Platelet-Derived Growth Factor Receptor Inhibition and Chemotherapy for Castration-Resistant Prostate Cancer with Bone Metastases

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Abstract

Purpose: To further assess preclinical and early clinical evidence that imatinib mesylate, a platelet-derived growth factor receptor (PDGFR) inhibitor, modulates taxane activity in prostate cancer and bone metastases, a randomized study was conducted.

Experimental Design: Men with progressive castration-resistant prostate cancer with bone metastases (n = 144) were planned for equal randomization to i.v. 30 mg/m^2 docetaxel on days 1, 8, 15, and 22 every 42 days with 600 mg imatinib daily or placebo, for an improvement in median progression-free survival from 4.5 to 7.5 months (two-sided α = 0.05 and β = 0.20). Secondary end points included differential toxicity and bone turnover markers, tumor phosphorylated PDGFR (p-PDGFR) expression, and modulation of p-PDGFR in peripheral blood leukocytes.

Results: Accrual was halted early because of adverse gastrointestinal events. Among 116 evaluable men (57 docetaxel + imatinib; 59 docetaxel + placebo), respective median times to progression were 4.2 months (95% confidence interval, 3.1-7.5) and 4.2 months (95% confidence interval, 3.0-6.8; P = 0.58, log-rank test). Excess grade 3 toxicities (n = 23) in the docetaxel + imatinib group were principally fatigue and gastrointestinal. Tumor p-PDGFR expression was observed in 12 of 14 (86%) evaluable bone specimens. In peripheral blood leukocytes, p-PDGFR reduction was more likely in docetaxel + imatinib – treated patients compared with docetaxel + placebo (P < 0.0001), as were reductions in urine N-telopeptides (P = 0.004) but not serum bone-specific alkaline phosphatase (P = 0.099).

Conclusions: These clinical and translational results question the value of PDGFR inhibition with taxane chemotherapy in prostate cancer bone metastases and are at variance with the preclinical studies. This discordance requires explanation.

The metastatic phenotype of prostate cancer is highly conserved and characterized by osteoblastic bone metastases, the burden of which correlates with morbidity and mortality from the disease (1). Clarification of the molecular basis of disease progression in bone is expected to yield therapeutic strategies that may significantly transform the natural history of the disease.

Several lines of evidence have implicated the platelet-derived growth factor receptor (PDGFR) in the progression of prostate cancer bone metastases. Amplification of conserved domains of tyrosine kinase receptors using degenerate primers identified PDGFR as the most commonly amplified transcript from pooled aspirate specimens from prostate cancer bone metastases (2). Immunohistochemical staining has shown high frequencies of PDGFR expression in prostate cancer bone metastases (2, 3). Our preclinical findings from an orthotopic model of prostate cancer bone metastases showed that PDGFR expression was up-regulated in PC3-MM2 cells and associated vascular endothelium within the bone microenvironment, suggestive of an organ-specific paracrine effect (4). Further, combining the PDGFR inhibitor imatinib mesylate (5) with

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taxane chemotherapy in this model led to improved therapeutic outcomes apparently explained by enhanced apoptosis in tumor vascular endothelial cells expressing PDGFR (4). Follow-up studies in this model using multidrug resistance-positive taxane-resistant prostate cancer cells suggested that imatinib overcame taxane resistance by a similar antivascular mechanism (6). Other studies have shown that PDGFR inhibitors can reduce tumor interstitial fluid pressure to enhance the delivery and improve the therapeutic index of cytotoxic chemotherapy (7, 8).

These preclinical findings led to a modular phase I study to establish the feasibility of combining imatinib and docetaxel in men with castration-resistant prostate cancer and bone metastases (9). Prolonged and unusual patterns of response were observed in a significant fraction of a heavily pretreated group of men (9, 10). With the hypothesis that imatinib favorably modulates docetaxel outcomes in castration-resistant prostate cancer and bone metastases, we designed a randomized, placebo-controlled study of docetaxel with or without imatinib with progression-free survival (PFS) as the primary end point. A provision for crossover to combination therapy was included for the docetaxel-placebo group at disease progression to assess whether imatinib could reverse drug resistance. We also sought to confirm the presence of phosphorylated PDGFR (p-PDGFR) in bone metastases, to explore the comparative inhibition of PDGFR phosphorylation by treatment arm, and the predictive value of p-PDGFR inhibition on therapeutic outcomes, using peripheral blood leukocytes as surrogate tissue, and to assess comparative effects on markers of bone formation and lysis.

Materials and Methods

Description of cohort

One hundred and sixteen men were accrued between April 2003 and July 2005 at five tertiary cancer care centers. Eligibility criteria included histologic evidence of adenocarcinoma of the prostate with radiological evidence of bone metastases, a serum testosterone level of ≤50 ng/dL, and evidence of disease progression as manifested by either successive increases in serum prostate-specific antigen (PSA) level, the appearance of new lesions on bone scan, progressive bidimensional disease, or worsening malignant bone pain. Other eligibility criteria included prior anti-androgen withdrawal, no more than two prior chemotherapy regimens (excluding taxanes), an Eastern Cooperative Oncology Group performance score of ≤2, a PSA level of at least 1 ng/dL, an absolute neutrophil count of 1,500/mm^3, a platelet count of 100,000/mm^3, a serum bilirubin level of ≤1.5 mg/dL, aspartate aminotransferase and alanine aminotransferase levels of <2× the upper limit of normal, and a creatinine clearance rate of >40 mL/min (calculated with the Cockcroft-Gault formula). Continued luteinizing-hormone releasing hormone agonist therapy was required in medically castrate men. Exclusion criteria included oxygen-dependent lung disease, chronic liver disease, peripheral neuropathy >grade 2, symptomatic congestive heart failure, angina or myocardial infarction in the last 6 months, and uncontrolled hypertension or diabetes mellitus. All patients provided written informed consent according to institutional guidelines, and an independent data and safety monitoring board was established. The study was supported by Novartis Pharmaceuticals and an Inter–Specialized Programs in Research Excellence grant from the National Cancer Institute.

Pretreatment evaluation and monitoring

All patients underwent a thorough history and physical examination that included pain score (worst pain and average pain in the last 24 h on a 0-10 scale). Baseline laboratory studies included complete blood counts, creatinine, bilirubin, transaminases, total alkaline phosphatase, bone-specific alkaline phosphatase (BAP), testosterone, and PSA levels; urinary levels of N-telopeptide cross-links of collagen type I (NTx); unilateral bone marrow biopsy and aspirate; and an optional peripheral blood sample for p-PDGFR monitoring. Histories were updated and physical examination, pain scores and measurements of complete blood counts, serum and urine chemistries, and PSA were repeated before each new cycle of therapy; serial peripheral blood samples for monitoring p-PDGFR status were repeated on the 1st day of cycle 2 in patients who consented to provide these optional research samples. Staging radiological studies included a chest X-ray, bone scan, and computed tomography of abdomen and pelvis, and these assessments were repeated every two cycles. On days 8, 15, and 22 of the cycle, complete blood count, creatinine, transaminases, and total bilirubin were measured to monitor toxicity.

Therapy

Patients were randomly assigned to receive docetaxel, 30 mg/m^2 administered i.v. over 60 min, on days 1, 8, 15, and 22 in 42-day cycles, with daily oral 600 mg imatinib mesylate or placebo. Premedication for docetaxel included i.v. 20 mg dexamethasone as a bolus dose 30 min before docetaxel with i.v. or oral 25 mg diphenhydramine and i.v or oral 20 mg famotidine. Toxicity was assessed according to the National Cancer Institute Common Toxicity Criteria version 2.0 and recorded before each cycle. An absolute neutrophil count of 1,500/mm^3 and a platelet count of 75,000/mm^3 were required to qualify for treatment at the beginning of each cycle. Grade 3 or 4 hematologic toxicity, troublesome grade 2 nonhematologic toxicity, or grade 3 or 4 nonhematologic toxicity required interruption of both drugs, with scheduled therapy resuming, on resolution to grade 2 or less toxicity, with a one-level dose reduction in both drugs. Patients needing more than two dose reductions or delays in scheduled therapy lasting more than 2 weeks because of toxicity events were removed from the study. Available drug levels for purposes of dose reduction were docetaxel at 25 mg/m^2 and imatinib or placebo at 400 mg (level -1) and docetaxel at 20 mg/m^2 and imatinib or placebo at 300 mg (level -2). After 87 patients were accrued to the trial, asymmetrical dose-limiting toxicity events between the arms led to modification of the starting dose of imatinib or placebo alone to 400 mg daily; accordingly, imatinib or placebo dose reduction provisions were modified to 300 mg (level -1) and 200 mg (level -2). Therapy was to continue until disease progression was established. Patients were removed from study for prohibitive toxicity, progressive disease, noncompliance, and patient or physician decision. Men with disease progression were unblinded as to treatment assignment, and men in the docetaxel-placebo group were offered the opportunity to ''cross over'' to docetaxel-imatinib combination therapy at the docetaxel dose level at the time of progression with a corresponding imatinib dose.

Treatment outcomes

For patients with measurable disease, an objective partial response was defined as a decrease of 50% or more in the product of the diameters of index lesions. Another measure of response was a decline in PSA level to 50% of baseline that was sustained for at least 6 weeks. Conversely, progressive disease was defined as an increase of 30% in the maximum diameter of any measurable lesion, an increase of more than 25% of the products of the lesion diameters, or the appearance of one or more unequivocal new lesions. Progressive disease was also defined as worsening symptoms attributable to disease progression or three consecutive increases of at least 1 ng/mL in PSA level, measured at least 2 weeks apart, any of which were >25% of nadir or baseline PSA. PFS was measured from the time of registration. Time of progression was defined as the time of appearance of symptoms attributable to disease progression, the first demonstrated clinical sign or radiological evidence of disease progression, or the time of first of consecutive PSA

increments that achieved 25% increase over baseline or nadir (or death during the study), whichever was earliest. Identical definitions were used for the cross-over study.

Statistical considerations

Primary end point. The study's primary objective was to compare the median PFS between the docetaxel + imatinib versus docetaxel + placebo groups. A group-sequential log-rank testing procedure with two interim tests was planned (11), with overall size 0.05 and power 0.80 to detect improvement in median PFS from 4.5 to 7.5 months. Planned accrual was 144 patients over 24 months with an estimated 7.3 months of additional follow-up for maximal trial duration of 31.3 months. Interim tests were planned at 41 and 82 events. Balanced randomization, using the Pocock-Simon method (12), was based on performance score (0 or 1 versus 2), hemoglobin (<11 versus ≥11 g/dL), alkaline phosphatase level (normal versus elevated), and number of prior treatments (0 versus 1 or 2). Data were analyzed on an intention-to-treat basis. For the crossover study, a PFS of 20% at 12 weeks after initiation of crossover therapy was specified as evidence of the ability of imatinib to reverse docetaxel resistance. PFS probabilities were estimated by the method of Kaplan and Meier (13) and compared between treatment groups by a log-rank test (14).

Secondary end points

Response and toxicity. The frequencies of 50% declines in PSA levels and objective responses in patients with measurable disease were compared by using Fisher's exact test (15). Raw toxicity outcomes were described in frequency tables.

Monitoring p-PDGFR expression: p-PDGFR expression in bone metastasis specimens. Unilateral biopsy samples obtained from the posterior superior iliac crest were fixed with formalin, decalcified with 5% formic acid, and embedded in paraffin. Four-micrometer-thick sections were sequentially treated with Borg decloaking solution (Biocare Medical) in a 70°C water bath for 2 h, 3% hydrogen peroxide in PBS for 12 min at room temperature, Cyto Q Fc receptor block (Innovex Biosciences) for 30 min at room temperature, and antibody to p-PDGFR-β (Tyr^{1021}) or p-PDGFR-α (Tyr^{720}; both from Santa Cruz Biotechnology) at a 1:100 dilution in protein-blocking solution at 4°C overnight, followed by secondary antibody (EnVision Plus, DAKO) diaminobenzidine (Open Biosystem) and Gill's #3 hematoxylin (Sigma Chemical Co.). Washes were done following each step. Tumor cells showing a membranous staining pattern and 2+ or greater intensity were considered positive for p-PDGFR-β or p-PDGFR-α; results were expressed in frequency tables. Megakaryocyte staining was used as a reference internal control for staining intensity (3+).

p-PDGFR expression in peripheral blood leukocytes. Venous blood samples were drawn at baseline and after 6 weeks of therapy (on the first day of cycle 2), collected into a sodium heparin Vacutainer and centrifuged at 1,200 × *g* for 20 min at 4°C within 30 min of collection. Packed cells were cryopreserved 10:1 with DMSO (Sigma Chemical) and frozen at -80°C. Frozen cells were gently thawed in a chilled 50-mL tube containing 10% DMSO in minimal essential medium and centrifuged at 500 × *g* for 5 min in a refrigerated centrifuge. The pellet was resuspended in thawing solution containing 1% paraformaldehyde, fixed for 20 min on ice, and centrifuged. The pellet was resuspended in fixative, and cytospin samples were made and fixed with acetone. Samples were rinsed with PBS and sequentially incubated with Cyto Q Fc receptor blocking solution for 30 min, 1:100 dilution of the p-PDGFR-β (Tyr^{1021}) antibody conjugated to cyanine-5 by Rockland Immunochemical Co. for 1 h, washed, counterstained with Sytox green (Molecular Probes/Invitrogen) for 10 min, mounted with fluorescence mounting medium, and examined by confocal microscopy. Fluorescence intensities of 2,000 individual peripheral blood leukocytes was measured by a laser scanning cytometer (Compucyte Corp.), and histograms were generated for analysis.

p-PDGFR monitoring. The objectives of this analysis were to estimate the probability [probability of a decrease in p-PDGFR over two measurement times [Pr(Decr)]], which the p-PDGFR levels within each patient and each treatment group decreased from baseline to the 1st day of cycle 2, and to assess the ability of the estimated Pr(Decr) values to predict the probability of 50% decline in PSA levels and PFS. Paired samples of p-PDGFR values from peripheral blood leukocytes from 88 men were available at baseline and on the 1st day of cycle 2. The large sample sizes (~2,000 cells each) of cell-specific p-PDGFR values obtained at both measurement points for each patient provide highly reliable within-patient estimators of Pr(Decr). Each patient's Pr(Decr) estimator was based on a Wilcoxon-Mann-Whitney statistic (16). Weighted averages of the within-patient Pr(Decr) estimators were computed to obtain group-level estimators for all patients, each treatment group, and patients with and without PSA response. The ability of treatment (imatinib versus placebo) and within-patient Pr(Decr) estimators to predict the probability of 50% decline in PSA level was assessed by logistic regression. Because preliminary goodness-of-fit analyses (17) showed that a Cox model for PFS gave a very poor fit to these data, the ability of treatment and Pr(Decr) to predict PFS was assessed by a log-normal time-to-event regression model, chosen from a set of several candidate models (18), based on additional goodness-of-fit analyses (19). Associations between categorical variables were assessed by Fisher's exact test (15) and its generalizations. PFS was compared between groups by using log-rank tests (16). All calculations were done in SPLUS (20).

Bone marker and bone pain outcomes. To assess changes in bone marker outcomes (pain score, urine NTx level, and serum BAP level), denoting baseline value as X and the value on the 1st day of cycle 2 as Y, the linear model $Y = a + bX + (c + dX) \times [\text{Imat}] + e$ was fit, where [Imat] = 1 in the docetaxel + imatinib group and 0 in the docetaxel + placebo group; a, b, c, and d are variables, and e is measurement error. Because the imatinib-versus-placebo effect under this model for a patient with baseline value X is $c + dX$, a 2-degrees-of-freedom χ^2 test of $c = 0$ and $d = 0$ was conducted for each bone marker to assess treatment effect on change between baseline evaluation and that on the 1st day of cycle 2.

Results

Description of trial contigencies. The first interim analysis after 42 events was reviewed by the data and safety monitoring board, which recommended continuing the trial. Disproportionate nonhematologic toxicity events in the experimental group after 87 patients had been registered led to a reduction in the starting dose of imatinib to 400 mg daily. After 116 patients had been registered, the principal investigator reported five cases of sigmoid diverticular perforations (four in the imatinib group and one in the placebo group) to the institutional review board. The institutional review board requested a second interim analysis, to include an examination of covariates that could have influenced these events, and referred the findings to the data and safety monitoring board. The data and safety monitoring board requested a futility analysis, the results of which suggested a low probability that a significant treatment difference would be revealed if the trial was continued to 144 patients. Those results, in combination with the toxicity events, led the institutional review board to recommend that the study be closed to further accrual. Hence, the results reported here are based on the 116 men registered and analyzed on an intent-to-treat basis.

Patient characteristics. One hundred sixteen men (57 in the imatinib group and 59 in the placebo group) were registered to the study, and 116 were randomly assigned to treatment groups; however, 1 patient randomized to the imatinib group was later found to be ineligible and was withdrawn before therapy (CONSORT, Appendix 1). Patient characteristics are shown in Table 1.

Table 1. Patient characteristics

Characteristic	Patients in placebo group (*n* = 59), *n* (%)	Patients in imatinib group (*n* = 57), *n* (%)
Age, y		
41-50	2 (3)	2 (4)
51-60	17 (29)	11 (19)
61-70	23 (39)	24 (42)
71-80	14 (24)	18 (32)
81-90	3 (5)	2 (4)
Race		
Caucasian, non-Hispanic	49 (83)	53 (93)
African-American, non-Hispanic	6 (10)	1 (2)
Hispanic	4 (7)	3 (5)
Stratification factors		
ECOG performance score		
0 or 1	55 (93)	54 (95)
2	4 (7)	3 (5)
Hemoglobin level, g/dL		
<11	9 (15)	7 (12)
≥11	50 (85)	50 (88)
Total alkaline phosphatase level		
Normal	32 (54)	31 (54)
Elevated	27 (46)	26 (46)
No. prior treatment regimens		
0	41 (69)	40 (70)
1 or 2	18 (31)	17 (30)

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

PFS, response, and overall survival. Eight-nine (77%) patients had disease progression or died, 46 (78%) in the placebo group and 43 (75%) in the imatinib group. The median PFS was 4.2 months (95% confidence interval, 3.0-6.8) in the placebo group compared with 4.2 months (95% confidence interval, 3.1-7.5) in the imatinib group (P = 0.58). PFS according to treatment group is shown in Fig. 1. PSA declines as defined were seen in 11 of 40 (28%) in imatinib group and 19 of 51 (37%) in the placebo group (P = 0.37), whereas partial response was noted in 2 of 24 (8%) and 3 of 23 (13%), respectively (P = 0.64). Thirty-three (28%) patients died, 15 (25%) in the placebo group and 18 (32%) in the imatinib group. Median survival time was not attained in the placebo group, but median survival time for the imatinib group was 20.9 months (95% confidence interval, 16.9 months, upper limit not attained; P = 0.23, log-rank test). Based on a Cox model stratified by performance score (0 or 1 versus 2), hemoglobin (<11 versus >11 g/dL), alkaline phosphatase (normal versus elevated), and number of prior regimens (0 versus 1 or 2), and including treatment (imatinib versus placebo) as the only covariate, the hazard ratio for death for patients taking imatinib compared with those taking placebo was 1.67 (95% confidence interval, 0.77-3.64; P = 0.20).

Toxicity. Adverse events (grades 1-5) are described in Table 2, which detail an excess grade 2 and 3 toxicity in the imatinib arm. Four deaths occurred during the study or within 30-day off-study: sudden cardiac death (placebo group), pneumonitis and disease progression (placebo group), diverticular perforation and peritonitis (imatinib group), and pneumonitis (imatinib group). The latter three were considered potentially related to therapy. In one man in the placebo group, a secondary malignancy identified in a worsening pleural effusion after removal from the study was identified as a pleural B-cell non-Hodgkin's lymphoma. Five sigmoid diverticular perforations were identified, four in the imatinib group and one in the placebo group, with one associated death from peritonitis. A study of potentially related covariates (use of high-dose steroids, aspirin, nonsteroidal anti-inflammatory drugs, cyclooxygenase-2 inhibitors, prior pelvic radiation, prior cumulative dose of docetaxel, prior gastrointestinal toxicity from docetaxel, smoking, pneumonitis, use of opioids, and hypertension) requested by the institutional review board indicated a possible association of the sigmoid diverticular perforations with concomitant high-dose steroids; three of the five men with perforations had received high-dose steroids (>10 mg prednisone equivalent) for the management of suspected spinal cord compression or pneumonitis. In two of these steroid-treated cases, there were no symptoms or signs referable to the abdomen. Pathologic evaluation of autopsy specimens and one surgical resection specimen revealed neither vascular abnormalities nor infarcts.

p-PDGFR monitoring and correlations. The distributions of the within-patient estimators, and the corresponding group-level estimators, of the probability of reduction in p-PDGFR [Pr(Decr)] are summarized in Table 3. The within-patient and group-level estimators indicate that, on average, p-PDGFR decreased in slightly less than half of the 88 patients tested, although the individual Pr(Decr) values were highly variable. The magnitudes of the Pr(Decr) were higher in the imatinib group (median, 0.487) than in the placebo group (median, 0.447; $P < 0.0001$) and were higher among men without a decline in PSA (median, 0.505) than in men with a decline in PSA (median, 0.406; $P < 0.0001$). A Kaplan-Meier estimate of PFS for the two groups of men with within-patient estimators of Pr(Decr) that were either above or below the median value of 0.46 indicated that that men with a higher Pr(Decr) had a shorter PFS (median, 4.1 months) than those with a lower Pr(Decr) (median, 5.7 months; P = 0.032). We used a fitted

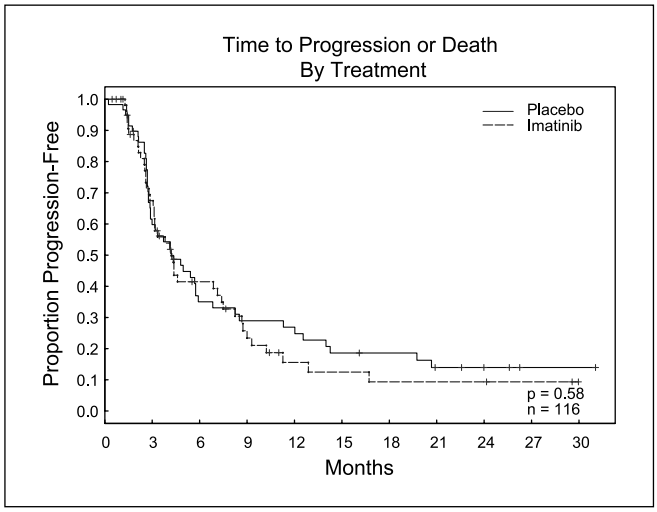

Fig. 1. Time to disease progression or death (PFS) according to treatment group (docetaxel + imatinib or docetaxel + placebo).

Table 2. Adverse events by treatment group

Adverse event	No. events	
	Docetaxel + imatinib group	Docetaxel + placebo group
Grade 5 toxicity		
Cardiovascular/general-other	0	1
Gastrointestinal-other	1	0
Pneumonitis	1	1
Total grade 5 events	2	2
Grade 4 toxicity		
Blood/bone marrow-other	0	1
Bone pain	0	1
CNS cerebrovascular ischemia	0	1
Creatinine phosphokinase	0	1
Gastrointestinal-other	1	1
Hyperglycemia	0	1
Leukocytes	1	0
Neutrophil/granulocytes	2	0
Thrombosis/embolism	1	0
Total grade 4 events	5	6
Grade 3 toxicity		
Fatigue	18	6
Gastrointestinal		
Diarrhea	8	4
Nausea	3	1
Gastrointestinal-other	7	2
Supraventricular arrhythmia	4	1
Ventricular arrhythmia	1	0
Neuropathy	1	0
Edema/effusion	3	1
Dyspnea	6	7
Neutrophils/granulocytes	1	3
Thrombosis/embolism	1	2
Conjunctival tearing	1	0
Stomatitis	1	1
Pneumonitis	1	1
Rash/desquamation	2	1
Cardiac ischemia/infarction	0	1
Hematuria	0	3
Hyperglycemia	1	4
Hemoglobin	0	1
Transfusions: RBC	3	2
Liver transaminases	2	0
Others (≤2 events)	19	19
Total grade 3 events	83	60
Grade 2 toxicity		
Anorexia	8	7
Diarrhea	10	10
Vomiting	6	2
Dyspnea	27	30
Edema	25	19
Fatigue	41	34
Nausea	12	9
Conjunctival tearing	15	17
Hyperglycemia	7	5
Neuropathy	6	3
Others (<5 events)	65	74
Total grade 2 events	222	210
Total grade 1 events	297	256

Abbreviation: CNS, central nervous system.

lognormal regression model to assess the ability of the within-patient estimator of Pr(Decr) to predict PFS and found that a higher Pr(Decr) was a marginally significant predictor of shorter PFS ($P = 0.076$).

p-PDGFR expression in tumor and bone marrow samples. Only 22 of 110 (20%) bone marrow biopsy samples collected at baseline contained tumor foci as identified by H&E staining (Fig. 2). Of those 22 samples, 14 (64%) had tumor evaluable for immunohistochemical analysis. Patterns of p-PDGFR isoform staining in the 14 samples were as follows: (*a*) p-PDGFR-β positive, p-PDGFR-α positive: 4 (29%); (*b*) p-PDGFR-β positive, p-PDGFR-α negative: 7 (50%); (*c*) p-PDGFR-β negative, p-PDGFR-α positive: 1 (7%); and (*d*) p-PDGFR-β negative, p-PDGFR-α negative: 2 (14%).

Bone marker outcomes. Evaluation of effects of imatinib versus placebo on changes in bone marker values between baseline and the 1st day of cycle 2 indicated lower urinary NTx levels ($P = 0.004$) and higher BAP levels ($P = 0.099$) in the imatinib group. The raw data and fitted lines for each bone marker in each treatment group are given in Fig. 3. No significant differences in pain score outcomes were noted between groups ($P = 0.449$ for average pain; $P = 0.281$ for worst pain).

Crossover from placebo to imatinib at disease progression. Of the 59 men randomly assigned to receive placebo, 46 were eligible for crossover to imatinib at the time of disease progression, and 23 were elected to do so. Patients who went off-study on the crossover study did so for the following reasons: disease progression ($n = 16$), toxicity ($n = 3$), patient decision ($n = 2$), physician decision ($n = 1$), and death from progressive disease ($n = 1$). The median PFS was 12 weeks (range, 1-53 weeks), and five men (22%) were progression-free beyond 12 weeks. Among six men with measurable disease, no partial responses were observed.. Although 6 of 15 (40%) patients had declines in PSA levels, these were either transient or did not exceed 50% of baseline (median, 31%; range, 13-60%). Urinary NTx levels declined at the 1st day of cycle 2 in 5 of 12 (42%) evaluable men, whereas none had declines in serum BAP levels.

Discussion

This is the first randomized study of chemotherapy and a PDGFR inhibitor in prostate cancer and, to our knowledge, in solid neoplasia. Notwithstanding evidence for a high frequency of expression of p-PDGFR in tumor, systemic p-PDGFR inhibition, and modulation of bone lysis markers, no evidence of therapeutic benefit, as principally judged by PFS, was identified for the combination of docetaxel and imatinib in castrate-resistant prostate cancer with bone metastases. Further, the addition of imatinib was associated with excess non-hematologic toxicity of a particular spectrum. Based on these results, a definitive phase III trial of the combination is not recommended.

No significant differences in PSA decline rates or objective responses in measurable disease could be detected between the treatment arms. The excess nonhematologic toxicity noted with imatinib compared with placebo was greater than anticipated, based on prior observations for each agent given as monotherapy at the respective starting dose levels. Although a pharmacokinetic interaction between docetaxel and imatinib has not been identified (21), a pharmacodynamic interaction remains plausible. These excess adverse events, especially grade 2 and 3 fatigue and gastrointestinal (including nausea, diarrhea, anorexia, and vomiting) or cardiovascular events (supraventricular arrhythmia), led to increased study drop-out rates, although

excess toxicity-related deaths were not identified. Sigmoid diverticular perforations, now reported in association with a variety of tyrosine kinase receptor inhibitors that may inhibit the vascular endothelial growth factor and/or PDGFR pathway (e.g., bevacizumab, sorafenib, and sunitinib; refs. 22–24), may well have a vascular basis but such pathology could not be defined on the two pathologic specimens obtained in this study. Steroid therapy has also been historically implicated as a cofactor in the pathogenesis of sigmoid diverticular perforation (25, 26) and high-dose steroids may have been involved in three of the five cases identified in this study confounding the association with imatinib. The masking effects of steroids on inflammatory signs and symptoms are an important clinical caveat relevant to the care of patients receiving this spectrum of agents and concomitant steroid therapy (27). Although there are no other reports of sigmoid diverticular perforations associated with studies involving docetaxel and imatinib to our knowledge, this experience serves as a cautionary note in future studies of the combination.

In our earlier modular phase I trial of the combination of imatinib and docetaxel (9), we used a lead-in period in which only imatinib was given to explore early biomarker effects of this agent and compare them against the outcomes of combination therapy in the same patient. The observation of long-term (median, 24 months) freedom from disease progression in a significant fraction (24%) of the heavily pretreated patients in that trial (10), but not among those in the study reported here, may simply reflect selection bias in the first trial that was accounted for in the second trial through randomization and risk group stratification. Alternatively, it is difficult to refute that the relatively long lead-in sequence of imatinib alone in the modular phase I trial before the combination phase of imatinib and docetaxel may have influenced the outcome. This design element was excluded from the subsequent randomized study on the premise that with chronic imatinib therapy, each subsequent cycle of docetaxel would have an effective lead-in with imatinib. Although the goal of hierarchically designed sequential modular phase I and randomized phase II trials is a more rapid assessment of biological agents in combination with chemotherapy, pitfalls such as the effect of a lengthy lead-in must be considered. Although the clinical schedule of the randomized phase II study mirrored the preclinical studies, we acknowledge the possibility of different qualitative outcomes with variations in dose, sequence, and schedule of the combination.

Table 3. Correlations of p-PDGFR with outcomes

Correlation	*n*	Pr(Decr) p-PDGFR (median)	*P*
Treatment group and reduction in p-PDGFR			
Imatinib	41	0.487	<0.0001
Placebo	47	0.447	
50% Decline in PSA and reduction in p-PDGFR			
50% Decline in PSA	30	0.406	<0.0001
No 50% decline in PSA	47	0.505	
Reduction in p-PDGFR and PFS			
Below median 0.46	44	5.7 mo	0.032
Above median 0.46	44	4.1 mo	

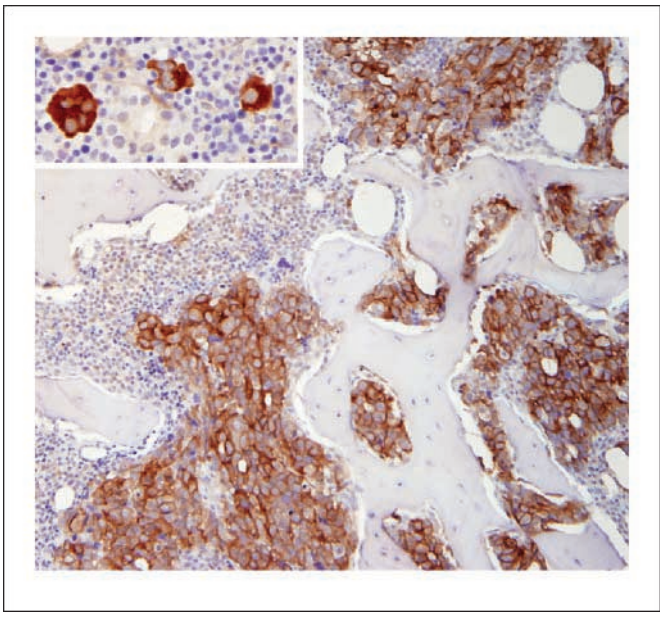

Fig. 2. Expression of p-PDGFR-β in bone metastases (megakaryocytes 3+ intensity, *inset*).

The translational studies indicate that the molecular target of interest in the study, p-PDGFR, was expressed at high frequency in tumor cells in the bone microenvironment. An important limitation in any study of prostate cancer and bone metastases is the inability to directly and reliably monitor modulation of tumor cell biology. The results of this study are a reminder of the relatively poor yield of informational material from the standard posterior superior iliac crest bone marrow biopsy (28, 29). Radiologically directed repetitive bone biopsies are expensive and can be difficult with varying yield. The expression profile of circulating tumor cells may vary considerably from that of tumor cells embedded in the bone microenvironment, and molecular heterogeneity is likely within the bone microenvironment as well. Changes in biomarkers in surrogate tissue may not mirror modulation of the tumor or its microenvironment.

With these limitations acknowledged, we used peripheral blood leukocyte p-PDGFR expression levels as a surrogate for systemic p-PDGFR inhibition to explore association between treatment arm and p-PDGFR status and correlations between p-PDGFR levels and treatment outcomes. Our data suggest a strong association between imatinib therapy and systemic p-PDGFR inhibition consistent with the known effect of the drug but we found that for the study population as a whole, increasing p-PDGFR inhibition was associated with a diminished probability of a PSA decline by 50% and a trend toward shorter PFS. These findings are of concern in light of a report that imatinib had antagonistic effects against docetaxel in PC3 cells (30). Although we have been unable to duplicate such *in vitro* findings, the implications of our

experimental results may require further scrutiny in studies involving PDGFR inhibitors combined with taxanes in other neoplasms (7, 8).

Changes in bone markers over time offer the possibility of interrogating organ-specific cellular machinery but have not been validated with respect to their association with important clinical outcomes. Prior studies of a variety of bone-targeting agents, such as bone-homing radioisotopes (31), potent bisphosphonates (32), or endothelin receptor antagonist therapy (33), have shown changes in bone markers or bone complications (e.g., pain or fracture) in men with castrate-resistant prostate cancer with bone metastases. However, definitive evidence of improved time to progression or overall survival has not been shown to date. Our bone marker findings suggest that imatinib targets the osteoclastic response (assessed by urine NTx excretion) preferentially over the osteoblastic response (assessed by serum BAP levels) in prostate cancer bone metastases. Declines in urinary NTx but not in serum BAP on crossover from placebo to imatinib support this apparent preferential effect.

These observations on disparate bone marker outcomes may offer clues to reasons for the discordance between our preclinical studies described earlier and these clinical results. Specifically, the PC3-MM2 orthotopic model of bone metastases is a highly proliferative, predominantly lytic phenotype with florid osteoclastic but sparse osteoblastic elements and a proclivity for extraosseous soft tissue progression (4). The predominant phenotype of metastases in men with castrate-resistant prostate cancer and bone metastases by contrast is mixed, with osteoblastic elements dominating the stromal microenvironment with limited extraosseous extension. A preferential osteoclastic targeting may be insufficient to disable the stromal-derived tumor cell survival pathways in the typical phenotype of progressive prostate cancer in bone. Indeed, osteoblastic inhibition as indicated by declines in BAP may have been impaired in the imatinib group. In this regard, at least one *in vitro* model involving coculture of LNCaP prostate cancer cells and a SV40-immortalized human osteoblast cell line supports the notion that osteoblasts can modulate the apoptotic threshold of prostate cancer cells to reduce sensitivity to chemotherapy (34).

Another discordance between the preclinical and clinical studies was the findings from the crossover from the placebo arm to imatinib at disease progression. This study served as a clinical parallel to our prior studies, which showed reversal of drug resistance with multidrug resistance PC3-MM2 cells with imatinib in an orthotopic model of bone metastasis via induction of apoptosis in tumor vascular endothelial cells expressing p-PDGFR (6). Although transient declines in PSA levels were observed in 40% of patients after crossover, disappointingly, none were particularly noteworthy in terms of magnitude or duration. It is to be determined whether the phenotypic limitations of the preclinical model relate to variations in vascular endothelial p-PDGFR status and/or the representative stromal phenotype as discussed earlier. Nevertheless, we might predict, from the results of the clinical trial, that the efficacy of taxane chemotherapy and imatinib observed in the PC3-MM2 lytic model of bone metastases would not be observed in an osteosclerotic model.

In summary, future preclinical studies of castrate-resistant prostate cancer with bone metastases should consider incorporation of more representative stromal phenotypes, including both osteoblastic and osteolytic components, with a dominant osteoblastic component. These models may provide a more robust platform for the interrogation of molecular pathways implicated in the epithelial-stromal cell interactions driving the lethal phenotype of prostate cancer and allow for useful bidirectional studies at the laboratory-clinic interface.

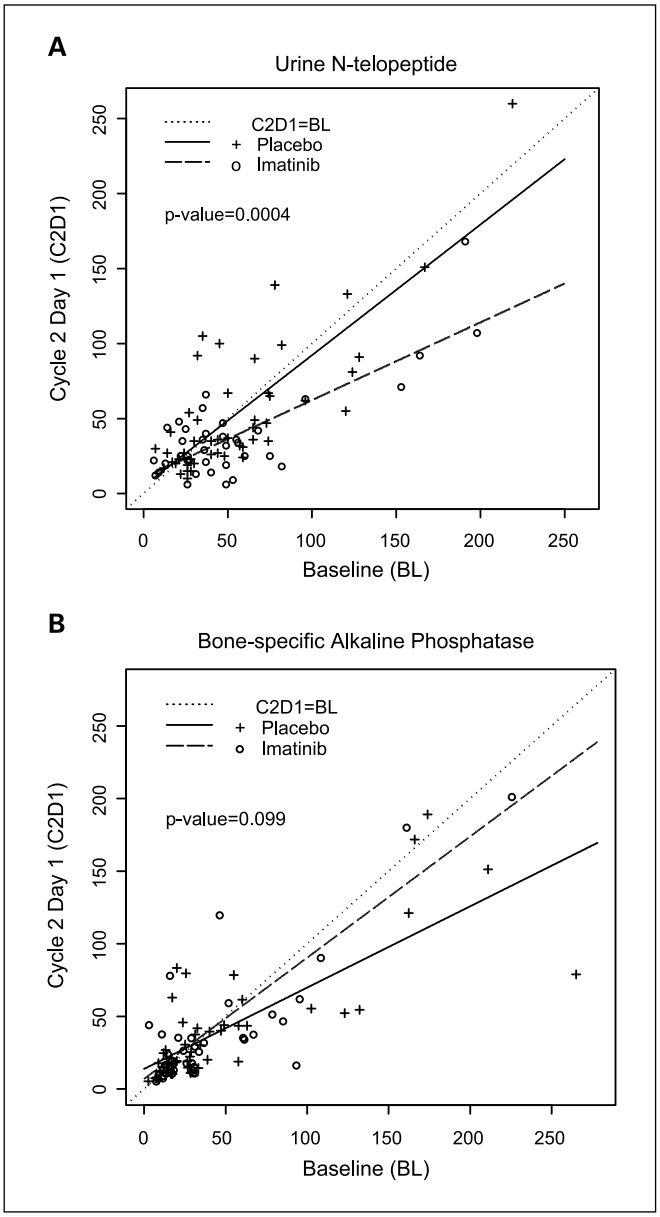

Fig. 3. Paired baseline (*BL*) and cycle 2 day 1 (*C2D1*) bone marker outcomes according to treatment group suggest that docetaxel + imatinib therapy affected NTx (*A*) and BAP (*B*) kinetics differently than docetaxel + placebo therapy.

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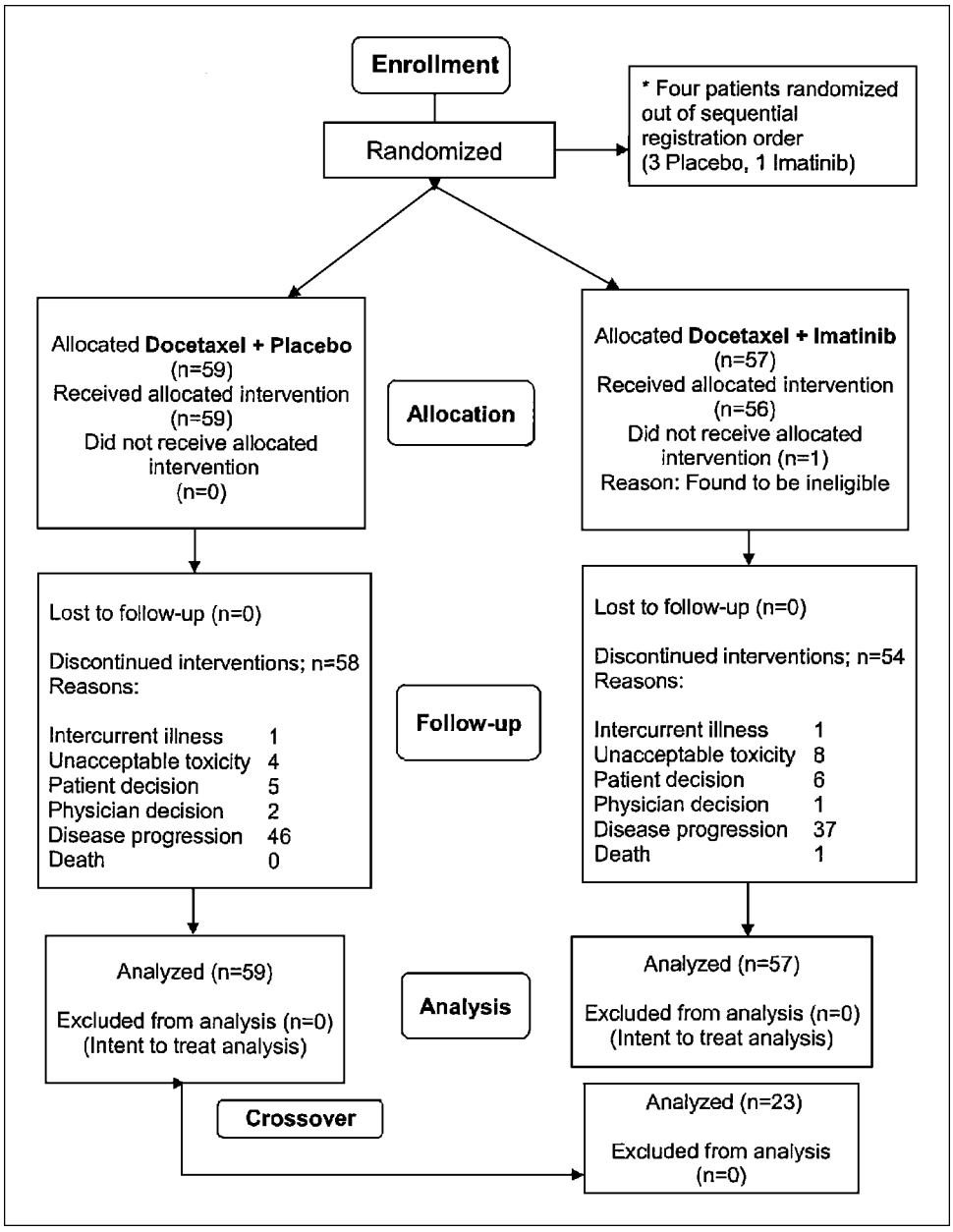

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