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A phase II trial of dasatinib in patients with metastatic castration-resistant prostate cancer treated previously with chemotherapy

Przemyslaw W. Twardowski[a], **Jan H. Beumer**[e], **C.S. Chen**[b], **Andrew S. Kraft**[f], **Gurkamal S. Chatta**[e], **Masato Mitsuhashi**[c], **Wei Ye**[a], **Susan M. Christner**[e], and **Michael B. Lilly**[d]

[a]Department of Medical Oncology and Therapeutics Research, City of Hope National Medical Center, Duarte

[b]Loma Linda University Cancer Center, Division of Hematology/Oncology, Loma Linda

[c]Hitachi Chemical Research Center

[d]Chao Family Comprehensive Cancer Center, Division of Hematology/Oncology, University of California, Irvine, California

[e]Department of Pharmaceutical Sciences, University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania

[f]Medical University of South Carolina Hollings Cancer Center, Division of Hematology/Oncology, Charleston, South Carolina, USA

Abstract

There is a need for efficacious therapies for metastatic castration-resistant prostate cancer (mCRPC) after disease progression on docetaxel. The *SRC* tyrosine kinase and its related family members may be important drivers of prostate cancer and can be inhibited by dasatinib. mCRPC patients, after one previous chemotherapy, started dasatinib at 70mg twice daily, amended to 100mg daily. The primary endpoint was the disease control (DC) rate, defined as complete response (CR), partial response (PR), or stable disease (SD) in prostate specific antigen (PSA), RECIST, bone scan, and FACT-P score. Up to 41 patients were to be accrued (two-stage design, 21+20) to rule out a null-hypothesized effect of 5 versus 20% (α=0.05, β=0.1). Secondary endpoints included progression-free survival, toxicity, and pharmacokinetic and pharmacodynamic correlatives. Of 38 patients, 27 were evaluable for response or toxicity. The median duration of therapy was 55 days (6–284). Five patients showed DC after 8 weeks of therapy (18.5% DC, 95% CI: 6.3–38.1%). One PR (3.7% response rate, 95% CI: 0.1–19.0%) was observed in a patient treated for 284 days. Twelve patients (43%) discontinued treatment for toxicity. Dasatinib induced a decrease in phytohemagglutinin-stimulated *CSF2*, *CD40L*, *GZMB*,

Correspondence to Przemyslaw W. Twardowski, MD, Department of Medical Oncology and Therapeutics Research, City of Hope National Medical Center, 1500 E Duarte Rd, Duarte, CA 91010, USA, Tel: +1 626 359 8111; fax: +1 626 301 8233; ptwardowski@coh.org.

Conflicts of interest

There are no conflicts of interest.

and *IL-2* mRNAs in blood cells, indicating target engagement. Decreases in plasma *IL-6* and bone alkaline phosphatase, and in urinary N-telopeptide, were associated with DC. Dasatinib has definite but limited activity in advanced mCRPC, and was poorly tolerated. The observation of a patient with prolonged, objective, clinically significant benefit warrants molecular profiling to select the appropriate patient population.

Keywords

dasatinib; prostate cancer; *SRC* inhibition

Introduction

Prospective clinical trials in patients with metastatic castration-resistant prostate cancer (mCRPC) have reported survival times of 16–25 months. Currently, one immunotherapy treatment (sipuleucel-T [1]), two chemotherapeutic agents (docetaxel [2] and cabazitaxel [3]), a systemic radionuclide (radium-223 [4]), the antiandrogen enzalutamide [5], and the androgen biosynthesis inhibitor abiraterone [6] confer an overall survival benefit in this setting. These treatments represent significant progress, but the prognosis of mCRPC patients remains poor. There is a need to investigate treatment strategies targeting different path-ways relevant in the biology of mCRPC. Recent evidence suggests a central role for the *SRC* family of nonreceptor tyrosine kinases (*SFK*) in the development, growth, and metastasis of many human cancers including mCRPC [7–9].

In mCRPC cells, activation of *SFKs* is constitutive, rather than ligand-regulated [10], and *SFKs* in turn regulate such diverse prostate cell pathways as *VEGF* production [11] and *FAK* signaling [9]. Dasatinib (SPRYCEL) is a multitargeted tyrosine kinase inhibitor with activity against *BCR-ABL*, *PDGFRβ*, and *KIT* kinases. It is approved for the treatment of chronic myelogeneous leukemia (CML) [12]. It inhibits all *SFKs* at low nanomolar concentrations [12]. Tissue culture studies have confirmed the ability of dasatinib to inhibit cell adhesion, migration, and invasion by malignant cells derived from prostate cancer [13]. Dasatinib has been evaluated in a phase II trial of patients with castration-resistant, chemotherapy-naive prostate cancer [14]. Lack of progression was observed in 43% of patients after 12 weeks of follow-up. A beneficial effect on the parameters of bone turnover was noted and the majority of these patients tolerated dasatinib well. Given these considerations, a phase II trial of dasatinib was conducted in patients with mCRPC treated with one previous chemotherapy regimen.

Patients and methods

Eligibility

Patients were required to have progressive mCRPC and had to have received at least one cycle of one (and only one) chemotherapy regimen for mCRPC with evidence of progression during or after discontinuation of chemotherapy. Progression was defined as an increasing prostate specific antigen (PSA) by at least 3 ng/ml from the chemotherapy nadir or new lesions on imaging studies. Castrated level of testosterone of less than 50 ng/dl was

maintained. ECOG performance status of 0–2, life expectancy of at least 8 weeks, and adequate hematologic, renal, and liver function were required.

Major exclusion criteria included uncontrolled heart disease within the previous 6 months, prolonged QTcF> 450 ms, concomitant use of drugs known to cause *torsades de pointes*, history of a significant bleeding disorder, concomitant use of therapeutic anticoagulants, pleural and pericardial effusions, and untreated CNS metastases.

The review boards of all participating institutions approved the study, which was carried out according to the provisions of the Declaration of Helsinki and the Good Clinical Practice Guidelines of the International Conference on Harmonization. All patients provided written informed consent to participate in the study.

Dasatinib treatment

Twenty-eight days constituted a treatment cycle. The initial starting dose of dasatinib was 70mg twice daily orally. The study was amended after six patients were treated to a starting dose of 100mg daily because of excessive toxicities, mainly pleural effusions and fatigue. Delays of up to 2 weeks in case of unresolved toxicities were allowed. Study treatment was continued until disease progression or unacceptable toxicity occurred. The occurrence of grade 3 or 4 hematologic or grade 3 nonhematologic toxicity required resolution of toxicity to grade 2 or less and subsequent dose reduction. The occurrence of grade 4 treatment-related nonhematologic toxicity resulted in the discontinuation of protocol treatment.

Efficacy and safety evaluation

Patients were evaluable for response if they were on therapy at day 56, the primary evaluation point. The disease control (DC) rate (primary endpoint) was evaluated as a composite endpoint of the treatment effect on four parameters: (a) PSA, (b) measurable disease (if present) by RECIST criteria (version 1.1), (c) bone scan, and (d) quality of life as measured by the FACT-P questionnaire. The FACT-P score was included as a potential indicator of treatment benefit, consistent with the Prostate Cancer Working Group 1 (PCWG1) [15] recommendations (current at the time of study initiation) that symptomatology may be a valid index of benefit of therapy. Assessments were performed before treatment, at the primary evaluation point, day 56, and every 8 weeks thereafter if the patient remained on therapy. Patients were considered to have DC if they were rated as having a complete response (CR), partial response (PR), or stable disease (SD) in each of these four parameters at day 56 or later. Specific criteria for rating the parameters of response, PSA level, RECIST-evaluable disease, bone scan changes, and FACT-P quality-of-life scores, are summarized in Table 1 and are based closely on the PCWG1 criteria. Lack of DC was defined as progressive disease (PD) in one or more parameters, with SD in the remaining parameters. A mixed response was defined as PR or CR in one parameter, with PD in another. Patients who showed a mixed response at day 56 continued on therapy until they showed either DC or lack of DC.

Progression-free survival (PFS) was a secondary endpoint. It was defined as the period from the initiation of dasatinib therapy until the documentation of PD in at least one of the four

parameters of the composite endpoint and no responses in any other parameters. Because of the relatively poor drug tolerance and relatively rapid PSA increases in most patients, it was not feasible to continue patients on treatment until there was radiographic evidence of disease progression. PFS was therefore based on the PSA level in most cases, consistent with the PCWG1 criteria [15]. Since then, updated recommendations of the Prostate Cancer Working Group 2 (PCWG2) [16] have emphasized time-to-event measurements as the primary endpoint for phase II trials in prostate cancer. Therefore, we reanalyzed the response data for our patients according to the PCWG2 criteria.

Patients were evaluable for toxicity if they took any dose of dasatinib. Toxicity was graded using the National Cancer Institute Common Toxicity Criteria for Adverse Events, version 3.0.

Statistical analysis

The primary goal of this single-arm phase II study was to evaluate the activity of dasatinib treatment on patients with mCRPC treated previously with chemotherapy. A true 56-day DC rate of 5% or less was considered clinically futile. A true DC rate of 20% or more was considered clinically significant. A maximum of 41 patients were to be accrued to rule out a null-hypothesized DC rate of 5% versus the alternative of a DC rate of 20% with α=0.05 and β=0.1. A Simon's two-stage optimal design was used for this study so that the trial could be stopped early if the treatment was ineffective (≤5% true DC rate). Twenty-one patients were to be initially entered; however, accrual could continue pending the evaluation of the first stage. If at least two patients with DC were observed among 21 evaluable patients in stage 1, an additional 20 patients would be entered for stage 2. If at least five patients with DC were observed among the total 41 evaluable patients, then the treatment would be considered not clinically futile. For an inactive agent (≤5% true DC rate), the probability of stopping at 21 patients was 71.7% and the probability of finding the drug ineffective (i.e. either stopping at stage 1 or stopping at stage 2 with less than 5 responses in 41 patients) was 94.6%.

The median PFS time and 95% confidence interval (CI) were calculated using the product limit method. Data for the patients who went off treatment early because of toxicity were censored at the last date of treatment. The exact Wilcoxon rank-sum test was used to compare baseline circulating tumor cells (CTCs) and changes in other biochemical parameters [plasma bone alkaline phosphatase (*BAP*), urinary N-telopeptide, and plasma *IL-6*] between patients with DC and those without DC. Changes in cytokine mRNAs with time were tested using the exact Wilcoxon signed-rank test.

Biochemical assays

Samples for correlative studies were collected before the start of treatment and again at each follow-up visit 2–6 h after that day's dose of dasatinib. Aliquots of clarified urine and plasma were stored at −80°C for analysis. BAP was measured in plasma using a functional immunoassay (Ostase; Quidel, San Diego, California, USA) as mIU/ml plasma. Plasma *IL-6* was quantified using a sandwich ELISA (hIL-6 Ultrasensitive ELISA; R and D Systems, Minneapolis, Minnesota, USA). Urinary N-telopeptide (*uNTx*) was expressed as nanomoles of collagen equivalents per millimole of creatinine following measurement of the analytes

with EIA or colorimetric assays (Wampole Laboratories, Princeton, New Jersey, USA and Quidel).

Circulating tumor cells

The Veridex CellSearch platform (Veridex, Raritan, New Jersey, USA) was used to detect CTCs. Results were expressed as the number of cells per 7.5 ml of blood. All assays were carried out by Dr Louis Fink at the Nevada Cancer Institute.

Plasma dasatinib concentration

Blood samples for the quantification of dasatinib plasma concentrations were collected in heparinized tubes before and every 14–28 days after the start of dasatinib 2–6 h after the daily dose. Plasma was prepared by centrifugation and immediately frozen at −80°C. Plasma concentrations of dasatinib were determined using a validated LC-MS/MS assay. Briefly, calibration samples were prepared over the concentration range of 3–1000 ng/ml and QC samples were prepared at 5, 200, and 800 ng/ml in control plasma. Mass spectrometric detection was carried out using a Waters (Milford, Massachusetts, USA) Quattromicro triple-stage, bench-top quadrupole mass spectrometer with electrospray ionization in the positive multiple reaction monitoring mode. The HPLC system and mass spectrometer were controlled by Waters MassLynx software (version 4.0; Waters), and data were collected using the same software. The analyte-to-internal standard ratio was calculated for each standard by dividing the area of each analyte peak by the area of the respective internal standard peak for that sample. Standard curves of the analytes were constructed by plotting the analyte-to- internal standard ratio versus the known concentration of analyte in each sample. Standard curves were fit by linear regression with weighting by 1/y2, followed by back calculation of concentrations. Under these conditions, the assay was linear, accurate (94.4–107.5%), and precise (coefficient of variation 3.3–10.3%) in the range of 3–1000 ng/ml.

Regulation of cytokine expression by dasatinib

To document the target engagement of biologically active concentrations of dasatinib, we measured cytokine mRNA expression in blood from dasatinib-treated patients. Briefly, heparinized whole blood was assayed immediately or stored overnight at 4°C before assay. Our studies showed no difference in cytokine expression between the two conditions (data not shown). Blood was treated with phytohemagglutinin (PHA) for 4 h at 37°C to induce cytokine expression in T lymphocytes, and then frozen at −80°C until assay. mRNAs were collected and analyzed as described previously [17].

Results

Patient characteristics

Patient demographics and clinical characteristics at diagnosis are summarized in Table 2. Twenty-one patients were to be initially entered; however, accrual could continue pending the evaluation of the first stage. Ultimately, 38 patients were enrolled between October 2007 and January 2010, of whom 27 were treated and evaluable for response or toxicity. Eight patients were never treated, in most cases because of effusions found on baseline scans, or

the development of intercurrent illness. Three patients were found to be ineligible after receiving initial treatment.

Clinical efficacy

The median duration of therapy in the evaluable patients was 55 days (range 6–284). Three patients had DC at 56 days in the first stage of the study, which justified continuation of the trial through the second phase, during which two additional patients had DC for a total of five patients (18.5% DC rate, 95% CI: 6.3–38.1%). One patient had a DC effect on the basis of a PR for PSA (86% reduction; Fig. 1), PR for RECIST criteria (Fig. 2), and SD for bone scan and FACT-P score, resulting in a response rate of 3.7% (95% CI: 0.1–19.0%). This patient remained on treatment for 284 days. Four patients had a DC effect on the basis of SD for all four parameters. Two patients were rated as having a mixed response at day 56 on the basis of an improvement in the FACT-P score with at least one PD score in another parameter. These patients continued on treatment for an additional 42 or 193 days until they were excluded from the study for PD. These patients were not considered to have DC. No patient was rated as having DC or lack of DC only on the basis of the FACT-P score. Figure 3 shows a PSA waterfall plot of a change in the PSA levels among patients evaluable for response at day 56.

As expected, five patients with DC showed markedly prolonged PFS (median PFS: 180 days, 95% CI: 79–284 days) compared with the 15 patients without DC (median PFS: 28 days, 95% CI: 28–29 days). The median PFS for all 27 patients was 37 days (95% CI of 28–79 days); data for the seven patients who went off treatment early because of toxicity were censored on the last date of treatment.

Analysis of these patients was in accordance with the recommendations of the then-current PCWG1 statement [15]. Since then, updated recommendations of the PCWG2 [16] have emphasized time-to-event measurements as the primary endpoint for phase II trials in prostate cancer. The response data for our patients according to the PCWG2 criteria are summarized in Fig. 4. Patients who were rated as DC had a significantly longer PFS than those who failed to achieve DC. This analysis shows that our original definition of DC produces outcomes similar to those of the more recent recommendations of PCWG2.

Toxicity

The most common attributable grade 3 and 4 toxicities included fatigue (19%), hyponatremia (8%), diarrhea (8%), dyspnea (8%), gastrointestinal bleed (8%), pleural and pericardial effusions (4%), elevated lipase (4%), and anemia (4%) (Table 3). The most common cause of discontinuation of dasatinib was PD (54%); however, 43% of patients were taken off the protocol because of toxicities, predominantly fatigue.

Biochemical assays

Serial samples were available for *BAP*, *uNT*, and *IL-6* assays in 14 patients evaluable for response, including four who had DC.

BAP—Figure 5a shows that the decrease in *BAP* on day 28 or later in patients with DC was significantly greater than the change in *BAP* among those without DC (median ratio between *BAP* nadir at 28 days or later and baseline 0.65 vs. 1.09, *P*=0.002).

uNTx—Patients with DC showed significantly greater decreases in urinary *uNTx* at day 28 or later than did those without DC (median ratio between *uNTx* at 28 days or later and baseline 0.67 vs. 1.43, *P*=0.014) (Fig. 5b).

IL-6—Patients who showed DC had greater decreases in plasma *IL-6* levels in the first 28 days of treatment than those without DC, and the difference was statistically significant (median ratio between *IL-6* at 28 days or earlier and baseline 0.27 vs. 0.81, *P*=0.036). However, the baseline *IL-6* level did not correlate with either outcome from dasatinib therapy or dasatinib toxicity (data not shown) (Fig. 5c).

Circulating tumor cells

Baseline CTC assays were available in 16 patients evaluable for response, including four who had DC. There was no significant difference in the baseline CTC counts between those with DC and those without (*P*=0.30) (data not shown). No sustained decrease in CTCs was observed in any patient during dasatinib treatment.

Bioassay for dasatinib effect

Expression of T-cell cytokines in response to PHA requires the activation of several dasatinib-inhibitable kinases, including members of the *SRC* family *(LCK*) as well as those of the *TEC* family (*BTK*, *TEC*). We adapted a high-throughput real-time PCR technique to serially measure mRNAs of the PHA-stimulated cytokines *CSF2*, *IL-2*, *CD40L*, and *GZMB* in the peripheral blood of dasatinib-treated patients [16]. Heparinized whole blood from a normal individual was first treated *ex vivo* with PHA, with or without exogenous dasatinib. The kinase inhibitor decreased the expression of the four target cytokines in a dose-dependent manner (Fig. 6 shows data for *CSF2*, *IL-2*, and *CD40L*). The half-maximal effect dose (ED_{50}) for cytokine inhibition occurred at clinically achievable concentrations (10–100 ng/ml; 20–200 nmol/l).

We serially measured the ability of PHA to stimulate cytokine expression in heparinized whole blood obtained from 13 patients taking dasatinib (Fig. 7a–d). The median levels of *CD40L*, *GZMB*, *CSF2*, and *IL-2* mRNA decreased by 77.0–93.5% during the first day of dasatinib treatment. This marked reduction in mRNA expression persisted during continued therapy for at least 56 days and was similar for each cytokine mRNA. Cytokine expression rapidly returned to baseline levels by 2 days after the discontinuation of dasatinib therapy (data not shown).

Plasma dasatinib levels were available for a subset of the samples used for cytokine analysis (*n*=30, total of 120 cytokine mRNA measurements). Figure 8a–d shows the relationship between cytokine suppression and plasma dasatinib levels. Samples were divided into quartiles on the basis of the plasma dasatinib level. All four cytokine mRNA analytes showed a general, inverse relationship between dasatinib concentration and level of cytokine

mRNA. The median cytokine expression did not differ significantly from pretreatment levels when the dasatinib plasma level was 5 ng/ml or less. However, the median levels of cytokine mRNA were progressively lower in each successive plasma dasatinib quartile. Using an E_{max} model as implemented in the ADAPT5 software for pharmacokinetic/pharmacodynamic systems analysis, we explored the quantitative relationship between plasma dasatinib levels and biologic effects in patients. The dasatinib level corresponding to the IC_{50} values ranged from 5.6 to 14.6 ng/ml, which agreed well with previous values for effective dasatinib blood levels in model systems [17].

Dasatinib produced the expected biologic effects in PBMNCs of almost all patients during treatment (Fig. 9 shows data for the dasatinib concentration and expression of *IL-2*). Among the 120 cytokine mRNA measurements for which the corresponding dasatinib plasma levels were known, there were 21 anomalous values – for example where there was no decrease in the mRNA expression of one or more cytokines during treatment. In 16 of these cases (16/21 or 76%), the corresponding plasma dasatinib values were less than 10 ng/ml, a level at which there is little apparent biologic activity. These low levels occurred despite the patients' claim of complying with the dosing regimen.

Discussion

Our trial fulfilled the prespecified efficacy criteria to enter the second stage of accrual and the prespecified number of five patients with DC was achieved. However, toxicity was high and tolerability was poor and the trial was terminated after 27 evaluable patients were accrued. Compared with previously published experience with dasatinib in prechemotherapy mCRPC patients [14], our patients showed worse tolerability of dasatinib despite a lower initial dose. Even the reduction in the starting dose from 70 mg twice daily to 100mg daily did not result in a significant improvement in tolerability and 46% of patients experienced dose delays or required dose reductions and 43% of patients discontinued therapy because of toxicity (vs. 15% in a study by Yu *et al*. [14]). Our patients were pretreated with chemotherapy and years of androgen deprivation. This may have resulted in decreased physiologic reserve as shown by the 44% frequency of ECOG PS of 0 compared with 60% of ECOG 0 patients in the aforementioned study [14]. Similar toxicities and short treatment times were observed in patients with advanced breast cancer treated with dasatinib following previous chemotherapy [18,19]. Recently reported results of the READY trial showed that the addition of dasatinib to docetaxel failed to prolong overall survival in patients with metastatic CRPC [20]. This negative outcome of a large randomized clinical trial indicates that the application of dasatinib to a broad population of patients with advanced PC is not an appropriate therapeutic strategy and more refined patient selection would be required to proceed with additional evaluation of dasatinib in this disease.

In an effort to explore potential predictive markers of clinical benefit of dasatinib in PC patients in our study, we have examined the relationship between clinical responses and various biological and biochemical parameters, as well as the relationship between dose and biological activity.

Our data show that whole blood from dasatinib-treated patients has impaired expression of proinflammatory cytokines after ex-vivo stimulation with PHA. This effect persisted for months during continued treatment with dasatinib, but resolved promptly following discontinuation of the inhibitor. The magnitude of the PBMNC defect showed a dose-related relationship with the plasma level of dasatinib, and could be reproduced by ex-vivo treatment of whole blood from normal donors with exogenous dasatinib at concentrations similar to those measured in the clinical specimens. These data are the first to show that treatment of cancer patients with dasatinib broadly impairs the ex-vivo function of T cells. As with all assays on surrogate cells, the direct relevance of these measurements to tumor cell growth inhibition is uncertain. These studies serve primarily to ensure that biologically active levels of dasatinib were achievable in these patients.

The critical targets for dasatinib's anticancer effects are widely assumed to be *SFK*s. However, dasatinib is a multikinase inhibitor, affecting the function of more than 60 kinases at nanomolar concentrations [21]. Recently, the *BTK* and *TEC* kinases, members of the *TEC* kinase family, have been found to be targets of dasatinib at clinically achievable concentrations [22,23]. *TEC* family kinase *BMX* mediates ligand-independent activation of the androgen receptor in PC cells and is capable of binding dasatinib [22]. Thus, inhibition of *TEC* family kinases could account for most of the observed clinical and biochemical activities of dasatinib. Supporting this hypothesis is the lack of activity in mCRPC of AZD0530 – a potent, oral *SFK* inhibitor [24]. Our bioassays do not distinguish between *SFK*-dependent effects and those mediated by *TEC* kinases.

Patients treated with dasatinib may show extensive interpatient and intrapatient variability in blood levels and elimination rates [25,26]. Our data indicate that mCRPC patients treated with dasatinib generally show a biologic effect in surrogate cells consistent with those expected from the measured plasma levels. However, impaired expression of cytokines *ex vivo* does not seem to correlate with DC.

Recently, attention has been focused on the problem of nonadherence during TKI therapy for CML. In the ADAGIO study of CML, patients with a suboptimal response had significantly higher mean percentages of imatinib not taken (23.2%) than did those with an optimal response (7.3%) [27]. Poor dose density has also been shown to significantly impair the achievement and maintenance of key cytological and molecular endpoints that predict good outcomes [27]. We have documented that the lack of the expected ex-vivo biologic effects of dasatinib in our patients' PBMNCs was usually related to low dasatinib blood levels. Although other interpretations are possible, these observations suggest that poor compliance may be a common feature of dasatinib therapy in these heavily pretreated patients with advanced prostate cancer, likely explained by the poor tolerability. Efforts to enhance the tolerability and compliance with dasatinib therapy might include the use of once-daily dosing regimens, optimized patient selection algorithms, or prophylactic low-dose glucocorticoid therapy to reduce toxicity [28].

We documented that a decrease in the plasma *IL-6* level by 50% or more during the first 4 weeks of dasatinib therapy was associated with DC. Many studies emphasize the predictive value of a decrease in the plasma *IL-6* level for clinical responses of cancer, including the

response of mCRPC to docetaxel therapy [29]. These data suggest that combinations of dasatinib with agents to lower the expression of, or block the effects of, *IL-6* could be advantageous. Many such agents have been identified. These include inhibitors of *IL-6* expression such as glucocorticoids, thalidomide, or lenalidomide [30] monoclonal antibodies to *IL-6* itself (siltuximab) [31].

In addition, agents that could block the downstream effects of *IL-6* include an antibody to the *IL-6* receptor (tocilizumab), as well as inhibitors of *JAK/STAT* signaling [31].

Supporting the previously made observation, we have noted that the clinical benefit (DC) of dasatinib was associated with decreasing levels of bone turnover markers consistent with the documented effect of dasatinib on reduction in osteoclastic activity mediated by *SRC* inhibition.

A key concept in the application of targeted therapeutics such as dasatinib is the selection of patients with the appropriate genetic target(s). Mendiratta *et al*. [32] reported a possible strategy for enriching for a patient population that has a higher probability of responding to dasatinib on the basis of a transcription-based '*SRC* signature' indicating high *SRC* activity and low androgen receptor signaling. *SFK*s and *BMX* kinases, potential dasatinib targets, are known to mediate ligand-independent activation of the androgen receptor and may be especially active in patients with CRPC.

In summary, dasatinib showed limited activity in mCRPC patients who were treated previously with chemotherapy and it was associated with considerable toxicity that limits its broad application in this population of patients. Observation of a case with a prolonged objective response and clinical benefit warrants further investigation of this agent in the less heavily pretreated patients and preferably in the context of research focusing on molecular profiling to identify tumors heavily dependent on *SFK* and *TEC* – family kinase signaling and in patients with bone metastases showing high osteoclastic activity.

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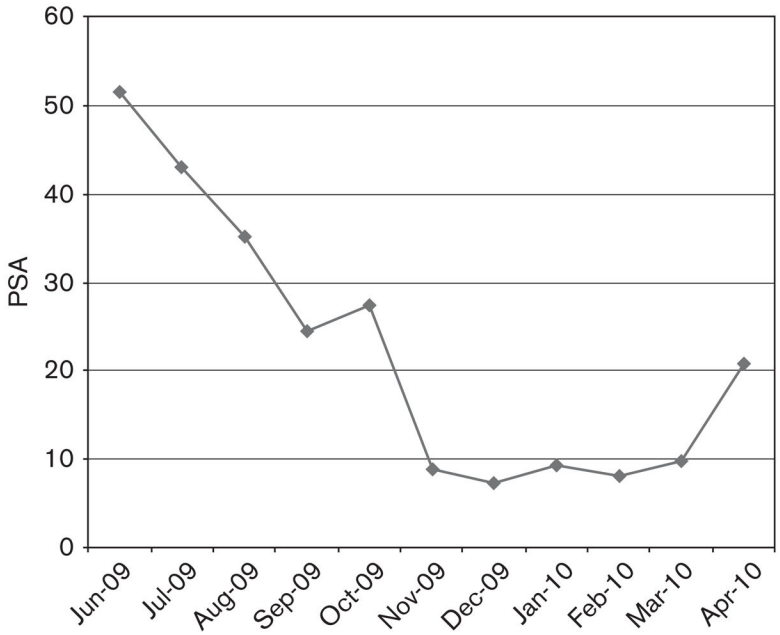

Fig. 1.
Confirmed partial prostate specific antigen (PSA) response during therapy.

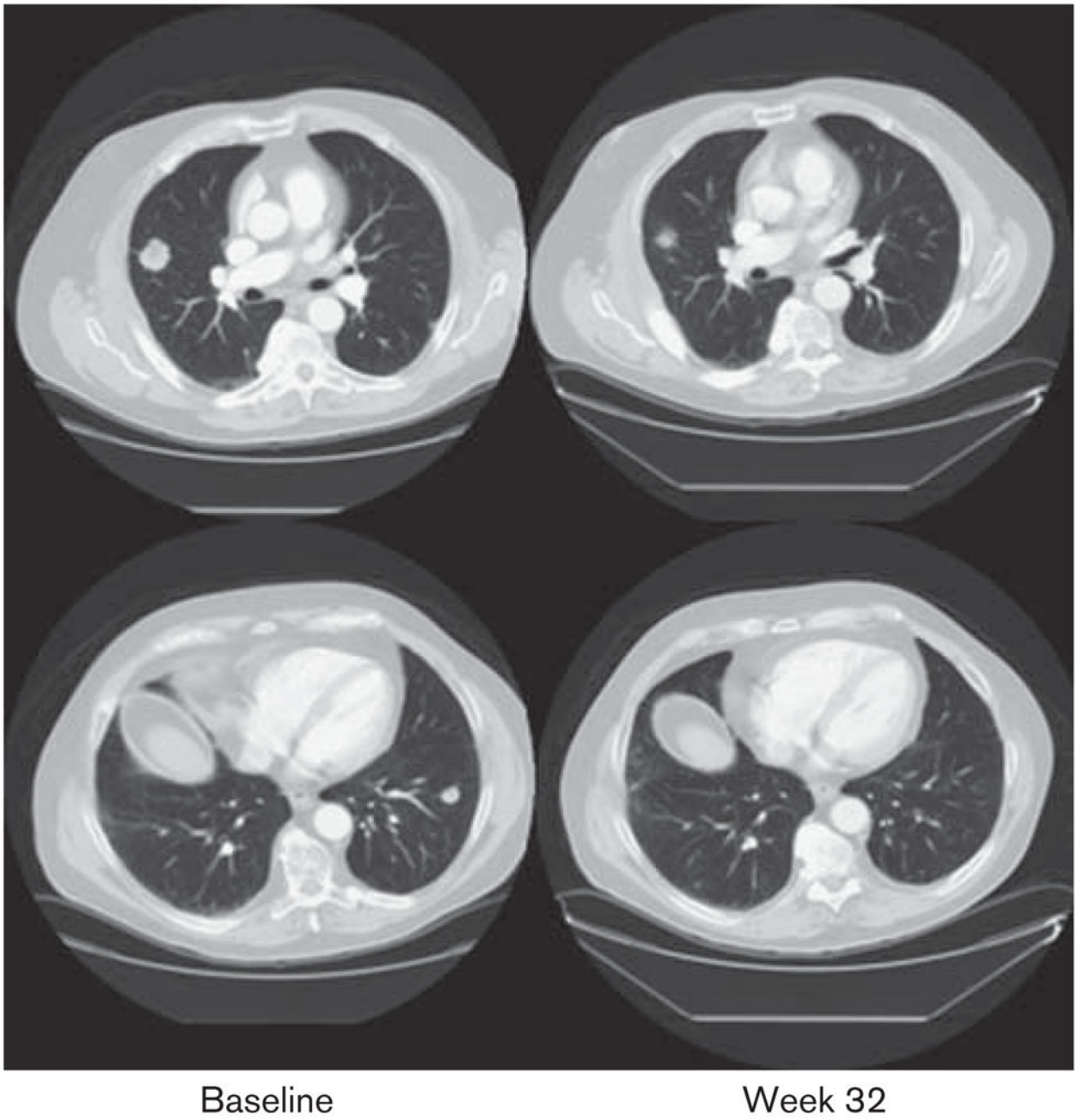

Fig. 2.

Computed tomography scans showing partial response of lung metastases by RECIST in the same patient with a prostate specific antigen response.

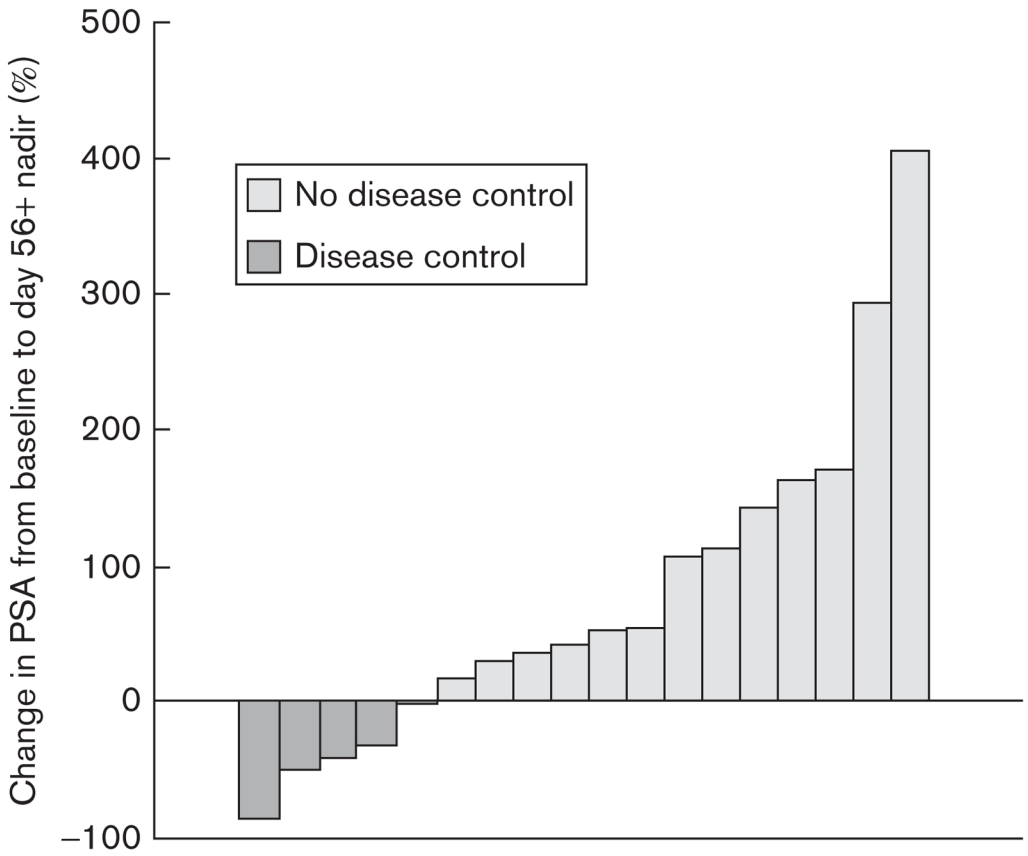

Fig. 3.

Waterfall plot of changes in prostate specific antigen (PSA) levels among patients with or without disease control on day 56.

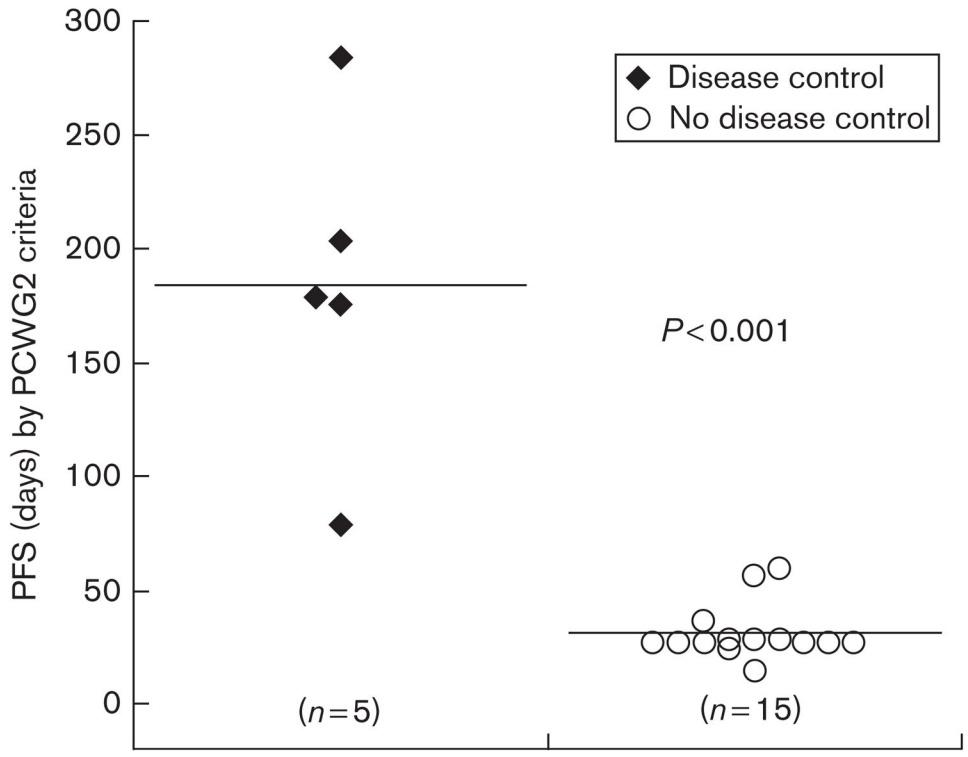

Fig. 4.

PFS according to the PCWG2 criteria in patients with DC or who failed to achieve DC. PCWG2, Prostate Cancer Working Group 2.

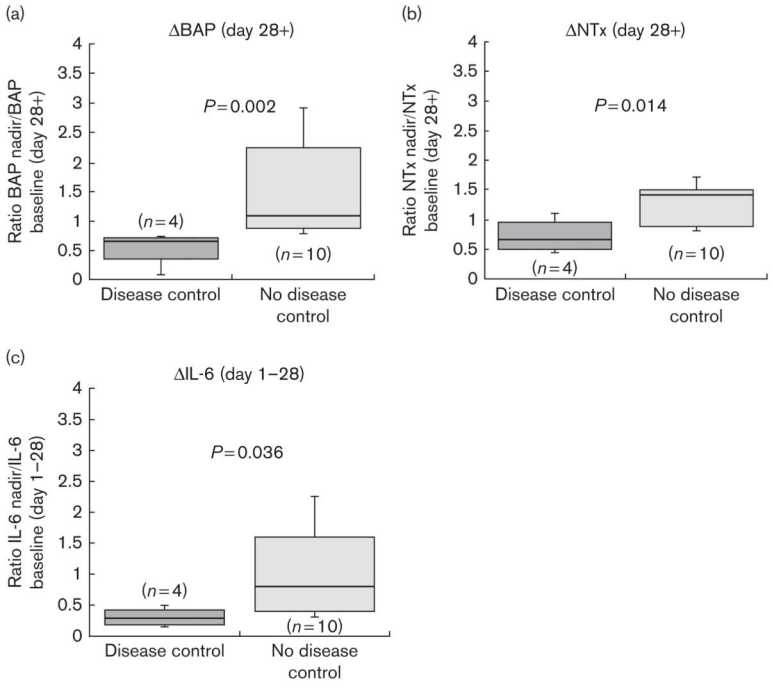

Fig. 5.

(a–c) Relationship between disease control and biochemical parameters in patients evaluable for response. (a) Plasma bone alkaline phosphatase; (b) urinary N-telopeptide; and (c) plasma *IL-6*.

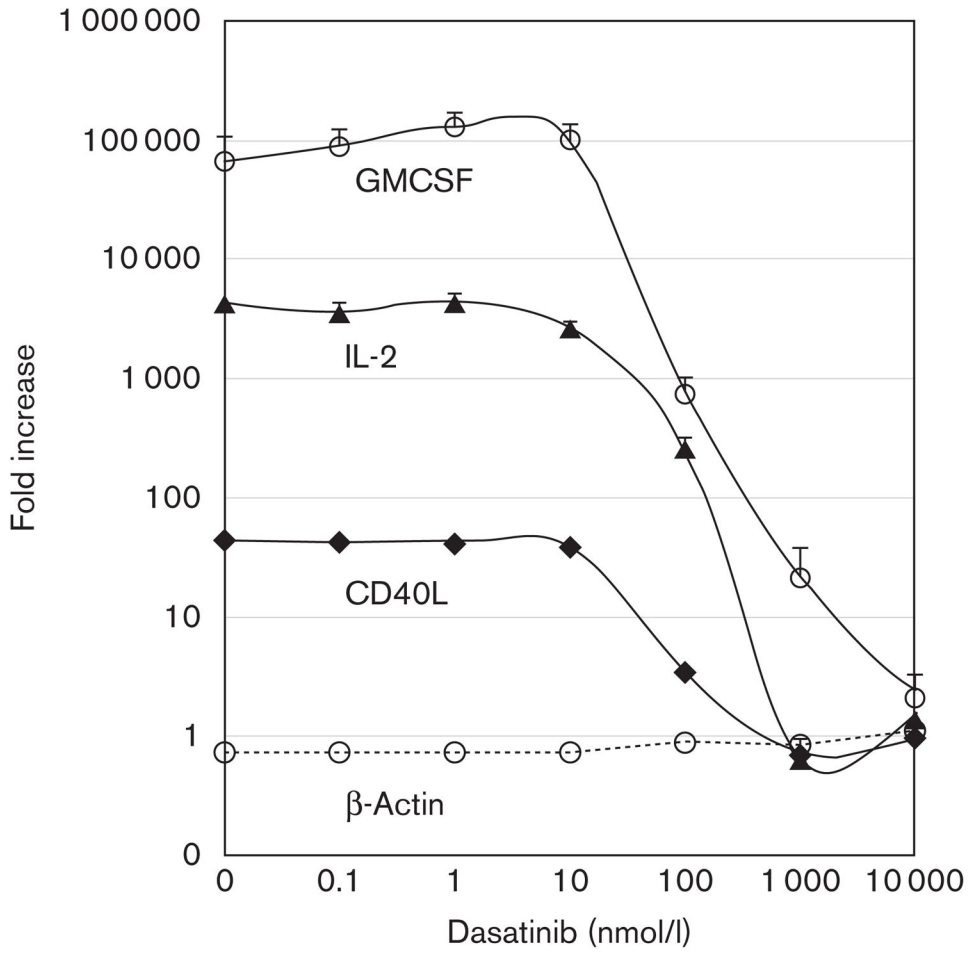

Fig. 6.

Ex-vivo inhibition of cytokine mRNA expression in PHA-stimulated whole blood. Blood from a normal volunteer was treated *ex vivo* with the indicated concentrations of dasatinib, and then stimulated with PHA for 2 h. mRNAs were then extracted and analyzed (*CSF2*, *IL-2*, *CD40L*). Each point represents the mean (±SD) of triplicate determinations from a representative experiment. Fold increase was calculated by $2^{\Delta Ct}$ [19]. PHA, phytohemagglutinin.

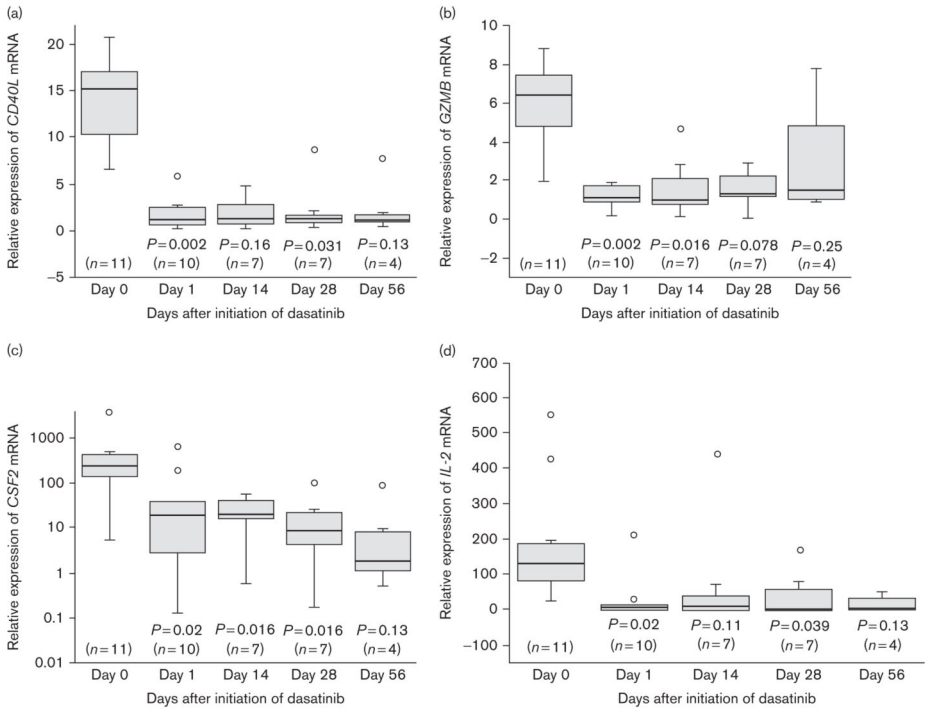

Fig. 7.

(a–d) Time course of cytokine mRNA suppression following institution of dasatinib therapy. Whole blood was obtained serially within 6 h of the daily dose of dasatinib, and then stimulated *ex vivo* with PHA×4 h, followed by cytokine mRNA assessment. (a) *CD40L*, (b)*GZMB*, (c) *CSF2*, (d) *IL-2*. *P*-values are for comparisons between day 0 and later days. For each sample, the cytokine mRNA measurements were normalized to that sample's level of ACTB mRNA (=1).

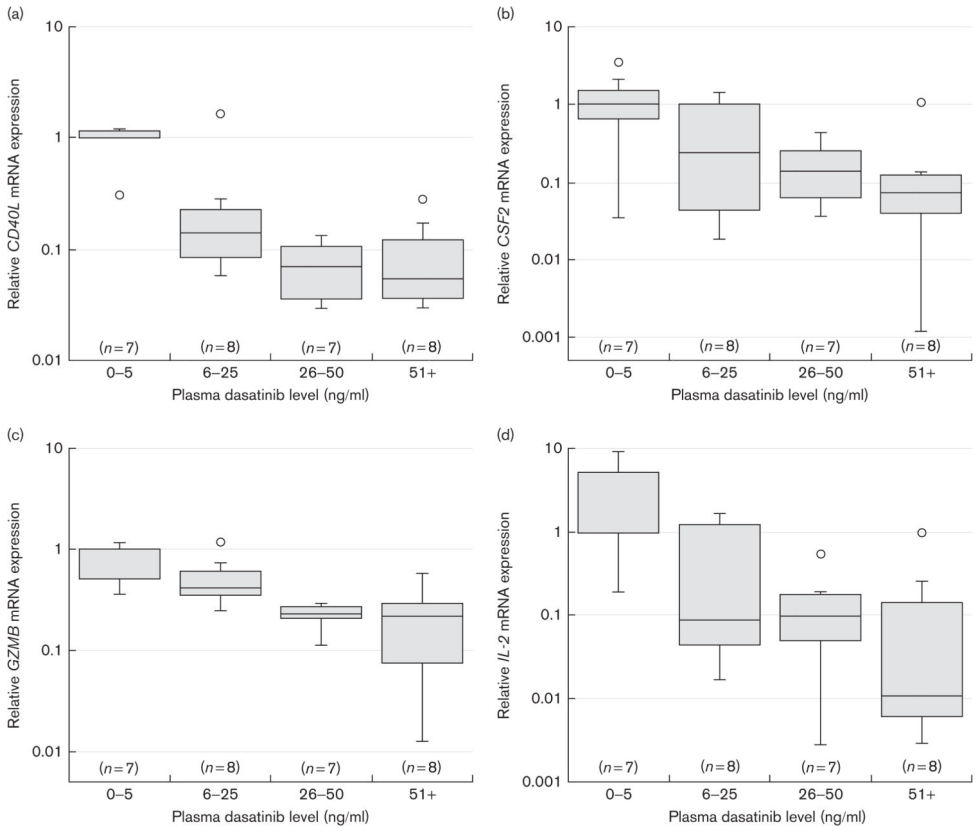

Fig. 8.

(a–d) A dose–response relationship between cytokine mRNA expression and plasma dasatinib level. Whole blood was obtained within 6 h of dasatinib administration, and then assayed for both cytokine mRNA expression [(a) *CD40L*, (b) *CSF2*, (c) *GZMB*, (d) *IL-2*], and dasatinib level. The range of dasatinib levels was divided into quartiles.

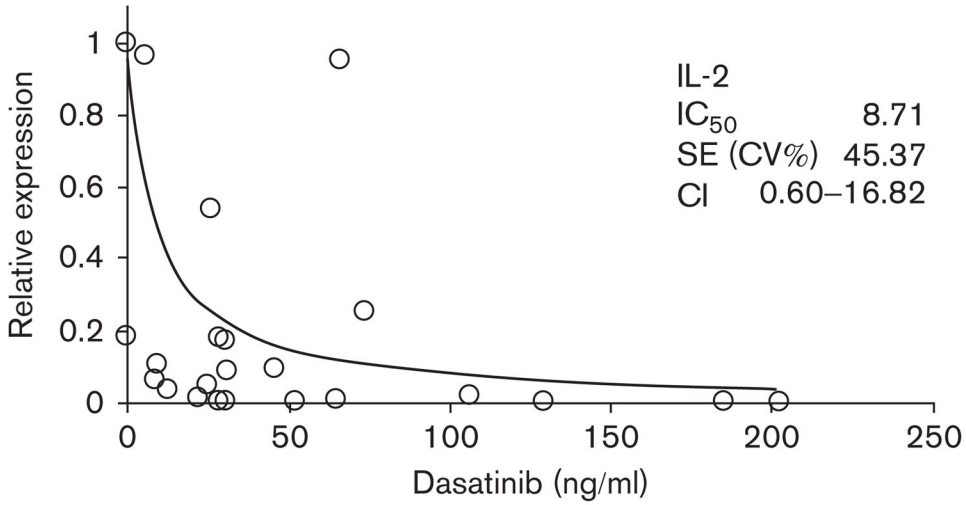

Fig. 9.

Estimation of IC_{50} values for dasatinib in inhibiting cytokine mRNA expression (IL-2) *in vivo*. Blood samples were obtained from patients taking dasatinib, and then analyzed for plasma dasatinib level and IL-2 mRNA expression. For each sample, the cytokine mRNA measurements were normalized to that sample's level of *ACTB* mRNA (=1). CI, confidence interval.

Table 1

Assessments of response to dasatinib in castration-resistant prostate cancer

Parameters	CR components	PR components	SD components	PD components
PSA[a]	< 0.2 ng/ml after RRP < 1.0 ng/ml after XRT < 4.0 ng/ml if prostate is intact	> 50% decrease from the pretreatment level	Not fulfilling the criteria for PR or PD	>25% increase from the pretreatment level, or >50% above dasatinib treatment nadir. PSA must be ≥5 ng/ml
RECIST	Disappearance	≥30% decrease in target lesion	Not fulfilling the criteria for PR or PD	≥20% increase in the size of target lesion
Bone scan	Complete disappearance of previous lesions	N/A	No more than one new lesion	Two new lesions
FACT-P	N/A	≥15% increase in the overall score	Not fulfilling the criteria for PR or PD	≥25% decrease in the overall score

CR, complete response; PD, progressive disease; PR, partial response; PSA, prostate specific antigen; SD, stable disease.

[a] All PSA-based assessments require a confirmatory level no more than 1 month later.

Table 2

Patient characteristics

	N **(%)**
Age [median (range)] (years)	68 (55–88)
PSA [median (range)] (ng/ml)	94 (0.2–2.085)
Race[a]	
Caucasian	14 (78)
African American	3 (17)
Asian	1 (5)
ECOG performance status[a]	
0	11 (44)
1	13 (52)
2	1 (4)
RECIST-evaluable disease[a]	
Yes	14 (56)
No	11 (44)
Site of metastases	
Bone	23 (85)
Lymph nodes	9 (33)
Lungs	2 (7)
Liver	1 (4)
Prior chemotherapy regimens	
Docetaxel	17 (65)
Docetaxel + atrasentan	4 (15)
Docetaxel + carboplatin	4 (15)
Etoposide	1 (4)
Zoledronic acid	6 (23)

PSA, prostate specific antigen.

[a]Race, ECOG PS, and RECIST status were not available on all patients.

Table 3

Grade 3 and 4 toxicities

	Toxicity [*N* (%)]	
	Grade 3	**Grade 4**
Fatigue/asthenia	5 (19)	–
Diarrhea	2 (8)	–
Dyspnea	2 (8)	–
Gastrointestinal bleed	2 (8)	–
Hyponatremia	1 (4)	1 (4)
Elevated lipase	1 (4)	–
Pleural/pericardial effusion	1 (4)	–
Anemia	1 (4)	–
Venous thrombosis	1 (4)	–