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ORIGINAL ARTICLE

Accelerated disease progression in prostate cancer and bone metastases with platelet-derived growth factor receptor inhibition: observations with tandutinib

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Abstract
Background Activated platelet-derived growth factor receptor (p-PDGFR) is frequently expressed in bone metastases of castration-resistant prostate cancer (CRPC). Phase II study of tandutinib was conducted to assess the effects of a continuously administered highly potent PDGFR inhibitor in this disease state.
Methods Men with progressive CRPC, bone metastases, and prior taxane chemotherapy were treated with oral tandutinib 500 mg twice daily until disease progression under a two-stage design with the 8-week freedom-from-progression (FFP) rate as the primary endpoint. The trial was designed to have 87% power to reject a null FFP rate of 10% when the true rate was 33% (type I error rate = 0.02). Secondary endpoints included tumor expression of p-PDGFR, bone marker (urine *N*-telopeptide, serum bone-specific alkaline phosphatase) kinetics, in vivo monitoring of PDGFR inhibition in peripheral blood leukocytes, and correlation with survival.
Results Among 18 patients registered (aged 47–81, median 66 years), 15 were evaluable for efficacy. Five of 6 evaluable tumors were p-PDGFR positive. Mean urine *N*-telopeptide declined from 123.7 (baseline) to 41.0 (Cycle 2 Day 1) nmol/mmol Cr ($P = 0.012$). Probability of decrease in peripheral blood leukocyte p-PDGFR >0.5 versus <0.5 was associated with progression-free survival of 6 versus 8 weeks ($P = 0.03$, log-rank) and overall survival, 26.6 versus 42.9 weeks, respectively ($P = 0.09$, log-rank).
Conclusions In vivo PDGFR inhibition with tandutinib correlated with accelerated disease progression. This observation raises the hypothesis that PDGF contributes to the homeostasis of bone metastases from prostate cancer.

Keywords Platelet-derived growth factor · Prostate cancer · Bone metastases · Tandutinib · PDGF

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Background

Effective therapeutic targeting of the diverse molecular mechanisms utilized by prostate cancer cells in the bone microenvironment toward survival and progression will likely translate into improved morbidity and mortality from the disease. The platelet-derived growth factor (PDGF) [1], which physiologically regulates the remodeling cellular machinery of bone through its effects on mesenchymal progenitors, osteoblast maturation, and osteoclast function, has been implicated in the progression of prostate cancer in the bone microenvironment.

With degenerate primers toward highly conserved domains of tyrosine-kinase receptor transcripts, the PDGF receptor (PDGFR) was among the most frequently amplified

transcripts from pooled bone marrow aspirates from men with bone metastases from prostate cancer [2]. In an orthotopic model of prostate cancer bone metastases, PDGF expression is upregulated specifically in tumor proliferating within the bone microenvironment and not in tumor cells distant from the bone interface [3] probably as a result of paracrine signaling of bone-derived peptides such as TGF-beta The PDGFR is also specifically expressed in these tumor cells and the endothelial cells of its vasculature, suggesting bone microenvironment-specific autocrine and paracrine effect of PDGF toward upregulation of its receptor, the PDGFR [3]. In this model, therapeutic inhibition of PDGFR with imatinib mesylate [4] resulted in enhanced endothelial apoptosis specific to PDGFR-expressing tumor, enhanced taxane efficacy, and reversal of taxane resistance [3, 5]. Immunohistochemical studies have demonstrated a high frequency of phosphorylated PDGFR expression in tumor cells in the bone metastases specimens from men with metastatic castrate-resistant prostate cancer (CRPC) [6].

There are no published observations on the effects of a continuously administered highly potent PDGFR inhibitor correlated with in vivo PDGFR inhibition in CRPC and bone metastases. Tandutinib is a small molecule inhibitor of receptor tyrosine kinases including Flt3, c-kit, colony stimulating factor-1 receptor with potent in vitro activity against PDGFR-beta [7, 8], the predominant isoform expressed in bone metastases from prostate cancer [6]. Tandutinib was developed through systematic modifications to a quinazoline of a type previously shown to inhibit PDGFR phosphorylation [7] and possesses an IC50 of 20 nM compared with an IC50 of 100 nM for imatinib versus PDGFR-beta. It has no appreciable activity against EGFR, FGFR, KDR, or several non-receptor kinases including Src and Abl [8]. Phase 1 clinical results with tandutinib in adults with acute myelogenous leukemia and myelodysplasia identified reversible muscle weakness and fatigue as the principal dose-limiting toxicities [9].

A two-stage Phase II trial in men with bone metastases from CRPC and prior taxane therapy was conducted with freedom-from-progression at 8 weeks as the primary endpoint. Secondary endpoints included the assessment of p-PDGFR expression in bone metastases, kinetics of bone turnover markers, pharmacodynamic measures of in vivo p-PDGFR inhibition, and correlations with progression-free survival and overall survival.

Methods

Patient population

Men with histologic evidence of adenocarcinoma of the prostate and radiological evidence of bone metastases were eligible. A castrate level of testosterone (<50 ng/dl) and evidence of progressive disease after anti-androgen withdrawal, when applicable, were required. Definition of progressive disease included worsening malignant bone pain, increasing bidimensional disease, the appearance of new lesions on bone radiographs or consecutive rises in prostate-specific antigen (PSA). For PSA progression, 2 consecutive rises in PSA, each at least 1 ng/ml and at least 2 weeks apart, were required. A minimum PSA of 5 ng/ml was required. Patients must have had at least one prior taxane-based regimen but no prior known PDGFR inhibitor therapy was permitted. Patients must have had absolute neutrophil count $\geq$1,500/mcL, platelets $\geq$100,000/mcL, total bilirubin within normal institutional limits, aspartate aminotransferase and alanine aminotransferase $\leq 2.5\times$ institutional upper limit of normal, creatinine clearance $\geq$40 ml/min/1.73 m^2, and the ability to understand and the willingness to sign a written informed consent document. Exclusions included patients who have had chemotherapy or radiotherapy within 4 weeks to entering the study or those who have not recovered from dose-limiting adverse events due to agents administered more than 4 weeks earlier and comedication with an agent that causes QTc prolongation, a mean QTc >500 ms (with Bazett's correction) in screening electrocardiogram, left ventricular ejection fraction (LVEF) <40%, myocardial infarction within 6 months of enrollment or New York Heart Association (NYHA) Class III or IV heart failure, uncontrolled angina, uncontrolled ventricular arrhythmias or electrocardiographic evidence of acute ischemia, inability to take oral medication, chronic liver disease, known or suspected primary muscular or neuromuscular disease, and brain metastases. The study was conducted under institutional review board guidelines and with the written informed consent of all participants (NCT00390468).

Evaluation at baseline and follow-up

History, physical examination, laboratory tests including complete blood count, serum creatinine, liver transaminases, bilirubin, PSA, and testosterone, bone-specific alkaline phosphatase, and urine *N*-telopeptide were completed at baseline and every 4 weeks. Electrocardiograms and echocardiograms assessed baseline cardiac function, and QT intervals were monitored every 4 weeks as asymptomatic QTc prolongation related to tandutinib therapy was suggested in prior Phase I studies [7, 9]. Radiological studies at baseline included chest X-ray, computerized tomography of abdomen and pelvis, and radionuclide bone scan and were repeated every 8 weeks. Patients were evaluated every 2 weeks for symptoms of toxicity or disease progression. A Brief Pain Inventory was administered at baseline and every 4 weeks to measure bone pain outcomes. Unilateral

posterior–superior iliac crest bone marrow biopsy was performed at study entry.

Immunohistochemistry for p-PDGFR expression in tumor cells in bone marrow biopsies: 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}) (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. Megakaryocyte staining was used as a reference internal control for staining intensity (3+).

In vivo p-PDGFR monitoring: Samples for in vivo p-PDGFR monitoring in peripheral blood leukocytes were drawn at baseline, after 2 weeks of tandutinib therapy, and at progression or removal from study and were subsequently batch-analyzed. Venous blood samples were collected into a sodium heparin Vacutainer and centrifuged at $1{,}200\times g$ 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 $500\times g$ 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 were measured by a laser scanning cytometer (Compucyte Corp.), and histograms were generated for analysis.

Therapeutics

Tandutinib was planned as an oral dose of 500 mg twice daily every day. Available dose levels for reduction were 400 mg bid (−1) and 300 mg bid (−2). For Grade 2 or higher gastrointestinal toxicity or muscle weakness, tandutinib was to be interrupted and dose reduction implemented at recovery to Grade 1 or less. For Grade 3 or higher (non-hematologic or Grade 4 (hematologic) adverse events related to tandutinib, lasting >5 days that did not resolve to Grade 2 or below despite maximum supportive care for ≤48 h, drug interruption and dose reduction were permitted if recovery to Grade 2 or less occurred. In the absence of recovery of toxicity to this level, patients were to be removed from study.

Definition of progression

Patients were withdrawn from the study for symptomatic or radiologic progression of disease, unmanageable toxicity, or physician/patient choice. Progression-free survival on study was defined as the time of initiation of therapy to the first determination of progression of disease by clinical or radiologic criteria that included appearance of one or more new bone lesions, worsening malignant bone pain, unequivocal progression of existing non-target lesions, and/or progression of measurable disease by RECIST criteria. The frequency of PSA declines from baseline was assessed but rises in PSA in the absence of symptomatic or radiological evidence of progression as above did not contribute toward a definition of progression.

Statistical methods

A Simon two-stage design with freedom-from-progression at 8 weeks as the primary endpoint was employed. This study was designed to detect a freedom-from-progression at 60 days of 30%. A sample size of 30 patients was estimated. If among the first 15 patients, 3 or more patients were free from progression at 8 weeks, then an additional 15 patients were to be enrolled. If 7 or more of 30 patients were free from progression at 8 weeks, the drug was to be considered for further study. If the true freedom-from-progression rate was 30%, then the power for declaring the drug efficacious was 78%. The type I error rate for a true freedom-from-progression rate of 10% was 0.02.

The frequency of p-PDGFR expression in bone marrow biopsy specimens, PSA declines by 50% sustained for 4 weeks, measurable disease outcomes by RECIST criteria, and quantitative and qualitative toxicities were assessed. Descriptive statistical analyses were carried out using boxplots, means, and standard deviations. Spearman correlation [10] was calculated to assess correlation between two continuous variables. The Fisher exact test [11] was used to assess association between categorical variables. Wilcoxon signed-rank tests for paired data [12] were used to assess the pain and bone marker changes between baseline and cycle 2 day 1. Progression-free survival and overall survival

functions were estimated using the Kaplan–Meier method [13]. The two-sided log-rank test [14] was used to examine the association between a categorical variable and time to progression or overall survival. All *P* values were derived from two-sided tests. All computations were performed using *SPLUS 2000 (1988-2000)*, Insightful Corporation [15].

Analysis of in vivo p-PDGFR monitoring: Dynamic changes from baseline in p-PDGFR, assayed in 2,000 individual peripheral blood leukocytes, obtained at baseline and on Cycle 2 Day 1, were reported as the probability of decrease in p-PDGFR. 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 the probability of decrease in p-PDGFR, each based on a Wilcoxon–Mann–Whitney statistic [12]. A value of this estimator greater than 0.5 implies that p-PDGFR is decreasing from baseline to C1D14, a value of the estimator less than 0.5 implies that p-PDGFR is increasing from baseline to C1D14, and a value of the estimator equal to 0.5 implies that p-PDGFR has no change. The ability of the probability of decrease in p-PDGFR values to predict progression-free survival and overall survival was assessed by a log-normal time-to-event regression model, as described previously [6].

Results

Patient characteristics

Table 1 provides a summary of patient characteristics, all of whom had prior taxane chemotherapy, indicating a largely symptomatic group of men with bone pain and high frequency of prior taxane resistance defined as disease progression during treatment with a taxane. A total of 18 patients were registered to the study; however, 3 patients were judged invaluable for efficacy because of prior PDGFR inhibitor therapy with imatinib mesylate ($n = 2$) or early adverse events including worsening baseline fatigue and bone pain resulting in removal from study within 3 days of initiating therapy ($n = 1$).

Table 1 Patient characteristics

Race/ethnic composition	Caucasian: 18 (100%)
Prior taxane resistance	13 (72%)
ECOG Performance Score (PS)	PS 0: 8; PS 1: 10
Other baseline variables	*Median/range*
Age	66 (47–81) years
Gleason score	9 (7–10)
PSA	45 (7.9–4,609.5) ng/ml
Bone-specific alkaline phosphatase[a]	26 (7.6–181) mcg/l
Urine *N*-telopeptide[b]	122 (34–292) nmol/mmol Cr
Average bone pain[c]	2 (0–6)
Worst bone pain[c]	3 (0–7)
Prior chemotherapy regimens	2 (1–4)

[a] Normal reference range: 7.6–14.9

[b] Normal reference range: 9–60

[c] Brief Pain Inventory Scale (0–10)

Progression events and observed responses

Two of 15 evaluable patients were progression-free at 8 weeks. One patient had a ≥50% PSA decline with time-to-disease progression of 40 weeks. The second patient had a 40% PSA decline and chose to discontinue therapy at 43 weeks for toxicity (fatigue). The study was closed to further accrual per design specifications. Thirteen patients exhibited progression by radiological criteria, all accompanied by PSA rises. One patient discontinued therapy for symptomatic progression alone. Time to progression ranged between 3 and 40 weeks with the median at 8 weeks.

Expression of p-PDGFR in bone marrow biopsies

All 18 patients had posterior–superior iliac crest bone marrow biopsies and aspirates at baseline and six of these had evaluable tumor. Five patients had p-PDGFR expression in tumor (Fig. 1a), and the patient with the longest progression-free survival lacked p-PDGFR expression in tumor (Fig. 1b).

Qualitative and quantitative toxicity

Table 2 demonstrates the high frequency of Grades 1–2 gastrointestinal toxicity and fatigue observed with tandutinib therapy in this population. With exception of malignant bone pain, adverse events independent of attribution are included. Scheduled administration of oral granisetron and dose reductions were effective in controlling troublesome nausea. Diarrhea was managed effectively with oral loperamide.

Bone pain and bone marker outcomes

Table 3 summarizes means and standard deviations of pain and bone marker variables. *P* values were based on Wilcoxon signed-rank test for paired data. Significant declines in mean urinary *N*-telopeptides were observed, but by contrast mean values of bone-specific alkaline phosphatase rose. No improvement in pain scores was observed.

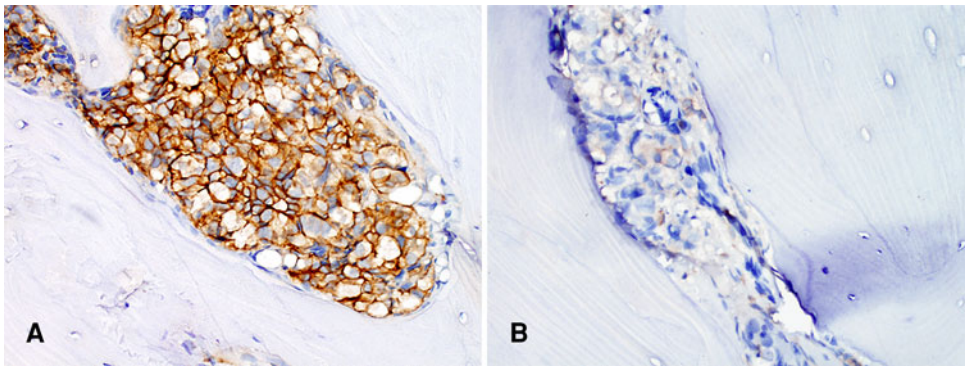

Fig. 1 Expression of p-PDGFR in bone metastasis of prostate cancer. **a** The tumor cells in one patient showed positive membranous immunoreactivity for p-PDGFR (×200). **b** The tumor cells in another patient showed negative immunoreactivity for p-PDGFR (×200)

Table 2 Qualitative and quantitative toxicity profile

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4	Total
Nausea	15	7	0	0	22
Vomiting	13	3	0	0	16
Diarrhea	9	3	0	0	12
Edema	13	0	0	0	13
Fatigue	13	7	3	0	23
Anemia	20	3	3	0	26
Neutropenia	1	0	0	0	1
Thrombocytopenia	2	1	0	1	4
Transaminase	7	0	0	0	7

Table 3 Bone pain and bone marker kinetics

	Mean (BL)	SD	Mean (C2D1)	SD	*P*
Average bone pain[a]	2.1	1.9	1.3	1.3	0.231
Worst bone pain[a]	2.6	2.4	2.8	2.7	0.773
Urine *N*-telopeptide[b]	123.7	67.1	50.6	41.0	0.012
Bone-specific alkaline phosphatase[c]	44.3	43.1	67.8	73.4	0.211

[a] Brief Pain Inventory Scale (0–10)

[b] Normal reference range: 9–60

[c] Normal reference range: 7.6–14.9; *BL* baseline, *C2D1* cycle 2 Day 1, *SD* standard deviation

Analysis of p-PDGFR dynamics

The probability of reduction of p-PDGFR at cycle 1 day 14 is described in Fig. 2. Dotted intercepts indicate the two patients with PSA declines with the lowest intercept denoting the patient with the freedom-from-progression to 40 weeks. A high correlation between the probability of p-PDGFR decrease at Cycle 1 Day 14 and at progression (11 paired samples were evaluable) was noted (Spearman correlation = 0.80, $P = 0.02$), suggesting the absence of tachyphylaxis of p-PDGFR inhibition with tandutinib, i.e., no apparent decline in p-PDGFR inhibition over time. The

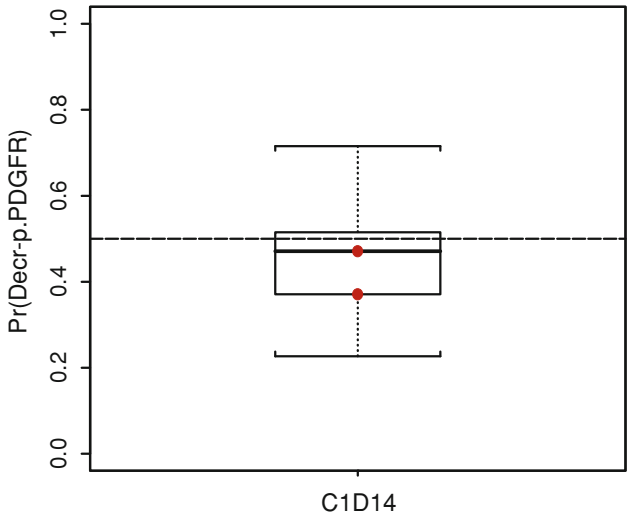

Fig. 2 Box-plot of probability of decrease in p-PDGFR [Pr(Decr-p-PDGFR) at Cycle 1 Day 14 (C1D14)

median (range) of probability of decrease in p-PDGFR was 0.471 (0.227, 0.716) at Cycle 1 Day 14 and 0.512 (0.104, 0.691) at progression.

Progression-free survival and correlations

The median follow-up is 29 weeks for 18 registered patients. The median progression-free survival was 7.9 weeks (95% CI: 7.7–8.1 weeks), while median overall survival was 36.1 weeks (95% CI: 24.9–60 weeks). When the probability of decrease in p-PDGFR at cycle 1 day 14 was <0.5, median progression-free survival was 8 weeks with median overall survival of 42.9 weeks. When the probability of decrease in p-PDGFR at cycle 1 day 14 was >0.5, median progression-free survival was shorter at 6 weeks ($P = 0.03$ in log-rank test; hazard ratio = 4.1 with 95% CI: 1.1–15.2) with median overall survival of 26.6 weeks ($P = 0.09$ in log-rank test; hazard ratio = 3.0 with 95% CI: 0.8–11.7) (Fig. 3a, b, respectively). Changes in bone pain, urine *N*-telopeptide, or bone-specific alkaline phosphatase at cycle 2 day 1 did not predict for progression-free survival.

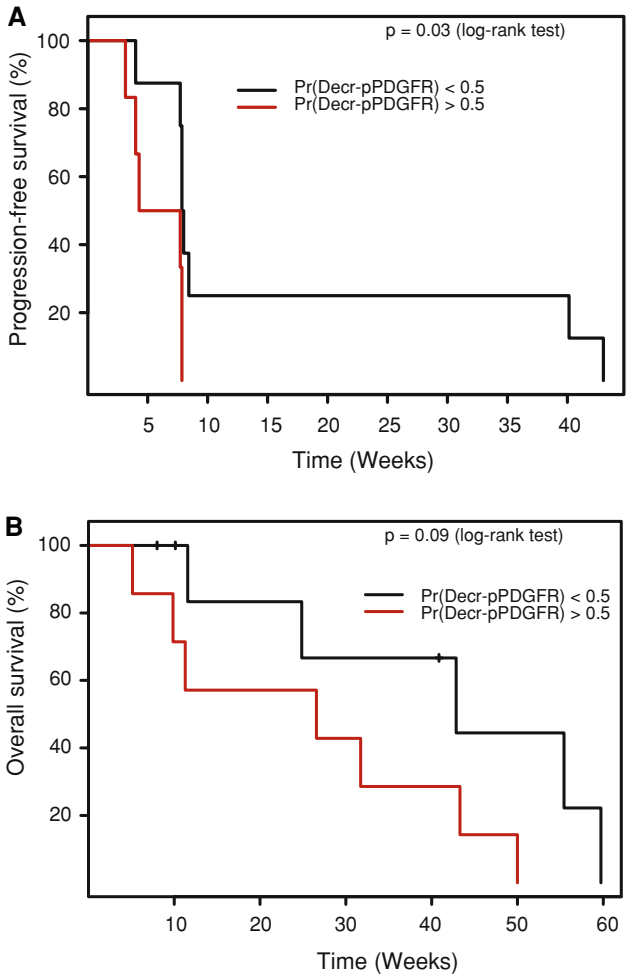

Fig. 3 Progression-free survival by probability of decrease in peripheral blood leukocyte p-PDGFR [Pr(Decr-pPDGFR]. **b** Overall survival by probability of decrease in peripheral blood leukocyte p-PDGFR [Pr(Decr-pPDGFR]

Discussion

Several lines of evidence indicated that the activated platelet-derived growth factor receptor is a strong candidate mediator of mechanisms of prostate cancer disease progression specific to the bone microenvironment. In this study with tandutinib, a continuously administered potent inhibitor of the PDGFR-beta, evidence of in vivo PDGFR inhibition correlated with shorter progression-free survival times in men with advanced castration-resistant prostate cancer and bone metastases.

Given the observations with single-agent tandutinib within the context of high frequency of p-PDGFR overexpression in prostate cancer cells within the bone microenvironment, the role of this signaling pathway, including a putative *negative* regulation of the progression of this disease state, requires reappraisal. Significant declines in urine *N*-telopeptide suggest effective targeting of the osteoclastic element of the bone remodeling machinery, while stable or increased levels of bone-specific alkaline phosphatase suggest that dominant stromal component of the neoplastic phenotype in bone, osteoblastic proliferation, is unaffected. Given that preclinical observations demonstrating antivascular effects of PDGFR inhibitor therapy, specifically imatinib, have been generated from PC-3 cell lines that generate lytic-dominant phenotypes in bone [3, 5], these clinical observations may refer to the need for more relevant osteosclerotic models of the disease. Variations in tumor vascular biology among these phenotypic variants in bone may be pertinent to the clinical observations with PDGFR inhibition. Major translational obstacles in the field have been the technical difficulty in phenotyping vasculature in clinical specimens of prostate cancer bone metastases as well as generating proliferative models of the typical osteoblastic-dominant phenotype of prostate cancer in bone for screening of experimental therapeutics within a suitable time-frame.

Several of these clinical observations with tandutinib are in line with those obtained with imatinib [4], a less potent PDGFR inhibitor, in men with castration-resistant prostate cancer and bone metastases. In a modular Phase I design of imatinib and docetaxel [16], a 4-week lead-in period with imatinib monotherapy was associated with PSA declines from baseline in 2 of 27 men with daily administration of 600 mg of oral imatinib, whereas median PSA and bone pain scores rose twofold from baseline over this time period. In vivo PDGFR monitoring and correlations with outcomes were not assessed in this study or in other disease states of prostate cancer in which single-agent PDGFR inhibitors were studied; these studies have also demonstrated low-frequency declines in PSA [17–20]. By contrast, in two prior combination studies of imatinib and docetaxel in which in vivo p-PDGFR monitoring was conducted, similar trends in outcomes were identified. In metastatic castration-resistant prostate cancer with bone metastases in which men were randomized between docetaxel and imatinib and docetaxel and placebo, a probability of decrease in in vivo p-PDGFR >0.5 following therapy correlated with shorter times to progression and/or overall survival [6, 21]. In the setting of high-risk localized prostate cancer with docetaxel and imatinib as neoadjuvant therapy [22], a similar trend toward shorter progression-free survival with a probability of decrease in in vivo p-PDGFR >0.5 was noted, although this was not statistically significant. Intriguingly, data from the docetaxel and placebo control arm of the randomized study demonstrated that an *increase* in p-PDGFR following taxane therapy correlated with improved progression-free survival and overall survival [21]. Taken together, these observations suggest that taxanes and platelet-derived growth factor receptor

inhibitors may work at cross-purposes through hitherto undetermined mechanisms involving PDGFR signaling in the tumor microenvironment.

The tandutinib and imatinib studies [6] together demonstrate that significant urine *N*-telopeptides declines are not a universal surrogate of clinical benefit in castration-resistant prostate cancer and bone metastases. Further, the contrasting outcomes with respect to bone-specific alkaline phosphatase with both these agents [6] are consistent with evidence that PDGFR inhibition promotes osteoblastic differentiation and inhibits osteoclastogenesis [23–27]. It is plausible that a therapeutic induction of osteoblast differentiation by PDGFR inhibitors results in a resistance mechanism mediated by osteoblast–epithelial interactions [28].

Although the case for PDGFR as a target for cancer therapy has been developed [29–31], other than tumors in which the PDGFR is translocated or harbors an activating mutation, PDGFR inhibitors alone have not proved useful in solid tumors. There is evidence from this study with tandutinib, from the association of low probability of in vivo PDGFR inhibition and absent p-PDGFR expression in tumor in a singular case of substantive PSA decline and prolonged freedom-from-progression, that low-frequency antitumor effects may be PDGFR-independent effects of the experimental agent. It is plausible that similar low-frequency PSA declines observed with imatinib monotherapy [16–20] have a similar explanation. These agents have diverse alternate kinase targets [8, 26] that may explain such activity.

A mechanistic understanding underpinning the correlative observations of peripheral blood leukocyte p-PDGFR dynamics and disease outcomes outlined here is beyond the scope of this study. The difficulties in obtaining serial informative biopsies containing tumor from men with prostate cancer and bone metastases on PDGFR inhibitor therapy imply that necessary correlation and validation of the effects of these agents (and p-PDGFR dynamics in peripheral blood leukocytes) on PDGF signaling in tumor, stroma, and vasculature will be challenging.

In summary, the data linking in vivo p-PDGFR inhibition with accelerated disease progression, from the single-agent tandutinib and combination imatinib studies, demonstrate that a more accurate understanding of the diverse PDGF signaling interactions in tumor epithelium, stroma, and vasculature, including a putative homeostatic function, is required.

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References

1. Heldin C-H, Westermark B (1999) Mechanism of action and in vivo role of platelet-derived growth factor. Physiol Rev 79:1283–1316
2. Chott A, Sun Z, Morganstern D et al (1999) Tyrosine kinases expressed in vivo by human prostate cancer bone marrow metastases and loss of the type 1 insulin-like growth factor receptor. Am J Pathol 155:1271–1279
3. Uehara H, Kim SJ, Karashima T et al (2003) Effects of blocking platelet-derived growth factor-receptor signaling in a mouse model of experimental prostate cancer bone metastases. J Natl Cancer Inst 95:458–570
4. Buchdunger E, Cioffi CL, Law N et al (2000) ABL protein-tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-kit and platelet-derived growth factor receptors. J Pharmacol Exp Ther 295:139–145
5. Kim SJ, Uehara H, Yazici S et al (2006) Targeting platelet-derived growth factor receptor on endothelial cells of multidrug-resistant prostate cancer. J Natl Cancer Inst 98:783–793
6. Mathew P, Thall P, Bucana CD et al (2007) Platelet-derived growth factor receptor inhibition and chemotherapy for castration-resistant prostate cancer with bone metastases. Clin Cancer Res 13:5816–5824
7. CTEP/Investigator's Brochure MLN518, Edition 3 (2004) Millenium Pharmaceuticals, Inc., Cambridge, MA
8. Pandey A, Volkots DL, Seroogy JM et al (2002) Identification of orally active, potent, and selective 4-piperazinylquinazolines as antagonists of the platelet-derived growth factor receptor tyrosine kinase family. J Med Chem 45:3772–3793
9. DeAngelo DJ, Stone RM, Heaney ML, Nimer SD, Paquette RL et al (2006) Phase I clinical results with tandutinib (MLN518), a novel FLT3 antagonist, in patients with acute myelogenous leukemia or high-risk myelodysplastic syndrome: safety, pharmacokinetics, and pharmacodynamics. Blood 108:3674–3681
10. Fisher LD, Belle GV (1993) Biostatistics: a method for the health sciences. Wiley, New York
11. Snedecor GW, Cochran WG (eds) (1980) Statistical methods, 7th edn. Iowa State University Press, Ames
12. Hollander M, Wolfe DA (1979) Introduction to the theory of nonparametric statistics. Wiley, New York
13. Kaplan EL, Meier P (1958) Nonparametric estimator from incomplete observations. J Am Stat Assoc 53:457–481
14. Mantel N (1966) Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 60:163–170
15. SPLUS 2000 (1988–2000) Insightful Corporation, Seattle, WA
16. Mathew P, Thall PF, Jones D et al (2004) Platelet-derived growth factor receptor inhibitor imatinib mesylate and docetaxel: a modular Phase I trial in androgen-independent prostate cancer. J Clin Oncol 22:3323–3329
17. Ko Y-J, Small EJ, Kabbinavar F et al (2001) A multi-institutional Phase II study of SU101, a platelet-derived growth factor receptor inhibitor, for patients with hormone-refractory prostate cancer. Clin Cancer Res 7:800–805
18. Rao K, Goodin S, Levitt MJ et al (2005) A phase II trial of imatinib mesylate in patients with prostate specific antigen progression after local therapy for prostate cancer. Prostate 62:115–122
19. Lin AM, Rini BI, Weinberg V et al (2006) A phase II trial of imatinib mesylate in patients with biochemical relapse of prostate cancer after definitive local therapy. BJU Int 98:763–769
20. Bajaj GK, Zhang Z, Garrett-Mayer E et al (2007) Phase II study of imatinib mesylate in patients with prostate cancer with evidence of biochemical relapse after definitive radical retropubic prostatectomy or radiotherapy. Urology 69:526–531

21. Mathew P, Thall PF, Wen S et al (2008) Dynamic change in phosphorylated platelet-derived growth factor receptor in peripheral blood leucocytes following docetaxel therapy predicts progression-free and overall survival in prostate cancer. Br J Cancer 99:1426–1432
22. Mathew P, Pisters LL, Wood CG et al (2009) Neoadjuvant platelet derived growth factor receptor inhibitor therapy combined with docetaxel and androgen ablation for high risk localized prostate cancer. J Urol 181:81–87
23. O'Sullivan S, Naot D, Callon K et al (2007) Imatinib promotes osteoblast differentiation by inhibiting PDGFR signaling and inhibits osteoclastogenesis by both direct and stromal cell-dependent mechanisms. J Bone Miner Res 22:1679–1689
24. Fitter S, Dewar AL, Kostakis P, To LB, Hughes TP, Roberts MM, Lynch K, Vernon-Roberts B, Zannettino ACW (2008) Long term imatinib therapy promotes bone formation in CML patients. Blood 111:2538–2547
25. Dib EHI, Gallet M, Mentaverri R, Sevenet N, Brazier M, Kamel S (2006) Imatinib mesylate (Gleevec®) enhances mature osteoclast apoptosis and suppresses osteoclast bone resorbing activity. Eur J Pharmacol 551:27–33
26. Vandyke K, Fitter S, Dewar AL, Hughes TP, Zannettino ACW (2010) Dysregulation of bone remodeling by imatinib mesylate. Blood 115:766–774
27. Brownlow N, Vaid M, Dibb NJ (2008) Tandutinib inhibits FMS receptor signalling, and macrophage and osteoclast formation in vitro. Leukemia 22:1452–1453
28. Logothetis CJ, Lin S-W Osteoblasts in prostate cancer metastasis to bone. Nat Rev Cancer
29. Pietras K, Sjoblom T, Rubin K et al (2003) PDGF receptors as cancer drug targets. Cancer Cell 3:439–443
30. Yu J, Ustach C, Kim HC (2003) Platelet-derived growth factor signaling and human cancer. J Biochem Mol Biol 36:49–59
31. Pietras K, Pahler J, Bergers G, Hanahan D (2008) Functions of paracrine PDGF signaling in the proangiogenic tumor stroma revealed by pharmacological targeting. PLoS Med 5:e19. doi:10.1371/journal.pmed.0050019