

Phase II studies of two different schedules of dasatinib in bone metastasis predominant metastatic breast cancer: SWOG S0622

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Abstract Bone metastases from breast cancer are common, causing significant morbidity. Preclinical data of dasatinib, an oral small molecule inhibitor of multiple oncogenic tyrosine kinases, suggested efficacy in tumor control and palliation of bone metastases in metastatic breast cancer (MBC). This clinical trial aimed to determine whether treatment with either of 2 dose schedules of dasatinib results in a progression-free survival (PFS) >50 % at 24 weeks in bone metastasis predominant MBC, to evaluate the toxicity of the 2 dosing regimens, and explore whether treatment results in decreased serum bone turnover markers and patient-reported “worst pain.” Subjects with bone metastasis predominant MBC were randomly assigned to either 100 mg of dasatinib once daily, or 70 mg twice daily, with treatment continued until time of disease progression or intolerable toxicity. Planned accrual was 40 patients in each arm. The primary trial endpoint was PFS, defined as time from registration to progression or death due to any cause. Median PFS for all eligible patients (79) was 12.6 weeks (95 % CI 9.1–16.7). Neither cohort met the threshold for further clinical interest. There were no significant differences in PFS by randomized treatment arm ($p = 0.85$). Toxicity was similar in both cohorts, with no clear trend in

serum biomarkers of bone turnover or patient-reported pain. Dasatinib was ineffective in controlling bone-predominant MBC in a patient population, unselected by molecular markers. Further study of dasatinib in breast cancer should not be pursued unless performed in molecularly determined patient subsets, or rational combinations.

Keywords Breast cancer · Phase II clinical trial · Bone metastasis · Tyrosine kinase inhibitors · Dasatinib

Introduction

Breast cancer is a common cause of morbidity and mortality in the United States and worldwide. Despite optimal use of adjuvant anthracycline and taxane chemotherapy, breast cancer recurrence occurs in up to 30 % of high-risk or node-positive patients within 8 years of diagnosis, resulting in 21 % breast cancer mortality [21]. In the United States, approximately 40,000 deaths are attributable to breast cancer each year, predominantly related to distant metastatic disease. One of the most common locations of metastatic relapse in breast cancer, regardless of cancer subtype, is in bone [23], and these are clinically important because skeletal related events from bony metastases lead to significant morbidity and mortality.

Dasatinib is an orally available tyrosine kinase inhibitor (TKI) that inhibits multiple oncogenic tyrosine kinases including BCR-ABL, SRC family kinases (SFKs), platelet derived growth factor (PDGF), and c-KIT. It is approved for use in Philadelphia chromosome-positive (Ph+) chronic myelogenous leukemia (CML) and acute lymphoblastic leukemia (ALL), and is also relevant in gastrointestinal stromal tumors (GIST; off-label use). SFK's

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have a role in multiple pathways of signal transduction, many of which are relevant to breast cancer, includes the following: (1) osteoclast proliferation, survival, and resorptive function; (2) transduction of cell proliferative and antiapoptotic signals; (3) angiogenesis and vascular permeability; and (4) cell motility and migration, cell–cell adhesion, anchorage independence, and other cytoskeletal events which characterize the invasive and metastatic phenotype. [12, 19, 22] Substantial preclinical data support the role of SFKs in human breast cancer, including the finding of elevated c-Src in breast tumors, as well as preclinical studies show that 7/23 breast cancer cell lines were sensitive to dasatinib (IC₅₀ <1 μM). Also, c-Src plays a role in mitogenic signaling via ER and PgR (in the presence or absence of ligand), as well as through EGFR, HER2, and p130Cas, which are found to be upregulated in breast cancer models of antiestrogen resistance. These preclinical effects of dasatinib suggest that it has potential for tumor control and palliation of bone metastases in patients with bone-predominant breast cancer metastasis.

Materials and methods

Study design

The study consisted of two parallel Phase II trials of two different doses and schedules of dasatinib. The original approved dose of dasatinib for CML was 70 mg twice daily (BID), and at the time of initiation of this study, exploration of dosing at 100 mg daily (QD) was just beginning (now standard). Therefore, this trial tested both 70 mg BID and 100 mg QD dosing in the bone metastasis predominant breast cancer population. Planned accrual was forty patients in each arm over 78 weeks (1.5 years) with an additional 24 weeks of follow-up.

The study was performed within SWOG, a cooperative group within the National Clinical Trials Network. The participating sites obtained institutional review board's approval. Informed, written consent was obtained from all patients prior to enrollment, and the study was registered on ClinicalTrials.gov Identifier: NCT00410813. Subjects were randomized to either Arm 1: dasatinib, 100 mg by mouth QD, or Arm 2: dasatinib, 70 mg by mouth BID. Randomization was stratified by use of trastuzumab at the time of registration. Treatment was given continuously until disease progression, unacceptable toxicity, symptomatic deterioration, or treatment delay in excess of 4 weeks.

The primary trial endpoint was progression-free survival (PFS), defined as time from registration to progression or death due to any cause. The progression was determined locally without the central review. Secondary trial

endpoints included the following: (1) Response Evaluation Criteria in Solid Tumors (RECIST) response (in those with measurable disease); (2) mucin-1 (MUC-1) antigen response; (3) circulating tumor cell (CTC) response; (4) incidence of Grade 3–4 toxicity; (5) change in serum bone turnover markers; and (6) change in the primary measure “worst pain” on the Brief Pain Inventory (BPI) at 8, 16, and 24 weeks. [8]

Patient population

Eligible patients were women or men with bone-predominant metastatic breast cancer. Bone-predominant breast cancer was defined as the presence of one or more bone metastases with or without nonbone (visceral or soft tissue) disease. The number of documented bone lesions was greater than or equal to the number of RECIST measurable visceral target lesions, and visceral disease was not causing symptoms to reduce the performance status. Eligible patients satisfied either criterion a or b: a. Measurable disease by RECIST criteria; b. Nonmeasurable disease only, with rising serum CA 15-3, CA 27-29, CEA, or CA-125 documented by two measurements taken at least 14 days apart with the more recent measurement within 42 days prior to registration. The second serum marker value was greater than the institution's upper limit of normal and had at least a 20 % increase over the earlier measurement.

Patients may have had 0 or 1 prior cytotoxic chemotherapy regimens for metastatic disease. Patients whose tumors were ER and/or PgR positive must have experienced progression of disease on at least one hormonal therapy in the metastatic setting.

Patients may have had previously treated and currently asymptomatic brain or CNS metastasis with radiation completed at least 8 weeks prior to registration. Patients could not receive concurrent antineoplastic therapy for breast cancer while on protocol treatment; one exception was that patients with HER2-positive breast cancer, who were on trastuzumab for at least 12 weeks could continue to take trastuzumab concurrently with dasatinib.

Patients who were on bisphosphonates must not have had a dose of the bisphosphonate within 3 weeks prior to the enrollment. Patients and their physicians agreed to hold bisphosphonates for the duration of study treatment in order to limit interaction between concurrent bisphosphonate administration and markers of bone metabolism. Concurrent therapy with RANKL inhibitors was not allowed.

Endpoint evaluations

Monitoring of toxicity occurred at a clinic visit on Weeks 4, 8, 16, 24, and every 8 weeks thereafter, with reporting of

serious adverse events (SAE) by the NCI Common Terminology Criteria for Adverse Events (CTCAE Version 4.0). CTCAE Version 3.0 was used for routine toxicity reporting. Efficacy was evaluated using CT scans, bone scans, and other assessments as required by RECIST 1.0 criteria. Each individual site was responsible for response and progression assessment; central review was not performed.

Blood biomarker evaluation

Serum biomarkers were tested centrally, and were not used for treatment decision making. Blood was drawn at the sites at baseline and Weeks 4, 8, 16, and 24 after

registration and shipped to the SWOG Solid Tumor Tissue bank for serum separation and storage. In addition, sites obtained the whole blood via one Cell Save™ tube at the same timepoints, which was shipped to Janssen Diagnostics laboratories for CTC enumeration with CellSearch® (Janssen Diagnostics, Raritan, New Jersey).

CA 15-3 and CEA measurements were performed in the University of Michigan clinical laboratories, using standard laboratory methods. Serum bone biomarker expression was analyzed using ELISA kits according to the manufacturers' instructions for expression of vascular endothelial growth factor (R&D Systems Inc., Minneapolis, MN), interleukin-6 (R&D Systems Inc.), dickkopf 1 (R&D Systems Inc.), bone alkaline phosphatase (IDS Inc., Gaithersburg, MD),

Fig. 1 Flow diagram of the progress through the randomized trial

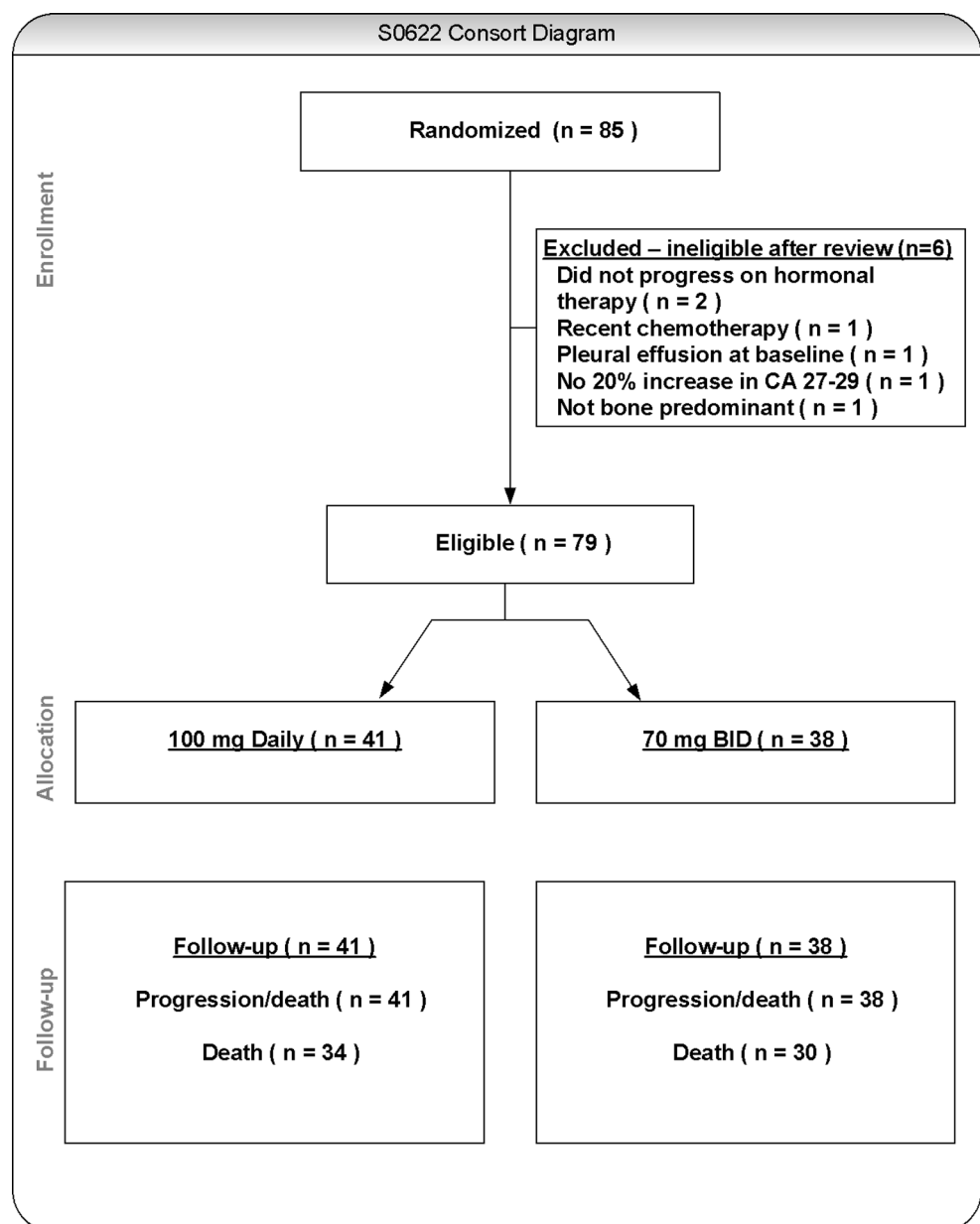


Table 1 Demographic and disease characteristics by treatment group at time of randomization

	Arm 1: 100 mg daily	Arm 2: 70 mg BID	Total
Randomized	43	42	85
Ineligible or withdrew consent	2 (4.6 %)	4 (9.5 %)	6 (7.1 %)
Analyzed	41	38	79
Age median (range)	59 (36–81)	64 (27–86)	60 (27–86)
Race			
White	38 (92.7 %)	35 (92.1 %)	73 (92.4 %)
Nonwhite	3 (7.3 %)	3 (7.9 %)	6 (7.6 %)
Gender			
Female	40 (97.6 %)	38 (100.0 %)	78 (98.7 %)
Male	1 (2.4 %)	0 (0.0 %)	1 (1.3 %)
Hormone receptor status			
Positive	36 (87.8 %)	34 (89.5 %)	70 (88.6 %)
Negative	5 (12.2 %)	4 (10.5 %)	9 (11.4 %)
HER-2 status			
Positive	2 (4.9 %)	3 (7.9 %)	5 (6.3 %)
Negative	34 (82.9 %)	35 (92.1 %)	69 (87.3 %)
Equivocal/unknown	5 (12.2 %)	0	5 (6.3 %)
Disease type			
Measurable	14 (34.1 %)	11 (29.0 %)	25 (31.6 %)
Nonmeasurable	27 (65.9 %)	27 (71.0 %)	54 (68.4 %)
Concurrent trastuzumab			
Yes (Stratum 1)	1 (2.4 %)	0 (0.0 %)	1 (1.3 %)
No (Stratum 2)	40 (97.6 %)	38 (100.0 %)	78 (98.7 %)

tartrate-resistant acid phosphatase 5b (IDS Inc.), osteocalcin (IDS Inc.), soluble receptor activator of nuclear factor kappa-B ligand (ALPCO, Salem, NH), osteoprotegerin (ALPCO), and N-terminal telopeptide (Alere Inc., Waltham, MA). Serum samples were analyzed in duplicate.

Statistical methods

Analysis of PFS

Estimates of PFS in this patient population selected for bone-dominant disease are difficult to obtain. We considered historical trials of second- and third-line hormonal therapy, assuming that these trials may include a preponderance of patients with “bone-predominant” disease and estimated that between 40–50 % of such patients treated with aromatase inhibitors or fulvestrant as second- or third-line hormonal therapy will have stable disease at 24 weeks [6, 7]. Therefore, the PFS target was chosen to be 50 % at 24 weeks, and the null hypothesis of PFS was 30 % at 24 weeks.

Planned accrual was forty patients in each arm over 78 weeks (1.5 years) with an additional 24 weeks of follow-up. This design had power of 0.90 for each arm given a

Type I error rate of 0.025 (1-sided). If PFS was favorable for both arms, the arm with the greatest tolerability would be the dose/schedule chosen to proceed to a Phase III study. A comparison of efficacy between the two arms was a secondary analysis conducted using a log-rank test for PFS and OS with estimation of the hazard ratio from Cox regression.

Secondary trial endpoints

Only patients with measurable disease at baseline were evaluated for RECIST response. Patients analyzed for overall MUC-1 antigen response, defined as previously described, [24] included those whose initial baseline MUC-1 antigen level was >2 X ULN. Patients analyzed for CTC response rate included those who had elevated CTCs (≥ 5 cells/7.5 ml) at baseline. CTC response was defined as the percentage of patients with initially elevated CTCs, whose CTC level drops to < 5 . The RECIST, MUC-1, and CTC response rates were estimated with exact 95 % two-sided confidence intervals using standard methods based on the binomial distribution. The analysis of changes in exploratory serum biomarkers associated with bone was descriptive in nature and based on all patients who started trial therapy.

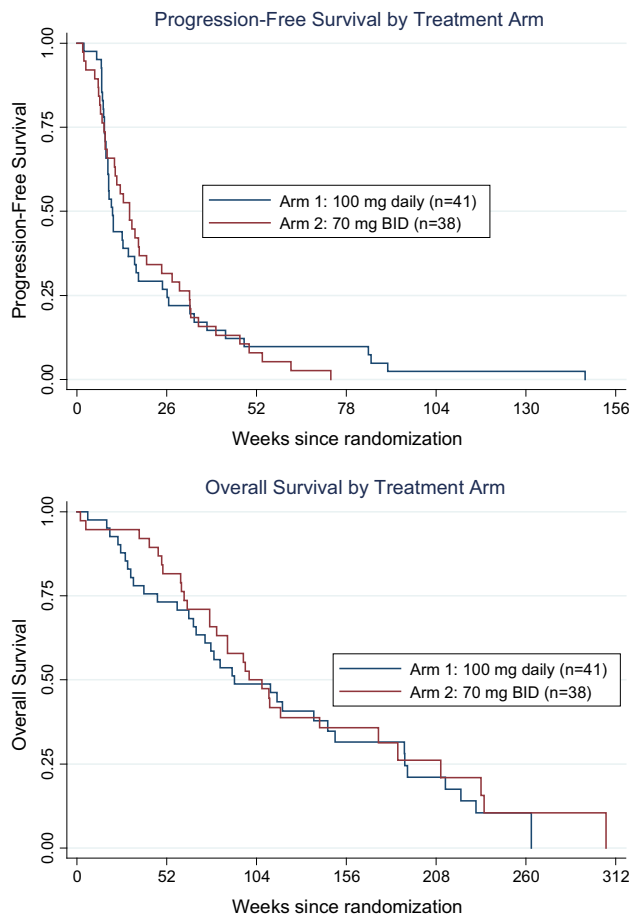


Fig. 2 Kaplan–Meier estimates of progression-free and overall survival in the patient population, by arm of enrollment

The toxicity was recorded at clinic visits scheduled at Weeks 4, 8, 16, 24, and then every 8 weeks thereafter. With a minimum of 25 patients per treatment arm, any toxicity occurring in >10 % of patients had a 93 % probability of being observed.

The Brief Pain Inventory (BPI) [8] is a patient-reported measure of pain including severity and impact of pain on daily functioning. The BPI was administered at randomization and weeks 8, 16, and 24 in either English or Spanish. Here we consider worst pain as a measure of severity and the average interference score. Additional exploratory analyses from baseline to Week 8, Week 16, and Week 24 were also performed.

Results

Consort diagram

This study was opened to accrual on March 15, 2007 and closed on December 15, 2010, after reaching full accrual with 85 registrations. Six patients were found to be

ineligible for the following reasons: no progression on hormonal therapy (2 patients); prior chemotherapy less than three weeks prior to registration; pleural effusion at baseline; no 20 % increase in CA 27–29; and not bone-predominant disease. (Fig. 1)

Description of enrolled population

Patients in the two arms were similar, as shown in Table 1. The median age was 60 years, 88.6 % of participants had hormone receptor positive (HR+) tumors, and 6.3 % had HER2-positive tumors. As per eligibility, all HR+ patients must have received at least one line of endocrine therapy in the metastatic setting, and only 0–1 prior lines of chemotherapy in the metastatic setting.

Analysis of PFS

All patients have had progression of disease or have died so there was no censoring of the primary endpoint. The overall median PFS was 10.3 weeks (95 % CI 8.4–16.7) in Arm 1 and 15.3 weeks (95 % CI 8.7–20.1) in Arm 2 (Fig. 2). Neither Arm met the a priori criterion of interest (50 % PFS at 24 weeks): Arm 1 29 % (95 % CI 16–43 %); Arm 2 34 % (95 % CI 20–49 %). There were no apparent differences in PFS between the two arms (log-rank $p = 0.85$), with HR for Arm 2 versus Arm 1 = 1.04 (95 % CI 0.66–1.65). Combining the Arm 1 and Arm 2 together, we observed an overall median PFS = 12.6 weeks (95 % CI 9.1–16.7).

Deaths have been recorded for 64 of 79 patients. Median overall survival was observed at 99.7 weeks (95 % CI 79.3–137.3), and there were no apparent differences between the randomized arms (Fig. 2): log-rank $p = 0.61$; HR = 0.88 (95 % CI 0.53–1.44).

RECIST response

Of 25 patients with measurable disease, only one patient had a partial response, so this outcome was not evaluated further.

Toxicity

Toxicities are summarized in Table 2. Among 79 patients evaluated for toxicity, three (one on Arm 1; two on Arm 2) reported Grade 4 toxicities. The toxicities included thrombocytopenia, pulmonary hypertension, and hypokalemia. Twenty-seven additional patients (9 on Arm 1; 18 on Arm 2) experienced Grade 3 toxicities as maximum degree. Seventeen patients discontinued protocol treatment early due to adverse events.

Table 2 Toxicity by cohort

ADVERSE EVENT	Dasatinib, 100 mg daily			Dasatinib, 70 mg bid		
	<i>(n = 41)</i>			<i>(n = 38)</i>		
	Grade			Grade		
	0–2	3	4	0–2	3	4
ALT	40	1	0	36	2	0
AST	40	1	0	37	1	0
Anorexia	41	0	0	37	1	0
Dehydration	41	0	0	37	1	0
Diarrhea	41	0	0	36	2	0
Dyspnea	40	1	0	34	4	0
Fatigue	41	0	0	32	6	0
Hemoglobin	41	0	0	37	1	0
Hypoalbuminemia	41	0	0	37	1	0
Hypokalemia	40	1	0	35	2	1
Hyponatremia	41	0	0	37	1	0
Infection, various	39	2	0	36	2	0
Left ventricular systolic dysfunction	39	2	0	37	1	0
Lung pain: chest wall, chest/thorax	39	2	0	38	0	0
Lymphopenia	41	0	0	37	1	0
Musculoskeletal pain, various	39	2	0	37	1	0
Nausea	41	0	0	37	1	0
Neuro pain: head/headache	40	1	0	37	1	0
Neutrophils	41	0	0	37	1	0
Platelets	39	1	1	37	1	0
Pleural effusion	40	1	0	36	2	0
Pneumonitis	40	1	0	38	0	0
Pulmonary hypertension	41	0	0	37	0	1
Pulmonary-other	41	0	0	37	0	1
Rash	41	0	0	37	1	0
Vomiting	41	0	0	37	1	0
Max grade any adverse event	31	9	1	18	18	2

Blood biomarkers

CTC response evaluation was limited to those patients who had measurements performed at baseline and at a subsequent time point. Due to diminishing numbers of samples collected at later time points, we reported only the analysis of those who had CTCs enumerated at baseline and 4 weeks. In total, 60 patients had CTCs measured at baseline, (33 < 5 cells/7.5 mg; 27 ≥ 5 cells/7.5 mg) and 51 had measurements at week 4 (31 < 5 cells/7.5 mg; 20 ≥ 5 cells/7.5 mg). Only 41 patients had measurements at both time points. Within this subset, 4 of 17 patients had reduction in CTCs from high to low (CTC response rate of 24 % (95 % CI 7–50 %)). As shown in other trials, CTC >5 cells/7.5 ml was negatively prognostic for both PFS and OS. For those with elevated CTC's at baseline, the PFS HR = 2.27 (95 % CI 1.29–3.98) and OS HR = 2.27 (95 % CI 1.22–4.21). Of 38 individuals

with high CA15-3 at baseline, 37 remained high at 4 weeks (mRR = 3 %). All 26 individuals with high CEA at baseline remained high at 4 weeks.

The analysis of serum biomarkers associated with bone focused on baseline, 4 week, and 8 week biomarkers. Observations at 16 and 24 weeks had very small numbers (*n* = 21 and 12, respectively) because of patient dropout due to disease progression and the estimated mean was very unstable, so are not included. Figure 3 depicts the mean values (and 95 % CI) of the markers at baseline, 4, and 8 weeks. No significant trend in biomarker levels over the three time points was observed. The baseline marker was dichotomized at the median and PFS compared for low or high values at baseline. There was a trend toward worse prognosis in patients with a baseline IL-6 level above the mean, but this did not reach statistical significance (data not shown).

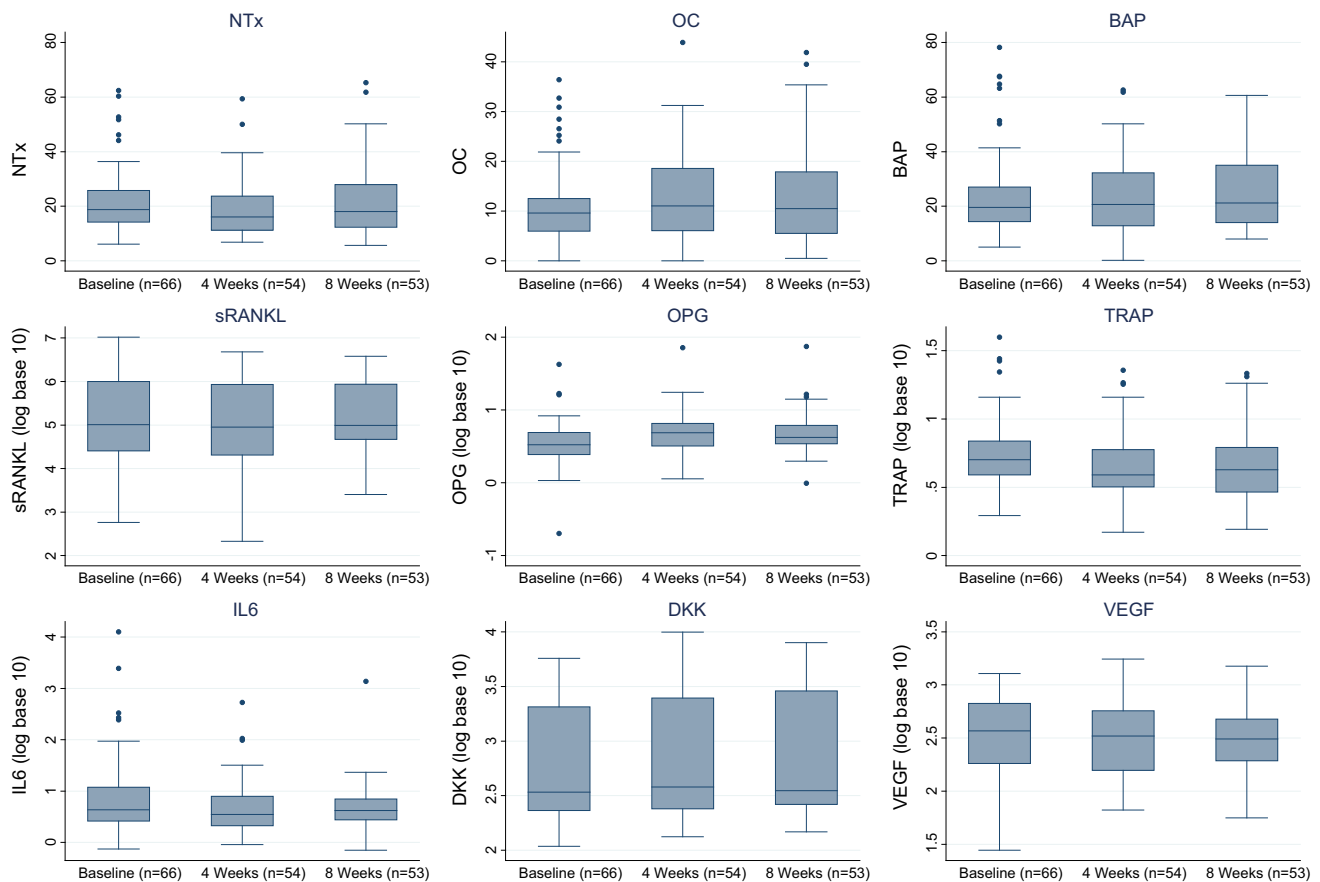


Fig. 3 Bone turnover markers (*box plots*) at 0, 4, and 8 weeks; combined Arm 1 and Arm 2

Brief pain inventory

All 79 patients completed the Brief Pain Inventory at baseline, but the numbers were reduced at 8, 16, and 24 weeks ($n = 68$; 37; and 41, respectively). Pain severity and pain interference did not differ significantly between the two treatment arms at 8, 16, or 24 weeks. Pain severity did not differ across the time points. Mean pain severity was 3.37, 3.82, 3.06, and 3.73 at 0, 8, 16, and 24 weeks based on a 10-point pain severity score (10 highest). Overall interference was significantly worse at 8 weeks compared to baseline ($p = 0.005$), but not at weeks 16 or 24. Mean interference levels were 2.05, 2.71, 1.71, and 2.34 at 0, 8, 16, and 24 weeks on a 10-point scale. Therefore, any interference due to pain induced by the start of treatment was transitory.

Discussion

Our original hypothesis was that dasatinib, a SFK would alter the course of bone-predominant breast cancer by inhibiting osteoclasts, reducing tumor cell invasiveness,

reducing cellular proliferation, and causing apoptosis. However, our trial results did not support this hypothesis.

As the measurement of response to the treatment in a bone metastasis predominant population can be challenging, our trial design included multiple methods of evaluation for anticancer activity: including progression-free survival; RECIST response rate [26]; and serial measurement of MUC-1 antigens [24, 28], CTC's [10, 25, 32], and patient-reported measures of pain utilizing the Brief Pain Inventory. [8] Although diminishing numbers of patients at later time points resulted in few measurements after 16 weeks, none of the measures pointed toward a significant benefit from treatment. Given that multiple surrogate measures for efficacy were negative, it is unlikely that the study falsely missed a positive signal.

In addition, based on our exploratory analysis of serum markers of bone turnover, we could not confirm significant activity of dasatinib in modulating either bone resorption or bone deposition in this patient population. Dasatinib was predicted to have significant effect on osteoclast proliferation and survival through its effects on RANKL [16]. We studied a panel of collagen markers of bone turnover based on abundant evidence that these are raised in the blood and

urine of a high percentage of patients with progressive skeletal metastases. [4, 9, 11, 14, 17, 29–31, 33] Although we observed a baseline elevation in bone metabolism marker levels in these patients with bone metastases [15, 29], treatment with dasatinib did not have a discernable effect on these markers. Antiresorptive agents, such as the bisphosphonate class of compounds and denosumab, a RANKL inhibitor, elicit a rapid and sustained suppression of these bone collagen breakdown markers in patients with skeletal metastases who are responding favorably to this form of therapy. [5, 13, 27, 31] A Phase II clinical trial of dasatinib in combination with zoledronic acid has suggested that a subset of patients with low grade, HR(+), and high baseline NTX levels may be more likely to experience a response to this combination regimen, which may warrant additional study [18].

Dasatinib has been evaluated as a single agent in other solid tumors, with low-reported RECIST response rates of 5–6 %. [2] In breast cancer specifically, TKI's have had limited activity when used as single agents in patients unselected for molecular aberrations. In contrast, use of TKI's can be effective when used in molecularly targeted breast cancer populations; lapatinib has demonstrated efficacy in patients with HER2 positive breast cancer [1], and neratinib has been suggested to have activity not only in HER2-positive breast cancer, but also in patients with tumors that harbor HER2 mutations [3]. Interestingly, in our trial there was a single patient with HER2-positive disease. This patient experienced a long progression-free survival for about 20 months. Although this patient's response evaluation is confounded by the concurrent use of trastuzumab, it is possible that dasatinib had some additive activity with the monoclonal antibody therapy. This hypothesis is supported by some preclinical models and is currently being explored in clinical trials of combined therapy [20].

In summary, further exploration of dasatinib as a single agent in bone-predominant metastatic breast cancer, unselected by additional predictive factors, is not warranted. Future evaluation of dasatinib in breast cancer could be pursued in molecularly determined patient subsets or in rational combinations.

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Compliance with ethical standards

Conflict of interest William E. Barlow, Evan T. Keller, Gabriel N. Hortobagyi, Jill M. Keller, Danika L. Lew, Carol M. Moinpour, Philip A. Dy, Anne F. Schott declare no conflict of interest; Catherine H. Van Poznak reports sponsorship of clinical trial to institution by the Bayer Pharmaceuticals; Daniel F. Hayes reports Stock Ownership: Oncimmune LLC, De Soto, KS, USA—stock options (7/20/09), Inbimotion, Barcelona, Spain—stock options (10/22/12); Lecture/Honorarium: Visiting Consultant for Lilly Oncology, Indianapolis, IN (11/7/14); Sponsored Clinical Research—Principle or co-Investigator: Merrimack Pharmaceuticals, Inc. (Parexel Intl Corp) (01/24/15-02/02/20), Eli Lilly Company (06/19/15-04/30/19), Janssen R&D, LLC (Johnson & Johnson) (12/23/08-04/28/18), Puma Biotechnology, Inc., (subcontract Wash Univ St. Louis to Univ Mich) (07/19/13-07/31/18), Pfizer (07/22/13-07/14/18), Astra Zeneca (11/01/14-10/31/16), Astra Zeneca (02/06/15-02/05/16; Royalties from licensed technology: Janssen R&D, LLC (Johnson & Johnson) (08/01/14); Patents: Title: A method for predicting progression-free and overall survival at each follow-up timepoint during therapy of metastatic breast cancer patients using circulating tumor cells. Filed 14 Mar 2005 with the European Patent Office, the Netherlands. Application No./Patent No. 05725638.0-1223-US2005008602. Applicant/Proprietor: Immunicon Corporation. Dr. Daniel F. Hayes is designated as inventor/co-inventor; Title: Diagnosis and Treatment of Breast Cancer. Patent No.: US 8,790,878 B2. Date of Patent: Jul. 29, 2014. Applicant Proprietor: University of Michigan. Dr. Daniel F. Hayes is designated as inventor/co-inventor; Title: Circulating Tumor Cell Capturing Techniques and Devices. Patent No.: US 8,951,484 B2. Date of Patent: Feb. 10, 2015. Applicant Proprietor: University of Michigan. Dr. Daniel F. Hayes is designated as inventor/co-inventor.

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