic settings. Intravenous bisphosphonate use was held for 2 weeks prior and 6 weeks after dasatinib administration due to the risk of hypocalcemia.

Patients were required to have normal heart function with left ventricular ejection fraction (LVEF) ≥ 50% and normal electrocardiogram. Eligibility required normal bone marrow and organ function as documented by the following parameters: granulocytes ≥ 1,500/µL; platelets ≥ 100,000/µL; hemoglobin ≥ 9 gm/dL; total bilirubin < 2 x upper limit of normal (ULN); AST, ALT, and alkaline phosphatase ≤ 2.5 x ULN; serum creatinine ≤ 3 x ULN. Serum sodium, potassium, magnesium, phosphate and calcium were all required to be ≥ lower limit of normal (LLN).

Patients with central nervous system (CNS) metastases were eligible only if there was a solitary unresectable brain metastasis or previously treated CNS disease with stability for at least 3 months prior to study enrollment. Patients with...
bone-only MBC were excluded. Patients with active pleural or pericardial effusion of any grade were excluded although patients with a history of effusions were allowed to participate. Patients were excluded if they had been treated in the past with dasatinib or had another malignancy other than breast cancer that required radiotherapy or systemic treatment within the past 5 years.

This study was approved by the Duke institutional review board as well as the local institutional review boards for all participating sites through the Duke Breast Cancer Working Group. Informed and written consent was obtained from all study patients prior to any study-related tests or treatments.

**Study Evaluations**

The pre-study evaluation, conducted within 14 days of registration, included history and physical, clinical tumor measurement, full staging with body and brain imaging (CT or MRI), assessment of cardiac function (echocardiogram or MUGA scan), EKG, blood cell counts, serum chemistries, coagulation parameters, and serum pregnancy test for women of childbearing potential.

During treatment with dasatinib, history and physical, toxicity assessment using common terminology criteria for adverse events (CTCAE version 3.0) grading, blood cell counts, and serum chemistries were assessed weekly during the first cycle and every 4 weeks thereafter. Metastatic site tumor biopsy was performed at enrollment and 4 weeks after initiating dasatinib. Urinary NTX level was assessed at baseline and every 4 weeks thereafter. EKG was performed monthly. Assessment of cardiac function, echocardiogram or MUGA scan, was
performed every 8 weeks. Clinical tumor measurement and imaging studies, consisting of CT scans and bone scan, were conducted every 8 weeks to assess disease response according to RECIST criteria. Patients with disease progression were removed from protocol therapy.

Tissue Biomarker Analysis

Site selection for the metastatic biopsies, including imaging as appropriate, was determined on a patient-by-patient basis by the treating physician. The least invasive site for biopsy was preferred in order to prioritize patient safety and minimize the risk for biopsy-related complications. The goal for tissue collection was at least 100 mcg of tumor-containing tissue, to be collected either via core needle biopsy or punch biopsy. For each patient, the same metastatic site was biopsied at baseline and week 4. For the week 4 biopsy, patients were instructed to hold the morning dose of dasatinib until 1 hour before biopsy procedure.

Tissue biomarker analysis was performed by a Quintiles Central Laboratory (formerly Targeted Molecular Diagnostics) in Westmont, IL. Fresh biopsies were placed in PhosphoGuard specimen collection kits (normal fixative designed to increase stability of phosphorylated antigens) and sent overnight to Quintiles’ Westmont laboratory. Upon arrival, biopsies were processed into FFPE blocks by standard methods (16). Tissue sections were mounted on charged slides (5µm) and processed by antigen retrieval using citrate buffer, pH6 (Dako, Carpinteria, CA) in a Decloaking Chamber (Biocare Medical, Concord, CA). The
phosphorylated rabbit polyclonal antibodies, and concentrations, were as follows: p-FAK (pY576, 1:50, Abcam, Cambridge, MA), p-pax (pY31, 1:25, Eptiomics, Burlingame, CA), and p-Src (pY418, 1:400, Invitrogen, Carlsbad, CA). The slides were stained with the phosphorylated antibodies on an automated staining platform (Dako). The phosphorylated antibodies were detected using the Envision+ dual link polymer-HRP and DAB+ chromagen (Dako). A positive breast tumor control was included with each run of the antibody assays. After antibody staining, the slides were counterstained manually with hematoxylin (Surgipath, Richmond, IL). Slides were scored by a board certified pathologist using a 1 score system (0, 1+, 2+, 3+). Image analysis was performed using an Aperio ScanScope XT using a positive pixel count algorithm to measure amount of staining and reported in optical density (OD) units (17).

**Statistical Methods**

The primary objectives of this study were to assess the 16-week progression-free rate and the tolerability of single agent dasatinib. Progression-free survival (PFS) was defined as the time from the start of treatment to disease progression or death due to any cause; patients who went off-treatment due to toxicity or voluntary withdrawal were censored. A two-stage "admissible" design was used to test the null hypothesis that the 16-week progression-free rate was ≤ 0.12 against the alternative hypothesis that the progression-free rate was ≥ 0.25 (18). At least 4 of the first 31 patients were required to be progression-free at 16 weeks in order for a second stage of 29 patients to be enrolled. At least 11 of the
60 patients had to be progression-free in order to reject the null. This design has a Type I error rate of 0.09 and a power of 0.90. PFS was described with a Kaplan-Meier plot. All toxicities were tabulated by type and grade. Of primary interest were the proportion of patients who developed pleural effusions and the proportion of patients who suffered any toxicities of grade 3 or higher. Relative change in biomarker level from baseline to week 4 was calculated as the change divided by the baseline value; relative change was described by calculating the median and range.

RESULTS

Patient Characteristics

The trial stopped accrual early in that none of the patients from the first stage of the statistical design was progression-free at 16 weeks. Thirty-one female patients were enrolled between December 2007 and June 2010; 25 of these patients were enrolled and treated at the main study site, Duke University Medical Center; 6 patients were enrolled and treated at participating regional cancer centers through the Duke Breast Cancer Working Group. The characteristics of the 31 patients are presented in Table 1. Of note, 13 patients (42%) had triple-negative (ER/PR/HER-2-negative) disease, 12 patients (39%) had HR-positive/HER-2-negative disease, 4 patients (13%) had HR-positive/HER-2-positive disease, and 2 patients (6%) had HR-negative/HER-2-positive disease.
Patients received a median of 2 prior lines of chemotherapy for MBC (range, 0 to 7). Twenty-four patients (78%) had previously been treated with an anthracycline; of these, 14 (45%) had been treated in the (neo)adjuvant setting only, 7 (23%) in the metastatic setting only, and 3 (10%) in both settings. Twenty-seven patients (86%) had previously been treated with a taxane; of these, 2 (6%) had been treated in the (neo)adjuvant setting only, 11 (35%) in the metastatic setting only, and 14 (45%) in both settings.

Safety

Toxicity data are available for all 31 patients. Table 2 gives the frequencies of all toxicities with incidence of >10% by grade, regardless of attribution; grade 3 toxicities, regardless of incidence or attribution are also included. The most common toxicities, with frequencies, were nausea (61% of patients), pleural effusions (52%), fatigue (52%), rash (52%), diarrhea (48%), and anorexia (42%). For each of these common toxicities, the majority were grades 1 and 2. Grade 3 toxicities were as follows: pain (12%), anorexia (10%), dyspnea (10%), pleural effusions (10%), pneumonitis (6%), elevated AST (6%), and DVT (6%). In addition, each of the following grade 3 toxicities was reported by 1 patient: fatigue, nausea, hyponatremia, thrombocytopenia, and cellulitis. There were no grade 4 or grade 5 toxicities. Four patients were hospitalized during dasatinib therapy for the following serious adverse events (SAE): 1. grade 3 non-occlusive thrombus associated with an in-dwelling catheter; 2. shortness of breath with ultimate diagnoses of grade 3 pneumonitis and grade 2 pleural
effusion; 3. nausea and vomiting with grade 3 hyponatremia; 4. non-neutropenic fever.

Given the high incidence of pleural effusions (52%), we reviewed each case of pleural effusion as well as the associated toxicities of pericardial effusions and pneumonitis; these are summarized in Supplementary Table. In total, 17 patients experienced pleural effusions and associated toxicities. Resolution of these toxicities was evaluable in 11 of these 17 patients (65%) and not evaluable in the remainder due to death or voluntary withdrawal from protocol therapy and related follow-up. All evaluable patients experienced resolution of pleural effusions with a median time to resolution following discontinuation of dasatinib of 7 days (range, 4 to 83). Toxicity resolution is depicted radiographically with representative series of CT scans for 2 patients in Supplementary Figure.

**Dose Adjustments and PFS**

Of the 15 patients in group A (starting dose dasatinib 50 mg twice daily), 12 patients completed the first cycle. Of these 12 patients, 8 patients (53% of group A) demonstrated tolerance of the starting dose and were escalated to 70 mg twice daily for cycle 2. Of note, one of these 8 patients developed toxicity at 70 mg dose and subsequently required dose-reduction back to starting dose. Similarly, of the 16 patients in group B (starting dose dasatinib 70 mg twice daily), 13 patients completed the first cycle. Of these 13 patients, 7 patients
(44%) demonstrated tolerance of the starting dose and were escalated to 100 mg twice daily for cycle 2.

Among the 31 patients on this trial, 1 patient was found to have disease progression at 16-weeks, 16 patients had disease progression prior to 16 weeks, 8 patients were taken off-treatment due to toxicity, and 6 patients voluntarily withdrew from treatment. These latter two groups of patients were censored in the analysis of PFS. Detailed description of the reasons and timing for study discontinuation is provided in CONSORT diagram, Figure 2. Among the 31 patients, the median time on dasatinib was 44 days (range, 19-115 days) and the median PFS was 7.9 weeks (55 days), Figure 3.

**Correlative Science**

All 31 patients had a baseline biopsy; 25 of these patients also had metastatic biopsy at week 4. Of these 25 patients, 20 of the paired metastatic biopsies were evaluable and 5 were not due to insufficient tissue quality. Of the other 6 patients, a week 4 biopsy was not performed for the following reasons: 1 patient experienced disease progression prior to 4 weeks, 2 were removed from protocol therapy due to toxicity, and 3 patients voluntarily withdrew. The biopsy sites were as follows: liver (10 patients, 32%), lymph node (9 patients, 29%), chest wall and other soft tissue nodules (12 patients, 39%).

For the 20 patients with evaluable biopsies at baseline and week 4, the median relative change from baseline in tissue biomarker levels were as follows (- indicates a decrease and + an increase): p-FAK -20% (range, -88% to +69%);
p-pax -6% (range, -55% to +47%); p-Src -13% (range, -70% to +95%), shown as box-plots in **Figure 4a**. Of these 20 paired biopsies, all displayed suboptimal evidence of Src inhibition, predefined as <80% inhibition of phosphorylation of both FAK and pax of week 4 biopsy phosphorylation levels compared to baseline phosphorylation levels. A patient example of the changes in p-FAK and p-pax as quantified by changes in OD units is provided in **Figure 4b**. Given that none of the 25 biopsy pairs demonstrated the pre-defined measures of optimal Src inhibition, 15 patients (60%) who met toxicity parameters were dose-escalated for cycle 2 while the other 10 patients (40%) remained at the starting dose due to cycle 1 toxicity limiting dose-escalation as per the study guidelines.

Twenty-two patients had urinary samples at baseline and week 4 that were evaluable for serial urinary NTX levels; the median change in urinary NTX levels during this time range was -19% (range, -62% to +60%). Although only 7 patients had urinary samples at week 4 and week 8 that were evaluable for urinary NTX levels, the median change in urinary NTX levels from week 4 to week 8 was comparable at -18% (range, -56% to +50%).

**DISCUSSION**

In this study, the lack of significant anti-tumor activity of dasatinib could be attributed to many factors, including the use of dasatinib as a single-agent, the number of prior therapies allowed, characteristics of our patient population or their breast cancer tumor characteristics, the small study size, and drug toxicities limiting the length of dasatinib exposure. These results are in contrast to two
recent phase II studies that also evaluated single-agent dasatinib for advanced breast cancer (19, 20). In the first study, Finn and colleagues treated 44 patients with triple-negative MBC, 66% of whom had received prior therapy for advanced disease, and demonstrated a clinical benefit rate (defined as partial response or stable disease lasting at least 16 weeks) of 9.3% (19). In the second study, Mayer and colleagues treated 68 patients with HER2-amplified and/or hormone receptor (HR)-positive MBC, 93% of whom had received prior therapy for metastases, and demonstrated a clinical benefit rate of 19%; interestingly, all patients who achieved some degree of disease control were HR-positive (20). Both studies demonstrated that tolerability of dasatinib was better when the dose was reduced from 100 mg twice daily to 70 mg twice daily. Thus, other studies have shown modest clinical benefit for use of single-agent dasatinib in women with metastatic breast cancer. In the current study, however, patients were eligible regardless of HER2 or HR status and no patients met the primary study endpoint of progression-free survival at 16 weeks.

Major limitations of this study were drug inefficacy and toxicity. Due to these limitations the median time on study of only 44 days, less than 2 cycles of dasatinib therapy. The majority of patients experienced disease progression prior to 16 weeks (52%) with toxicity (26%) being the second most common reason for study discontinuation. These results are comparable to those reported in a recent phase II trial of sorafenib, an orally-available tyrosine kinase inhibitor which also has multiple targets including vascular endothelial growth factor receptors, PDGFR, and c-Kit, which was stopped after the first cohort of 23 patients due to
lack of efficacy; patients received treatment for a median of two 28-day cycles and no patients experienced a partial or complete response to therapy (21).

In this study, the correlative science was focused on dasatinib’s mechanism as a Src inhibitor and there were no clinically meaningful changes in the tissue levels of p-FAK, p-pax, and p-Src from baseline biopsy to week 4 biopsy. Regarding tissue quality, 25 patients underwent paired metastatic biopsies; 5 of these paired samples were not evaluable due to insufficient tissue quality. Of these 5 inevaluable pairs, 2 were ultrasound-guided liver biopsies and 3 were punch biopsies of chest wall and other soft tissue nodules. Recalling that of the 25 patients who underwent paired metastatic biopsies, 10 patients had paired ultrasound-guided liver biopsies and 12 patients had paired punch biopsies of the chest wall and other soft tissue nodules, the failure rate of these two sites and methods is comparable at 20% and 25% respectively. While these are small numbers, our results do not suggest that either site (liver or soft tissue nodules) or method (ultrasound-guided biopsy or punch biopsy) influenced the success rate for acquisition of tissue of sufficient quality for biomarker analysis.

For multi-targeted tyrosine kinase inhibitors such as dasatinib, establishing appropriate tissue biomarker endpoints is challenging in that the drug may affect multiple targets of more or less clinical relevance and the dose levels and length of exposure required to achieve significant biomarker change may be undefined. We are seeking to establish biomarkers of dasatinib activity through additional correlative investigation including full genomic analyses.
Regarding Src inhibition as a potential treatment strategy for bone metastases, Yu and colleagues conducted a phase II study of single-agent dasatinib in 47 patients with metastatic castrate-resistant prostate cancer and showed disease stability among 19% of patients at 24 weeks (22). Of interest, 87% of the study population had evidence of bone disease at enrollment and bisphosphonate therapy was prohibited for 3 weeks prior to enrollment and during protocol therapy. Of 41 evaluable patients, 51% demonstrated at least 40% reduction in urinary NTX by week 12 compared to baseline. In the context of bone-predominant MBC, Moulder and colleagues are conducting an ongoing trial of dasatinib and zolendronic acid (23). In the current study, reductions in urinary NTX were also observed over time. However, this remained an exploratory objective as bone metastases were not required for enrollment and sample size for urinary NTX analysis was limited by the short duration of therapy for the majority of patients; only 7 patients had evaluable urinary samples at week 8.

The results of this trial demonstrate that with new targeted therapies come new challenges in clinical research. Potential strategies to optimize research strategies with targeted therapies may include defining a homogenous population of patients who are most likely to benefit from a given drug with the classic example being trastuzumab for HER2-positive patients. Although discovery and validation of tissue biomarkers may be essential towards this goal, there is also a need to improve on the process by which tumor biopsies are utilized. For example, the definition of \textit{in vivo} drug effects with tumor biopsies may be limited...
by multiple factors such as tumor heterogeneity and the potential for discordance between the primary and metastatic sites (24).

To address these and other concerns, Pintilie and colleagues have identified two major sources of error in tissue biomarker studies, inherent tumor heterogeneity and technical difficulties with the assay, and they have proposed methods to minimize each of these errors (25). Error due to tumor heterogeneity may be reduced by averaging the results of replicate biopsies from multiple tumor sites whereas technical difficulties may be understood by averaging the results of replicate biopsies from the same tumor site.

In conclusion, although dasatinib did not show clinical activity in an unselected group of patients with heavily-treated MBC, this study demonstrates that multiple research biopsies with real-time pharmacodynamic tissue biomarker analysis can be conducted in the context of a phase II trial and provides a design framework that may serve as a starting point for future studies of novel targeted agents.

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BMS-354825 potently inhibits multiple selected oncogenic tyrosine kinases and
possesses broadspectrum antitumor activities in vitro and in vivo [abstract 675].


**Figure 1: Study schematic**

**CYCLE 1**
- Starting dose
  - (Dasatinib 50 or 70 mg PO BID)
- Continue 4 weeks

**CYCLE 2**
- Optimal Src inhibition*
  - Hold dose constant
  - Dasatinib 50 mg PO BID
- Suboptimal Src inhibition**
  - AND tolerating drug
    - Escalate dose
    - Dasatinib 70 or 100 mg PO BID

* Optimal Src inhibition defined as ≥80% inhibition of phosphorylation of both FAK and pax compared to baseline phosphorylation levels.

** Suboptimal Src inhibition defined as <80% inhibition of phosphorylation of either FAK or pax compared to baseline phosphorylation levels.
Figure 2: CONSORT diagram

Signed consent (34 patients)

Ineligible (3)

Baseline biopsy and starting dose (31)

Off-protocol (6):
PD (2), toxicity (2), withdrew (2)

Week 4 biopsy (25)

Dose escalation (15)

Off-protocol (5):
PD (4), withdrew (1)

No dose escalation due to toxicity (10)

Off-protocol (9):
PD (1), toxicity (6), withdrew (2)

Week 8 restaging (1): Off-protocol for PD (1)

Week 8 restaging (10): SD (4)
Off-protocol for PD (6)

Week 16 restaging (1):
Off-protocol for PD (1)

Off protocol (3):
PD (2), withdrew (1)

PD = progressive disease
Figure 3: Kaplan Meier plot of time to progression
Figure 4a: Box-plots of biomarkers

- p-FAK
- p-pax
- p-Src

Marker

Percentage Change

-100 -80 -60 -40 -20 0 20 40 60 80 100
Figure 4b: Example of OD Photos

Baseline

p-FAK, OD= 49

p-pax, OD= 22

Week 4

p-FAK, OD= 27

p-pax, OD= 10

OD= optical density
Table 1: Patient Characteristics

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<td>10%</td>
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<tr>
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<td>7</td>
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<td><strong>Prior taxane</strong></td>
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<td>Neoadjuvant or adjuvant only</td>
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<tr>
<td>Metastatic only</td>
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<td>(Neo)adjuvant and metastatic</td>
<td>14</td>
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</tr>
<tr>
<td>None</td>
<td>4</td>
<td>13%</td>
</tr>
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</table>
Table 2: Frequencies of all toxicities with incidence of >10% by grade, regardless of attribution; grade 3 toxicities, regardless of incidence or attribution are also included.

<table>
<thead>
<tr>
<th>Grade 1 no.</th>
<th>Grade 1 %</th>
<th>Grade 2 no.</th>
<th>Grade 2 %</th>
<th>Grade 3 no.</th>
<th>Grade 3 %</th>
<th>Total no.</th>
<th>Total %</th>
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<tr>
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<tr>
<td>Fatigue</td>
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<td>23%</td>
<td>8</td>
<td>26%</td>
<td>1</td>
<td>3%</td>
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<tr>
<td>Anorexia</td>
<td>6</td>
<td>19%</td>
<td>4</td>
<td>13%</td>
<td>3</td>
<td>10%</td>
<td>13</td>
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<tr>
<td>Fever (non-neutropenic)</td>
<td>3</td>
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<td>2</td>
<td>6%</td>
<td>3</td>
<td>10%</td>
<td>5</td>
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<tr>
<td>Flushing</td>
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<td>16%</td>
<td>2</td>
<td>6%</td>
<td>7</td>
<td>23%</td>
<td>16</td>
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<tr>
<td>Insomnia</td>
<td>5</td>
<td>16%</td>
<td>2</td>
<td>6%</td>
<td>1</td>
<td>3%</td>
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<td>10</td>
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<td>16%</td>
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<td>3%</td>
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<tr>
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<td>8</td>
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<td>3</td>
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<td>3%</td>
<td>19</td>
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<tr>
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<tr>
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<tr>
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<td>16</td>
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<td>Pericardial effusion</td>
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<td>19%</td>
<td>6</td>
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<tr>
<td>Pain, bone</td>
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<td>6%</td>
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<td>5</td>
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<tr>
<td>Pain, chest wall</td>
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<td>6%</td>
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<tr>
<td>Pain, extremity</td>
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<td>Pain, joint</td>
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<tr>
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<td>Edema, facial</td>
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</tbody>
</table>
Clinical Cancer Research

Phase II Trial of Dasatinib in Patients with Metastatic Breast Cancer Using Real-Time Pharmacodynamic Tissue Biomarkers of Src Inhibition to Escalate Dosing

Christina I Herold, Vijaya Chadaram, Bercedis L Peterson, et al.

Clin Cancer Res  Published OnlineFirst August 2, 2011.

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