Predictors of Prostate Cancer Tissue Acquisition by an Undirected Core Bone Marrow Biopsy in Metastatic Castration-Resistant Prostate Cancer—A Cancer and Leukemia Group B Study

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Abstract **Purpose:** Analyzing metastatic prostate cancer tissue is of considerable importance in evaluating new targeted agents, yet acquiring such tissue presents a challenge due to the predominance of bone metastases. We assessed factors predicting a successful tumor harvest from bone marrow biopsies (BMBx) in castration-resistant metastatic prostate cancer patients.

Material and Methods: Data from Cancer and Leukemia Group B study 9663 were reviewed. Bone marrow biopsies were obtained from 184 patients who underwent an office-based, unguided bone marrow biopsy of the posterior iliac crest.

Results: Forty-seven of the 184 patients (25.5%) had a positive bone marrow biopsy. When considered in a multivariate logistic regression analysis, lower hemoglobin levels, higher alkaline phosphatase, and higher lactate dehydrogenase levels were associated with a higher likelihood of a positive BMBx. The median survival time was 11 months (95% confidence interval, 8.0-14) among patients with a positive BMBx compared with 23 months (95% confidence interval, 19-27) with a negative BMBx. The median time to progression and time to prostate-specific antigen progression-free survival were also significantly decreased among positive BMBx patients. No patients with a positive BMBx survived beyond 3 years, whereas 11 of the 137 patients with a negative BMBx survived beyond 5 years.

Discussion: Using common laboratory values, a specific patient cohort can be defined from whom the yield of a nonguided BMBx would be high enough to justify this approach. For studies that require broader entry criteria, a more directed approach with image guidance is recommended.

The current paradigm for the success of targeted therapy in oncology depends on the ability to analyze genetic alterations specific to tumor cells. This translational strategy has aided the development of therapies as diverse as tamoxifen and trastuzumab (Herceptin) in breast cancer and imantinib (Gleevec) in chronic myelogenous leukemia and gastrointestinal stromal tumor. Moreover, it was recently discovered that mutations in the epidermal growth factor receptor predict response to gefitinib (Iressa; refs. 1, 2) in non-small-cell lung cancer. In these diseases, tissue is either easy to acquire (chronic myelogenous leukemia and breast cancer) or material from the primary diagnostic procedure remains relevant and can be used for biological analysis (gastrointestinal stromal tumor and non-small-cell lung cancer).

Prostate cancer presents a challenge to the implementation of targeted therapy. On one hand, the disease is remarkably heterogeneous in genotype as well as phenotype, even between different metastases within the same patient, as shown by a recent rapid autopsy study by Shah et al. (3). Yet successful tissue acquisition is limited by the frequent absence of local disease (having often been eradicated in the distant past) and the predominance of bone metastasis, which are both difficult to sample and challenging to process.

The use of bone marrow biopsy in metastatic prostate cancer as a method of tissue acquisition was first described in 1936 by Rohr and Hegglin (4). There is a literature describing various techniques and success rates for this procedure in prostate cancer patients (5-10). All these published studies are small, from a single center, and do not describe predictors of success. In this report, we took advantage of the largest multi-institutional study to date that evaluated undirected bone marrow biopsies in metastatic prostate cancer to assess for factors that predicted a successful tumor harvest.

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Table 1. Baseline characteristics for patients with available data enrolled on CALGB study 9663 by bone marrow status

	Patients with negative bone marrow (n = 137)	Patients with positive bone marrow (n = 47)	Total (n = 184)	P*
Age (y)†	71 (64-77)	72 (65-78)	71 (64-77)	0.46
Years since diagnosis†	4 (2-6)	3 (1-6)	3 (2-6)	0.22
Gleason score of tumor				
2-4 (%)	9 (6)	1 (2)	10 (6)	
5-7 (%)	64 (47)	21 (45)	85 (46)	0.07
8-10 (%)	53 (39)	25 (53)	78 (42)	
Unknown	11 (8)	0 (0)	11 (6)	
Performance status				
0 (%)	131 (96)	39 (83)	170 (92)	
2 (%)	4 (3)	8 (17)	12 (7)	<0.01
Unknown	2 (1)	0 (0)	2 (1)	
Disease measurability				
Measurable	48 (35)	12 (26)	60 (33)	0.28
Metastases‡				
Any (%)	113 (82)	46 (98)	159 (86)	0.01
Bone (%)	107 (78)	46 (98)	153 (83)	<0.01
Lymph node (%)	42 (31)	10 (22)	52 (29)	0.45
Lung (%)	6 (4)	1 (2)	7 (4)	0.53
Liver (%)	7 (5)	0 (0)	7 (4)	0.01
Laboratory values†				
Hemoglobin, g/dL	13 (12-14)	11 (10-13)	13 (12-14)	<0.01
PSA, ng/mL	44 (15-118)	156 (42-509)	65 (20-165)	<0.01
Alkaline phosphatase, units/L	116 (83-176)	258 (149-538)	131 (93-259)	<0.01
Lactate dehydrogenase, units/L	200 (174-293)	254 (185-419)	209 (175-357)	0.03
Radiotherapy (%)	68 (50)	21 (45)	89 (49)	0.48
Prostatectomy (%)	33 (31)	12 (33)	45 (31)	0.84
Hormonal therapy (%)	133 (98)	45 (98)	178 (97)	0.44

*P values are calculated using Fisher's exact tests for the categorical variables and Wilcoxon test for the continuous variables.
†Median (interquartile range).
‡Patients may have had more than one metastatic site.

Patients and Methods

Patient population. Data from the Cancer and Leukemia Group B (CALGB) study 9663 were considered. This was a companion study of CALGB 9583, a phase III trial of antiandrogen withdrawal with immediate or sequential ketoconazole in men with androgen-independent prostate cancer, and required a bone marrow core biopsy at study entry (11). Metastatic disease with progression despite castrate levels of testosterone, prior antiandrogen therapy, and a minimum prostate-specific antigen (PSA) level of 5 ng/mL were required. Bone marrow biopsies were obtained from 164 patients enrolled on CALGB 9583 and from 20 patients enrolled on other CALBG chemotherapy trials (CALGB 9480, suramin; CALGB 9680, high dose mitoxantrone; CALGB 9780, docetaxel; refs. 12, 13). Patient registration and data collection were managed by the CALGB Statistical Center. Data quality was ensured by careful review of data by CALGB Statistical Center staff and by the study chairperson.

Tissue collection. Written Institutional Review Board–approved informed consent was obtained from all patients to do a bone marrow aspirate and biopsy from the posterior iliac crest. No image guidance was used. Investigators were provided an instructional video for sample procurement and shipment to a central laboratory. Marrow biopsies were immediately cut in half; the inner half was snap-frozen and used for androgen receptor analysis (for more details, see Taplin et al. 14) and the other half was placed in formalin and shipped for routine pathologic evaluation. A hematopathologist (B.A.W.) quantified marrow prostate cancer by light microscopy.

Statistical analysis. The primary end point was bone marrow biopsy tumor infiltration with two categories: yes (positive) or no (negative). The χ^2 test and Fisher's exact test (15) were used to compare differences between the two groups based on baseline clinical variables. In addition, the Wilcoxon test was used to compare the two groups on continuous laboratory variables. Logistic regression model was used to

Table 2. Multivariate logistic regression modeling the probability of positive bone marrow status

	Odds ratio (95% CI)	P
PSA, ng/mL	1.04 (0.99-1.10)	0.134
Hemoglobin, g/dL	0.64 (0.48-0.84)	0.002
Alkaline phosphatase, units/L*	1.35 (1.11-1.64)	0.002
Lactate dehydrogenase, units/L*	1.29 (1.06-1.56)	0.009

*Odds ratios are calculated when risk factors increase by 100 units.

Table 3. Median clinical outcomes by bone marrow biopsy

Bone marrow status	*n*	Overall survival (mo)		Progression-free survival (mo)		PSA progression-free survival (mo)	
		No. patients died	Median (95% CI)	No. patients progressed	Median (95% CI)	No. patients progressed	Median (95% CI)
Positive	47	47	11.2 (8.0-14.0)	47	2.15 (1.39-2.91)	47	3.90 (2.48-5.79)
Negative	137	120	22.9 (18.9-27.0)	127	2.41 (1.88-3.04)	127	4.89 (3.60-6.91)
*P**		<0.0001		0.02		0.01	

*Log-rank *P* value.

predict the probability of having a positive bone marrow biopsy (BMBx; ref. 16). We calculated odds ratios and 95% confidence intervals (95% CI) for independent variables in the model. The predictive accuracy of the model was assessed by the area under the receiver operating characteristic curve (17). Statistical analysis was done by CALGB statisticians.

Exploratory analyses were done to evaluate the importance of bone marrow status in predicting overall survival, progression-free survival, and PSA progression-free survival. Overall survival was measured from the date of randomization/study entry to date of death due to any cause. Progression-free survival was defined from the date of randomization/study entry to date of progression or death due to any cause, whichever occurred first. PSA progression-free survival was defined using the PSA consensus criteria (18) or death, whichever occurred first. Patients alive or lost to follow-up were considered as censored. The Kaplan-Meier product-limit method was used to estimate overall survival, progression-free survival, and PSA progression-free survival by the two groups (19) and the log-rank test was used to compare the two groups on these clinical outcomes (20).

Results

Patient characteristics. There were 194 patients enrolled on CALGB study 9663 between activation (January 1997) and closure (March 2001), of which 184 had a valid patient identification number that could be used to match the clinical database. Table 1 displays the baseline characteristics for these 184 patients by bone marrow biopsy result. Of the 184 patients, 47 (25.5%) had a positive bone marrow biopsy result, as defined by the presence of prostate cancer cells detected by light microscopy. No significant statistical differences were observed between two groups based on age distribution, years since diagnosis, Gleason grade, disease measurability, and prior therapies. Patients with a positive bone marrow biopsy had worse performance status compared with patients with negative bone marrow biopsy. Moreover, patients with positive bone marrow biopsy were more likely to have metastases, lower hemoglobin levels, and higher PSA, alkaline phosphatase, and lactate dehydrogenase levels.

Univariate association of risk factors for bone marrow involvement. In univariate analysis, patients with performance status of 2 were almost seven times more likely to have positive marrow biopsy (95% CI, 1.92-23.50) than patients with a performance status of 0. The presence of bone metastases, lower hemoglobin levels, and higher alkaline phosphatase, lactate dehydrogenase, and PSA levels were all associated with a probability of having a positive bone marrow biopsy (data not presented).

Multivariate analysis for bone marrow involvement. Table 2 displays a multivariate logistic regression analysis that identified several risk factors that predict the probability of having a positive bone marrow biopsy. The area under the receiver operating characteristic curve was 80%. Lower hemoglobin levels and higher alkaline phosphatase and lactate dehydrogenase levels were associated with a higher likelihood of a positive bone marrow biopsy.

Association of bone marrow status by clinical outcomes. Table 3 shows the estimates of median survival time, median time to progression, and time to PSA progression in 184 patients by bone marrow status. The median survival time is 11 months (95% CI, 8.0-14) among patients with a positive bone marrow biopsy compared with 23 months (95% CI, 19-27) among patients with a negative bone marrow biopsy (Fig. 1; $P < 0.0001$). No patients with a positive BMBx survived beyond 3 years whereas 11 of the 137 patients with a negative BMBx survived beyond 5 years. Furthermore, the median time to progression (2.41 versus 2.15 months; $P = 0.02$) and time to PSA progression (4.89 versus 3.90 months; $P = 0.01$) were significantly decreased among patients with a positive bone marrow biopsy as well (Figs. 2 and 3).

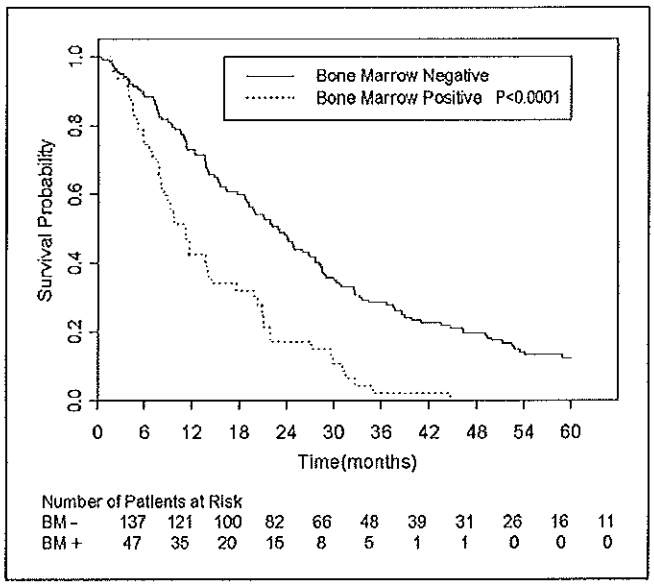

Fig. 1. Kaplan-Meier survival estimates by bone marrow biopsy result. Solid line, patients without detectable prostate cancer in their bone marrow biopsy at baseline; dotted line, patients with detectable prostate cancer in their bone marrow biopsy at baseline.

Discussion

As the importance of tissue analysis in cancer clinical trials and drug development grows, analysis of methods of tissue collection is needed. It is particularly relevant in prostate cancer where disease heterogeneity is profound and tissue analysis is limited by the interval between initial diagnosis and recurrence and the preponderance of bone-only disease.

Several factors affect biopsy yield in metastatic prostate cancer and should be considered. They include the use of image guidance (real time, non-real time, none), type of biopsy and number of attempts (fine needle aspiration, core biopsy), and eventual processing or analysis (immunohistochemistry, DNA analysis, RNA analysis, etc.). In prostate cancer, there is a paucity of data to guide implementation of these various options.

Historically, bone marrow biopsies were used in the diagnosis of metastatic prostate cancer to avoid overtreatment locally (21). In 1983, Varenhorst et al. (22) showed that technetium-99 hydroxymethylene diphosphonate bone scans were more sensitive than random bone marrow biopsies and the procedure fell out of favor for staging.

Some recent literature has readdressed this subject as the importance of tissue acquisition for research has emerged. Brown et al. (23) compared the yield of bone marrow biopsy with bone marrow aspiration in 20 patients with metastatic prostate cancer. Standard light microscopy with immunohistochemistry was their gold standard for tumor involvement. Of note, they used bone scans to guide their biopsy side and site although this guidance was not real-time. These investigators had a 75% success rate with bone marrow biopsy as compared with a 0% success rate with bone marrow aspiration. This superior yield from biopsy as opposed to aspiration is consistent with the literature (24) although bone marrow aspiration yield is typically higher than 0%.

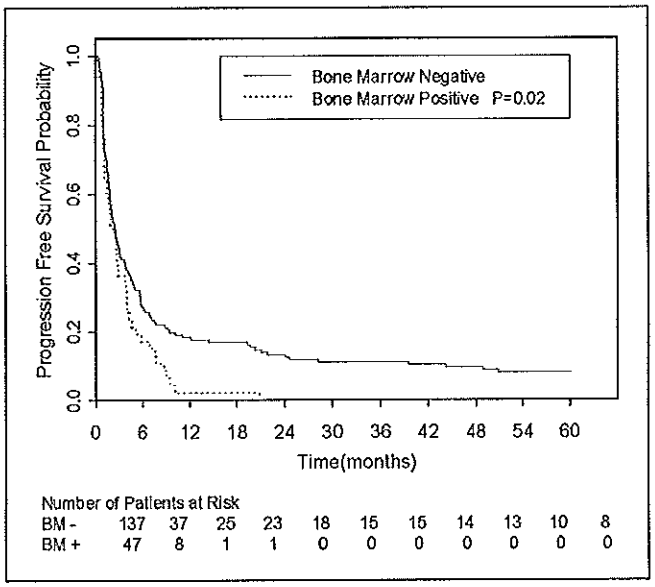

Fig. 2. Kaplan-Meier progression-free survival probability by bone marrow biopsy result. Solid line, patients without detectable prostate cancer in their bone marrow biopsy at baseline; dotted line, patients with detectable prostate cancer in their bone marrow biopsy at baseline.

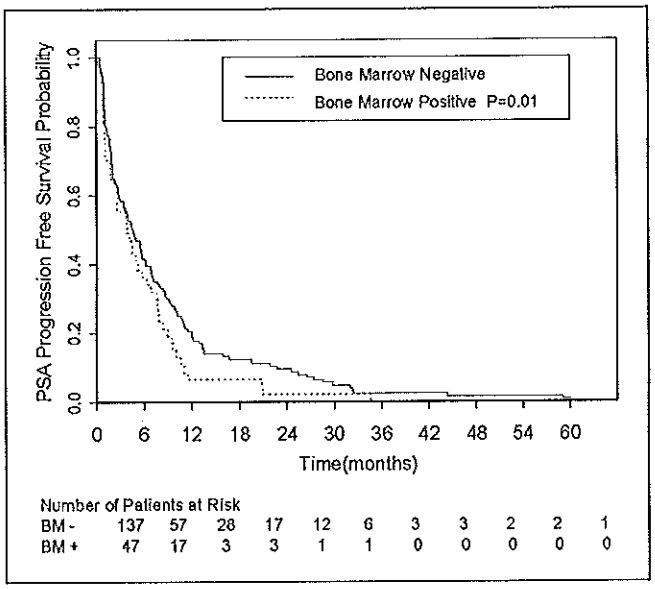

Fig. 3. Kaplan-Meier PSA progression-free survival probability by bone marrow biopsy result. Solid line, patients without detectable prostate cancer in their bone marrow biopsy at baseline; dotted line, patients with detectable prostate cancer in their bone marrow biopsy at baseline.

Several reports have considered the yield of undirected bone marrow biopsies in this setting, with yields ranging from 36% to 83%, although many of these studies predate the use of modern histopathologic techniques and all predate the PSA era and an improved understanding of prognostic factors (6, 9, 10). Moreover, the largest trial consisted of only 41 patients. Although this report contains many more patients, our bone marrow biopsy yield was far inferior (25.5%). This fact is explained by a few factors: no image guidance, multiple investigators and multiple institutions involved, and low-stage disease (minimum required PSA of 5 ng/mL). Despite these facts, a 25.5% yield may be too low for regular incorporation into phase I and II clinical trials.

Our analysis of factors that predict for a positive undirected bone marrow biopsy yield found that lower hemoglobin levels and higher lactate dehydrogenase and alkaline phosphatase levels were predictive of improved yield. Performance status and serum PSA were both predictive in the univariate analysis but fell out of the multivariate model. Although absolute PSA level was not a predictive factor in multivariate analysis, it should be noted that no patient with a PSA < 42 ng/mL had a positive core biopsy. In general, patients with more advanced disease were more likely to have a positive bone marrow biopsy. Thus, it is not surprising that they also had a statistically significantly shorter median survival time.

Our study was limited by several factors. First, we did not have information on whether there was evidence of bone involvement (by bone scan) of the area that underwent bone marrow biopsy. One would suppose from other data that a biopsy that was at least influenced by a site of predominant iliac involvement would influence yield. Future trials should specify and collect this information prospectively.

Second, the role of prior radiation therapy on the yield of undirected bone biopsies may be underestimated in this analysis. Whereas in the initial report of these data pelvic

irradiation was associated with a negative biopsy, when more data were available this association became nonsignificant. It remains possible that larger doses of whole pelvic radiation may influence the yield of this procedure.

Undirected bone marrow biopsy for the collection of metastatic prostate cancer tissue has the advantage of being a simple office procedure that is well tolerated with minimal morbidity. Moreover, as indicated by our data, this procedure has the advantage of applicability as multiple centers and investigators were involved in this study. This report is the largest to date describing the yield of this procedure and should be validated in a prospective clinical trial.

From this experience and our multivariate analysis, we suggest that using common laboratory values, a specific patient cohort can be defined from which the yield of this procedure may be high enough to justify incorporation into a protocol. For studies for which a biopsy is one part of a larger effort and therefore the trial cannot be designed around such characteristics, a more directed approach with image guidance (either real-time or before the procedure) is recommended.

Appendix A

The following institutions participated in this study: CALGB Statistical Office, Durham, North Carolina (Stephen George, Ph.D., supported by grant CA33601); Dartmouth Medical School-Norris Cotton, Lebanon, New Hampshire (Marc Ernstoff, M.D., supported by grant CA04326); Georgetown University Medical Center, Washington, District of Columbia (Edward P. Gelman, M.D., supported by grant CA77597); Mount Sinai School of Medicine, New York, New York (Lewis Silverman, M.D., supported by grant CA04457); Rhode Island Hospital, Providence, Rhode Island (William Sikov, M.D., supported by grant CA08025); SUNY Upstate Medical University, Syracuse, New York (Stephen L. Graziano, M.D., supported by grant CA21060); The Ohio State University, Columbus, Ohio (Clara D. Bloomfield, M.D., supported by grant CA77658); University of California at San Diego, San Diego, California (Stephen Seagren, M.D., supported by grant CA11789); University of California at San Francisco, San Francisco, California (Alan Venook, M.D., supported by grant CA60138); University of Chicago Medical Center, Chicago, Illinois (Gini Fleming, M.D., supported by grant CA41287); University of Illinois at Chicago, Chicago, Illinois (David Gustin, M.D., supported by grant CA74811); University of Maryland Cancer Center, Baltimore, Maryland (David Van Echo, M.D., supported by grant CA31983); University of Massachusetts Medical Center, Worcester, Massachusetts (Mary Ellen Taplin, M.D., supported by grant CA37135); University of Minnesota, Minneapolis, Minnesota (Bruce A. Peterson, M.D., supported by grant CA16450); University of Missouri/Ellis Fischel Cancer Center, Columbia, Missouri (Michael C Perry, M.D., supported by grant CA12046); University of Nebraska Medical Center, Omaha, Nebraska (Anne Kessinger, M.D., supported by grant CA77298); University of Tennessee Memphis, Memphis, Tennessee (Harvey B. Niell, M.D., supported by grant CA47555); Vermont Cancer Center, Burlington, Vermont (Hyman B. Muss, M.D., supported by grant CA77406); Wake Forest University School of Medicine, Winston-Salem, North Carolina (David D. Hurd, M.D., supported by grant CA03927); Washington University School of Medicine, St. Louis, Missouri (Nancy Bartlett, M.D. supported by grant CA77440).

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References

1. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350:2129–39.
2. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:1497–500.
3. Shah RB, Mehra R, Chinnaiyan AM, et al. Androgen-independent prostate cancer is a heterogeneous group of diseases: lessons from a rapid autopsy program. Cancer Res 2004;64:9209–16.
4. Rohr K, Hegglin R. Tumorzellen im sternalpunkat. Deutsch Arch Klin Med 1936;179:61–79.
5. Chua DT, Ackermann W, Veenema RJ. Bone marrow biopsy in patients with carcinoma of the prostate. J Urol 1969;102:602–6.
6. Crisp J. Random bone marrow biopsy in the staging of carcinoma of the prostate. Br J Urol 1976;48:265–7.
7. Mehan DJ, Broun GO, Jr., Hoover B, Storey G. Bone marrow findings in carcinoma of the prostate. J Urol 1966;95:241–4.
8. Nelson CM, Boatman DL, Flocks RH. Bone marrow examination in carcinoma of the prostate. J Urol 1973;109:667–70.
9. Spiers AS, Deal DR, Kasimis BS, Miller BR. Evaluation of the bones and bone marrow in patients with metastatic carcinoma of the prostate: radiologic, cytologic and cytogenetic findings. J Med 1982;13:303–7.
10. Sy FA, Gursel EO, Veenema RJ. Positive random iliac bone biopsy in advanced prostatic cancer. Urology 1973;2:125–7.
11. Small EJ, Halabi S, Dawson NA, et al. Antiandrogen withdrawal alone or in combination with ketoconazole in androgen-independent prostate cancer patients: a phase III trial (CALGB 9583). J Clin Oncol 2004;22:1025–33.
12. Levine EG, Halabi S, Roberts JD, et al. Higher doses of mitoxantrone among men with hormone-refractory prostate carcinoma: a Cancer and Leukemia Group B study. Cancer 2002;94:665–72.
13. Savarese DM, Halabi S, Hars V, et al. Phase II study of docetaxel, estramustine, and low-dose hydrocortisone in men with hormone-refractory prostate cancer: a final report of CALGB 9780. Cancer and Leukemia Group B. J Clin Oncol 2001;19:2509–16.
14. Taplin ME, Rajeshkumar B, Halabi S, et al. Androgen receptor mutations in androgen-independent prostate cancer: Cancer and Leukemia Group B Study 9663. J Clin Oncol 2003;21:2673–8.
15. Agresti A. A survey of exact inference for contingency tables. Stat Sci 1992;7:131–53.
16. Agresti A. Categorical data analysis. 1st ed. New York: Wiley; 1990.
17. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988;44:837–45.
18. Bubley GJ, Carducci M, Dahut W, et al. Eligibility and response guidelines for phase II clinical trials in androgen-independent prostate cancer: recommendations from the Prostate-Specific Antigen Working Group. J Clin Oncol 1999;17:3461–7.
19. Kaplan E, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457–81.
20. Peto R, Pike MC, Armitage P, et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. Analysis and examples. Br J Cancer 1977;35:1–39.
21. Welch J, Mackinnery C. Experience with aspiration biopsies of the bone marrow in the diagnosis and prognosis of carcinoma of the prostate gland. Am J Clin Pathol 1964;41:509–12.
22. Varenhorst E, Alund G, Lindstrom E, Manson JC. Bone marrow aspiration biopsy and bone scanning in the staging of prostatic cancer. Br J Urol 1983;55:534–7.
23. Brown RS, Dogan A, Ell PJ, Payne HA, Masters JR, Harland SJ. The comparative values of bone marrow aspirate and trephine for obtaining bone scan-targeted metastases from hormone-refractory prostate cancer. Prostate Cancer Prostatic Dis 2002;5:144–51.
24. Bearden JD, Ratkin GA, Coltman CA. Comparison of the diagnostic value of bone marrow biopsy and bone marrow aspiration in neoplastic disease. J Clin Pathol 1974;27:738–40.