Title

Bone turnover biomarkers identify unique prognostic risk groups in men with castration resistant prostate cancer and skeletal metastases: Results from SWOG S0421



Authors

Lara, PN

Plets, M

Tangen, C

et al.

Publication Date

2018

DOI

10.1016/j.ctarc.2018.04.005

Peer reviewed

HHS Public Access

Author manuscript

*Cancer Treat Res Commun*. Author manuscript; available in PMC 2019 July 15.

Published in final edited form as:

*Cancer Treat Res Commun*. 2018 ; 16: 18–23. doi:10.1016/j.ctarc.2018.04.005.

# Bone turnover biomarkers identify unique prognostic risk groups in men with castration resistant prostate cancer and skeletal metastases: results from SWOG S0421

**PN Lara Jr.**, **M Plets**, **C Tangen**, **E Gertz**, **NJ Vogelzang**, **M Hussain**, **PW Twardowski**, **MG Garzotto**, **JP Monk**, **M Carducci**, **A Goldkorn**, **PC Mack**, **I Thompson**, **M Van Loan**, and **DI Quinn**

University of California Davis Comprehensive Cancer Center, Sacramento, CA (PNL, PCM); SWOG Statistical Center, Seattle, WA (MP, CT); US Department of Agriculture, Western Human Nutrition Research Center, University of California Davis, Davis, CA (EG, MVL); US Oncology, Las Vegas, NV (NJV); Northwestern University, Chicago, IL (MH); City of Hope National Medical Center, Duarte, CA (PWT); Oregon Health & Science University, Portland, OR (MGG); Ohio State University, Columbus, OH (JPM); Johns Hopkins University, Baltimore, MD (MC); USC Norris Comprehensive Cancer Center, Los Angeles, CA (AG,DIQ); Cancer Therapy and Research Center, UTHSC, San Antonio, TX (IT)

## Abstract

**Background.—**Skeletal metastases often occur in men with castration-resistant prostate cancer (CRPC) where bone biomarkers are prognostic for overall survival (OS). In those with highly elevated markers, there is preferential benefit from bone-targeted therapy. In the phase IIIS0421 docetaxel+/− atrasentan trial, clinical covariates and bone biomarkers were analyzed to identify CRPC subsets with differential outcomes.

**Subjects and Methods.—**Markers of bone resorption [N-telopeptide-NTx; pyridinoline-PYD] and formation [C-terminal collagen propeptide-CICP; bone alkaline phosphatase-BAP] were measured in pre-treatment sera. Bone biomarkers and clinical covariates were included in a Cox model for OS; bone markers were added in a stepwise selection process. Receiver operating characteristic(ROC) curves were constructed for risk factor models +/− bone markers. Significant variables were allowed to compete in a classification and regression tree (CART) analysis. Hazard ratios(HR) were calculated by comparing OS in each of the terminal nodes to a reference group in a Cox model.

**Results.—**750 patients were included. Each bone marker significantly contributed to the risk factor-adjusted OS Cox model, with higher levels associated with worse OS. BAP (HR=1.15,p=0.008), CICP (HR=1.27,p<0.001), and PYD (HR=1.21,p=0.047) in combination

Address for editorial correspondence: Primo N. Lara, Jr., M.D., Professor of Medicine, University of California Davis Comprehensive Cancer Center, 4501 X Street, Sacramento, CA 95817, Tel: 916-734-3771; Fax: 916 734 7946, pnlara@ucdavis.edu. Address for other inquiries: Southwest Oncology Group Chair's Office, Group Chair's Office, OHSU Knight Cancer Institute, Marquam II, 2611 SW 3rd Avenue MQ280, Portland, OR 97201, Tel. 503.505.4198, arlauskp@ohsu.edu.

Clinical Trials Registry: ClinicalTrials.gov: NCT00134056

Presented in part at the 2016 European Society of Medical Oncology Congress in Copenhagen, Denmark, October 7–11, 2016

**Conflict of Interest Statement:** The authors declare no potential conflicts of interest.

were significantly associated with OS. Prognostic accuracy was improved by addition of bone markers to clinical covariates. CART analysis selected CICP, BAP, hemoglobin, and pain score for the final OS model, identifying five prognostic groups.

**Conclusions.—**Elevated serum bone biomarker levels are associated with worse OS in bone-metastatic CRPC. Bone biomarkers can identify unique prognostic subgroups. These results further define the role of bone biomarkers in the design of CRPC trials.

### Keywords

Prostate Cancer; Bone Turnover; Bone Metabolism; Biomarker; Prognostic Marker; Bone Metastases

## Background

Metastatic castration resistant prostate cancer (CRPC) is the terminal state of the prostate cancer disease trajectory.[1,2] Of the 26,700 American men who are estimated to succumb to prostate cancer in 2017[3], the majority is attributable to CRPC. In men with CRPC, skeletal metastasis is often observed and is a frequent source of morbidity such as bone pain and fracture.[4] In these patients, the homeostatic balance between bone formation and resorption is frequently disrupted, with predominance of osteoblastic activity manifesting as sclerotic bony disease. Furthermore, the concurrent use of androgen deprivation therapy enhances bone turnover in these patients, resulting in osteopenia and osteoporosis.

Markers of bone turnover can be clinically assessed using circulating biomarkers in serum.[5] Our group previously evaluated these biomarkers in the context of a large placebo-controlled prospective phase III trial of the bone-targeted agent endothelin-A antagonist atrasentan in combination with docetaxel.[6] We reported that elevated levels of these blood-based bone biomarkers have significant independent prognostic value for overall survival in men with CRPC.[7] Importantly, we identified a subset of CRPC patients with highly elevated markers who preferentially benefit from bone-targeted therapy, providing a potential pathway for a precision medicine approach in this disease.[7]

In order to further refine the potential role of bone biomarkers in the clinical evaluation of men with CRPC and in the design of trials testing bone targeted therapies in these patients, we assessed the individual and collective contributions of each of the bone resorption and formation markers to OS in the context of baseline covariates. Furthermore, we sought to identify unique subsets of patients defined by their baseline bone marker levels and clinical features using classification and regression tree methods.

## Methods

S0421 was a two-arm, randomized phase III trial, open label for docetaxel and double-blind placebo-controlled for atrasentan (ClinicalTrials.gov NCT00134056). Patients were assigned to receive docetaxel 75mg/m$^2$ intravenously over 1 hour every 21 days with prednisone 10 mg orally daily with or without atrasentan 10 mg daily orally. The trial was placebo-controlled for atrasentan. As previously reported, atrasentan failed to improve progression-

free survival or OS in the overall population.[6] Patients registered to S0421 were consented to provide serial serum specimens for the bone marker studies. All patients in this trial had metastatic CRPC with imaging evidence of bone metastasis. Bisphosphonate therapy was permitted but must have commenced before registration; initiation of bisphosphonates was not permitted within the first four cycles of study therapy. The study protocol was approved by the institutional review board or the National Cancer Institute Central Institutional Review Board or both. Blood (serum) samples were collected using standard venipuncture techniques. Fifteen milliliters of whole blood were drawn pretreatment (after registration but before receiving the first dose of protocol therapy) and on the day of docetaxel infusion in weeks 4, 7, and 9. Whole blood was collected in red-top vacutainer tubes and allowed to clot for approximately 30 minutes. Serum was separated from cells within 45 to 60 minutes of venipuncture by centrifugation at 3000× for 10 minutes. Serum was equally aliquoted into four cryotubes and shipped to the SWOG biobank at Nationwide Children's Hospital in Columbus, OH. Specimens were subsequently shipped to the USDA lab at UC Davis for bone marker analysis.

Markers for bone resorption (*N-telopeptide [NTx] and pyridinoline [PYD]*) and bone formation (*C-terminal collagen propeptide* [CICP] and *bone alkaline phosphatase* [BAP]) were measured in pre-treatment sera collected from men enrolled in the SWOG S0421 trial, as previously described.[7] Briefly, CICP was measured by a sandwich enzyme-linked immunosorbent assay (Quidel Corp, San Diego, CA) using a microtiter plate coated with monoclonal anti-C1CP antibody. Bone-specific alkaline phosphatase (BAP) activity in serum was measured using the Microvue BAP enzyme-linked immunosorbent assay (Quidel Corp) with a monoclonal anti-BAP antibody coated on a microtiter plate to capture BAP in the sample. N-telopeptide (NTx) was measured by a competitive enzyme-linked immunosorbent assay (Wampoles Laboratories, Princeton, NJ) using a 96-well microplate. Pyridinoline (PYD) was measured in serum using a competitive enzyme immunoassay in a microtiter plate format (Quidel Corp).

Bone markers were first added individually to a multivariate Cox regression model for OS that contained traditional risk factors of CRPC with a low fraction of missing data. Then, holding all risk factors in the Cox model constant, each bone marker was evaluated univariately and in a multivariate fashion. (A criteria to stay of $p< 0.05$ was used to select which combination of the four bone markers best contributes to the model. Receiver operating characteristic (ROC) curves were estimated for the traditional risk factor model predicting survival at 24 months, and also with the addition of the multivariate bone markers. The AUC was compared between the two models. Classification of patients by their survival status at 24 months and their predicted probability of death (low, medium/low, medium/high, or high) from the logistic regression model for the risk factor model and the multivariate bone marker model were compared to assess re-classification of risk based on the addition of bone marker data.

All four bone markers (BAP, CICP, NTx, PYD) and all risk factors used in the OS Cox model were allowed to compete as input variables in a regression tree analysis, with overall survival as the outcome. The binary partitioning of the bone marker levels in the regression tree was performed by the C-TREE function in the R package *party*. This function uses

recursive binary partitioning to choose the cut point of quantitative variables, such as bone marker measures and HgB levels. The optimal binary split is identified as the observed bone marker measure where the logrank test statistic is maximized. A final classification and regression tree (CART) was constructed. C-TREE was used to construct the regression tree due to its permutation based significance testing which avoids selection bias towards input variables with many possible cut-points. Since this method utilizes permutation tests, and splitting is based on statistical stopping rules, pruning was not conducted. Hazard ratios and corresponding 95% CIs were calculated by comparing overall survival of patients in each of the nodes 2–5 relative to node 1 (reference group) in a Cox proportional hazards model. Kaplan-Meier curves for each terminal node were constructed.

## Results

S0421 registered 1038 eligible patients with CRPC. Of these, 855 submitted serum for the bone biomarker studies. Of 855 men, 778 had usable specimens at baseline. In total, 750 patients with evaluable bone marker and clinical data were included. Patient characteristics for this cohort are summarized in Table 1.The following risk factors were considered of interest, were included in the multivariate OS Cox models and were candidates in regression tree analyses: age (in years), performance status (0–1 vs 2), hemoglobin (Hgb, g/dL), type of progression determining unresponsiveness to hormone therapy at baseline (measurable disease /non-measurable disease vs rising PSA), worst pain score at study entry as measured by the Brief Pain Inventory (<4 vs ≥4), race (black vs other), PSA at study entry (ng/mL), visceral disease present (yes vs no) and treatment arm (placebo vs atrasentan). Although treatment arm was not significant in the model (p=0.90), we chose to keep it for completeness.

Each bone marker significantly contributed to the risk factor Cox model univariately, with higher levels associated with worse OS. A selection model adjusted for clinical risk factors showed that the best combination of bone markers was with BAP (Hazard Ratio [HR]=1.15; 95% CI (1.04,1.27); p=0.008), CICP (HR=1.27; 95% CI (1.11, 1.45); p<0.001), and PYD (HR=1.21; 95% CI (1.00, 1.47); p=0.047).

In ROC analysis, the AUC of clinical risk factors for predicting 24 month OS was found to be 0.73; this modestly improved with the addition of CICP (AUC=0.76) or BAP (AUC 0.75) or combination BAP/CICP/PYD (AUC=0.76). These results are shown in Table 2 and the multivariate bone marker model in Figure 1. In Table 3, patients were cross-classified by their survival status at 24 months and the predicted probabililty (4 categories) of their death at that time point both for the risk factor model and the model that also included bone markers. . The addition of bone markers to the model correctly shifted some of the men who had died into a high probability category and conversely, some of those who were alive were shifted to a lower probability category. Approximately 9% more men who had died by 24 months were re-assigned to the highest risk category and approximately 10 % more men who were alive at 24 months were re-assigned to the lowest risk category when the 3 bone marker combo (BAP + CICP + PYD) was added to the predictive model.

CART analysis selected CICP, BAP, Hgb, and pain score for the final model (Figure 2), and identified five prognostic groups with differential OS outcomes (Table 4 and Figure 3). Kaplan-Meier curves for each terminal node were constructed to demonstrate the OS differences across each identified subset. Patients in Node 1 had a Hgb <= 11.3 g/dL. Patients in Nodes 2–5 all had Hgb levels above 11.3 g/dL. Patients in Nodes 3–5 all had CICP levels > 6.8 ng/mL (only patients in Node 2 had CICP <= 6.8 ng/mL). Patients in Nodes 4–5 had a pain score of <4 while patients in node 3 had a pain score >= 4. Finally, patients in Node 4 had BAP levels <= 90.9 u/L while those in Node 5 had BAP > 90.9 u/L. Node 1 had the worst outcome (median OS of 12.4 months), while Node 2 had the best median OS of 31.6 months. Patients in Node 2 and Node 4 (median OS of 27.1 months) had significantly longer OS than patients in Node 1 (both comparisons p<0.001).

The effect of treatment within each node group was evaluated with an interaction term. The addition of atrasentan to docetaxel did not alter OS in any of the nodes (4 degreee of freedom residual Chi-square, p=0.53), a finding in keeping with the negative primary outcome for S0421.

## Discussion

Precision cancer care, where patients with molecularly defined subsets of tumors are treated with a specific targeted agent that is predicted to yield a high tumor response rate, is now an established paradigm in many solid tumors such as non-small cell lung cancer, colorectal cancer, and malignant melanoma, among others. For example, in patients with metastatic non-small cell lung cancer of the adenocarcinoma subtype, it is standard-of-care to assess the status of selected oncogenic drivers to define the optimal systemic therapy approach.[8] However, such an approach remains investigational for the vast majority of patients with CRPC.[9] One exception is the ongoing active investigation into the predictive value of DNA damage repair gene alterations for treatment with PARP inhibitors or platinum-based chemotherapy[1011]; however, as of this writing, there is still no regulatory approval for any systemic therapy for prostate cancer that is defined by unique molecular subsets. Existing biomarkers in the CRPC context such as prostate specific antigen (PSA) levels or circulating tumor cells (CTCs) are currently employed to refine prognostication but not to assign specific treatments.

Most men with metastatic CRPC will have skeletal involvement at some point in their disease trajectory. Thus, there has been high interest in the drug development community to pursue therapies directed towards this patient population. As a result, many systemic agents directed towards bony metastatic disease have become commercially available over the past decade. These include drugs that reduce skeletal-related events such as bone pain or fracture (e.g., zoledronic acid, denosumab) as well as radio-isotopes (e.g., Radium 223).[12] Many other bone-targeted therapies have failed to yield sufficient activity in randomized phase III trials, including endothelin antagonists (e.g., atrasentan, zibotentan) and SRC-inhibitors (e.g., dasatinib), to warrant regulatory approval.[61213]

There are still no practical means in the clinic to identify biomarker-defined subsets of patients that can guide bone-targeted therapy selection. Interestingly, skeletal metastatic

disease can be clinically assessed and monitored using blood- or urine-based bone turnover biomarkers. This context offers an opportunity to define patient subsets responsive to bone-targeted therapies. For example, patients with bone metastases treated with denosumab were shown to have rapid and sustained reduction in bone turnover biomarkers including urinary N-telopeptide regardless of prior bisphosphonate therapy.[14] Similar effects were seen in a subset of men with bony metastatic prostate cancer.[15] Even in patients with non-metastatic prostate cancer, denosumab was shown to induce rapid inhibition of bone turnover biomarkers.[16] The radioisotope Radium-223 has also been shown to significantly reduce bone alkaline phosphatase levels in men with bone-metastatic CRPC. However, marker decline did not appear to be a surrogate for survival.[17] In contrast, our data from S0421 CRPC subjects treated with docetaxel/prednisone +/− atrasentan not only reinforced the strong prognostic value of baseline circulating markers of bone metabolism, but showed that in an enriched subset of men with the highest bone marker levels, atrasentan can improve survival.[7] In addition, our updated CART analysis incorporating bone biomarkers with baseline clinical covariates (CICP, BAP, Hgb, and pain score) identified five prognostic subgroups of CRPC patients with differential survival outcomes. It must be noted that although the primary analysis on the prognostic role of each of the four bone biomarkers was prospectively pre-specified at the time of S0421 initiation, the subsequent ROC and CART analyses reported here were performed post-hoc and should be considered a limitation of the study.

We believe that these data can be employed by clinical investigators in the design and conduct of future CRPC trials either as an enrichment strategy for bone-targeted therapies or as a stratification factor at randomization. Presently, our group is evaluating the prognostic and predictive role of these bone biomarkers in the context of metastatic hormone sensitive prostate cancer as part of SWOG S1216, a phase III trial of androgen deprivation therapy with or without the CYP17 inhibitor orteronel.[18]

## Acknowledgments

Research reported in this publication was supported by the National Cancer Institute of the National Institutes of Health under Award Numbers R01CA120469, U10CA180888, U10CA180819, U10CA180846 and U10CA180830; and in part by Abbott Laboratories and Sanofi-Aventis, LLC. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or Abbott Laboratories and Sanofi-Aventis, LLC.

## REFERENCES

1. Cornford P, Bellmunt J, Bolla M, et al. EAU-ESTRO-SIOG Guidelines on Prostate Cancer. Part II: Treatment of Relapsing, Metastatic, and Castration-Resistant Prostate Cancer. European urology 2017;71:630–42. [PubMed: 27591931]
2. Goldkorn A, Ely B, Tangen CM, et al. Circulating tumor cell telomerase activity as a prognostic marker for overall survival in SWOG 0421: a phase III metastatic castration resistant prostate cancer trial. International journal of cancer 2015;136:1856–62. [PubMed: 25219358]
3. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA: a cancer journal for clinicians 2017;67:7–30. [PubMed: 28055103]
4. Fizazi K, Carducci M, Smith M, et al. Denosumab versus zoledronic acid for treatment of bone metastases in men with castration-resistant prostate cancer: a randomised, double-blind study. Lancet 2011;377:813–22. [PubMed: 21353695]

5. Lara PN Jr., Stadler WM, Longmate J, et al. A randomized phase II trial of the matrix metalloproteinase inhibitor BMS-275291 in hormone-refractory prostate cancer patients with bone metastases. Clinical cancer research : an official journal of the American Association for Cancer Research 2006;12:1556–63. [PubMed: 16533781]
6. Quinn DI, Tangen CM, Hussain M, et al. Docetaxel and atrasentan versus docetaxel and placebo for men with advanced castration-resistant prostate cancer (SWOG S0421): a randomised phase 3 trial. The Lancet Oncology 2013;14:893–900. [PubMed: 23871417]
7. Lara PN Jr., Ely B, Quinn DI, et al. Serum biomarkers of bone metabolism in castration-resistant prostate cancer patients with skeletal metastases: results from SWOG 0421. Journal of the National Cancer Institute 2014;106:dju013. [PubMed: 24565955]
8. Gandara DR, Riess JW, Kelly K, Li T, Mack PC, Lara PN, Jr. Evolution and Increasing Complexity of the Therapeutic Landscape in Advanced Non-Small-cell Lung Cancer. Clinical lung cancer 2017;18:1–4. [PubMed: 28082049]
9. Mullane SA, Van Allen EM. Precision medicine for advanced prostate cancer. Current opinion in urology 2016;26:231–9. [PubMed: 26909474]
10. Mateo J, Boysen G, Barbieri CE, et al. DNA Repair in Prostate Cancer: Biology and Clinical Implications. European urology 2017;71:417–25. [PubMed: 27590317]
11. Goodall J, Mateo J, Yuan W, et al. Circulating Free DNA to Guide Prostate Cancer Treatment with PARP Inhibition. Cancer discovery 2017.
12. Suzman DL, Boikos SA, Carducci MA. Bone-targeting agents in prostate cancer. Cancer metastasis reviews 2014;33:619–28. [PubMed: 24398856]
13. Lara PN, Jr., Evans CP. Dasatinib and docetaxel in advanced prostate cancer. The Lancet Oncology 2013;14:1248–9. [PubMed: 24211164]
14. Body JJ, Lipton A, Gralow J, et al. Effects of denosumab in patients with bone metastases with and without previous bisphosphonate exposure. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 2010;25:440–6.
15. Fizazi K, Bosserman L, Gao G, Skacel T, Markus R. Denosumab treatment of prostate cancer with bone metastases and increased urine N-telopeptide levels after therapy with intravenous bisphosphonates: results of a randomized phase II trial. The Journal of urology 2009;182:509–15; discussion 15–6. [PubMed: 19524963]
16. Smith MR, Saad F, Egerdie B, et al. Denosumab and changes in bone turnover markers during androgen deprivation therapy for prostate cancer. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 2011;26:2827–33.
17. Sartor O, Coleman RE, Nilsson S, et al. An exploratory analysis of alkaline phosphatase, lactate dehydrogenase, and prostate-specific antigen dynamics in the phase 3 ALSYMPCA trial with radium-223. Annals of oncology : official journal of the European Society for Medical Oncology 2017;28:1090–7. [PubMed: 28453701]
18. Van Hook K, Huang T, Alumkal JJ. Orteronel for the treatment of prostate cancer. Future oncology 2014;10:803–11. [PubMed: 24799061]

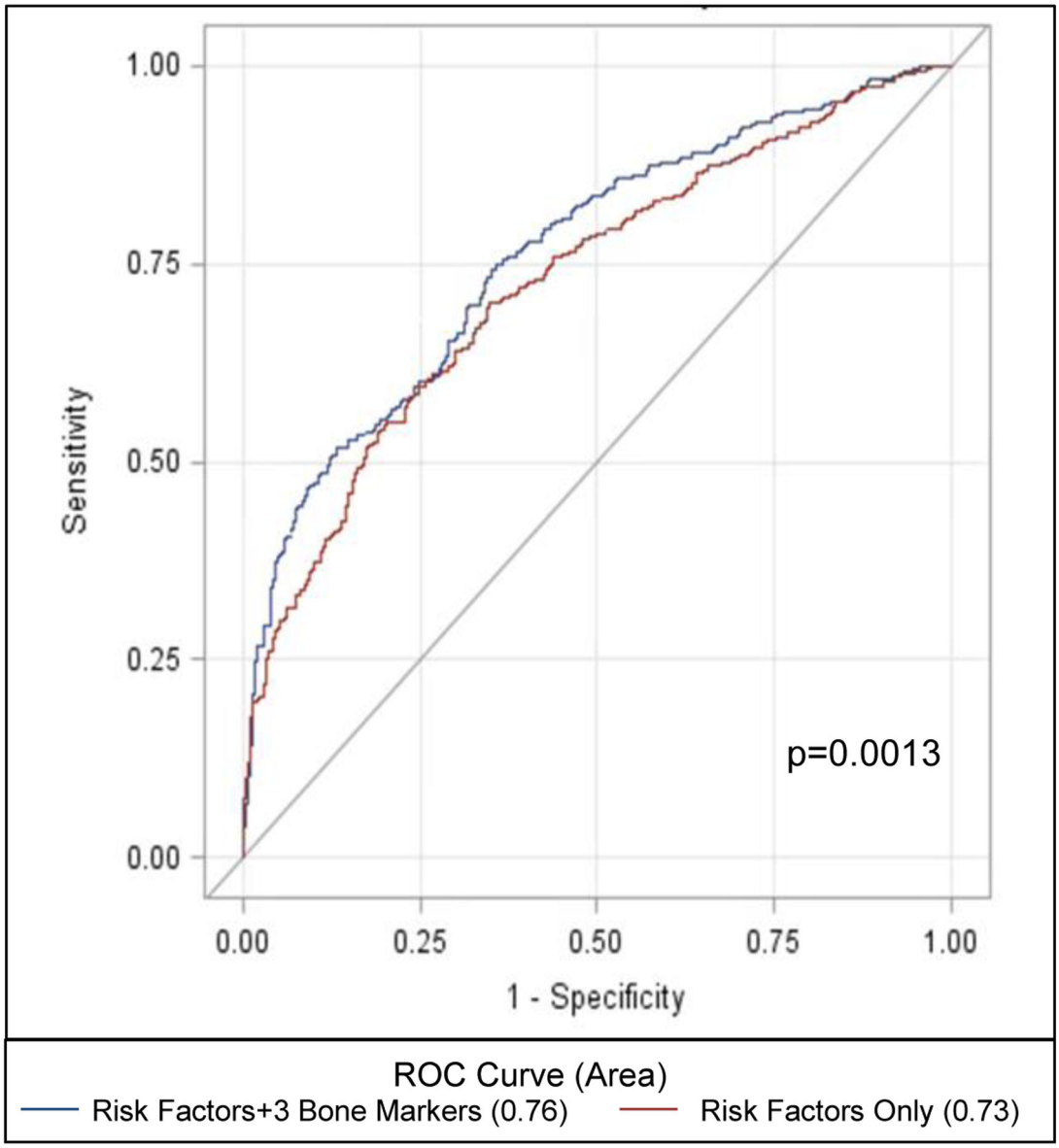


**Figure 1:**
Receiver Operating Characteristic (ROC) Curves for clinical covariates with (red curve) and without (blue curve) the three significant bone markers predicting 2-year survival.

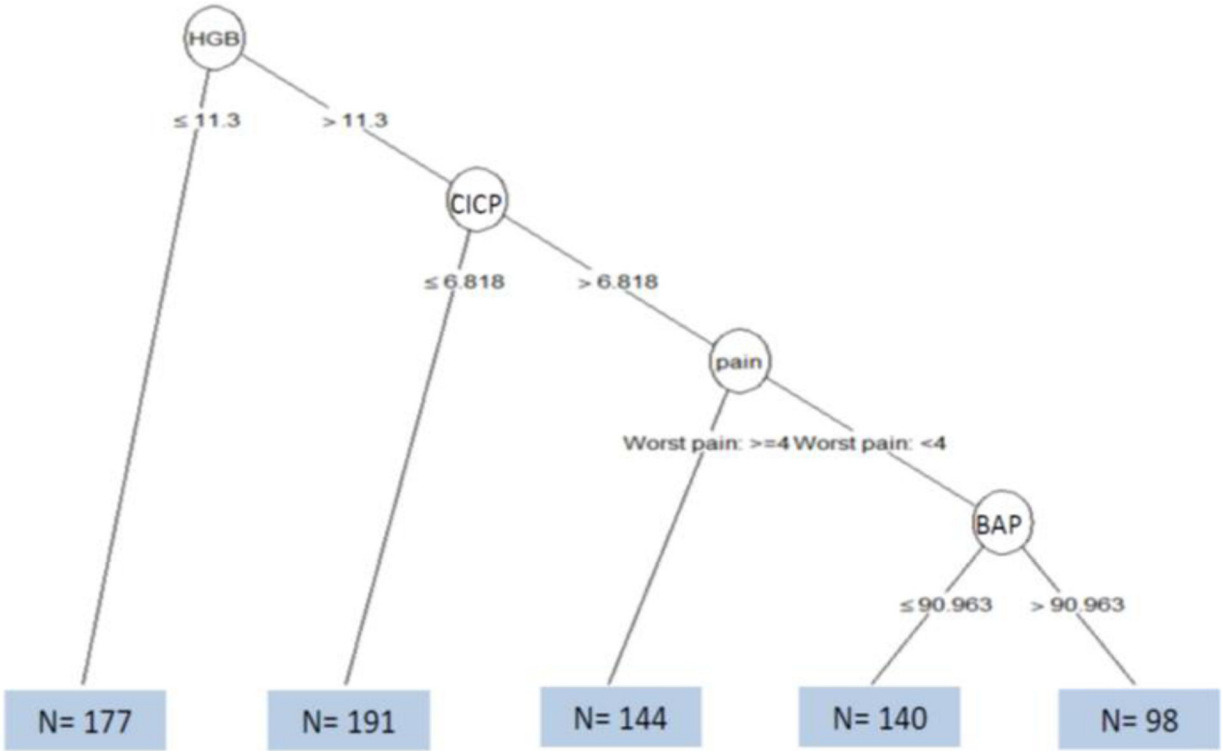


**Figure 2:**
Final Classification and Regression Tree (CART).

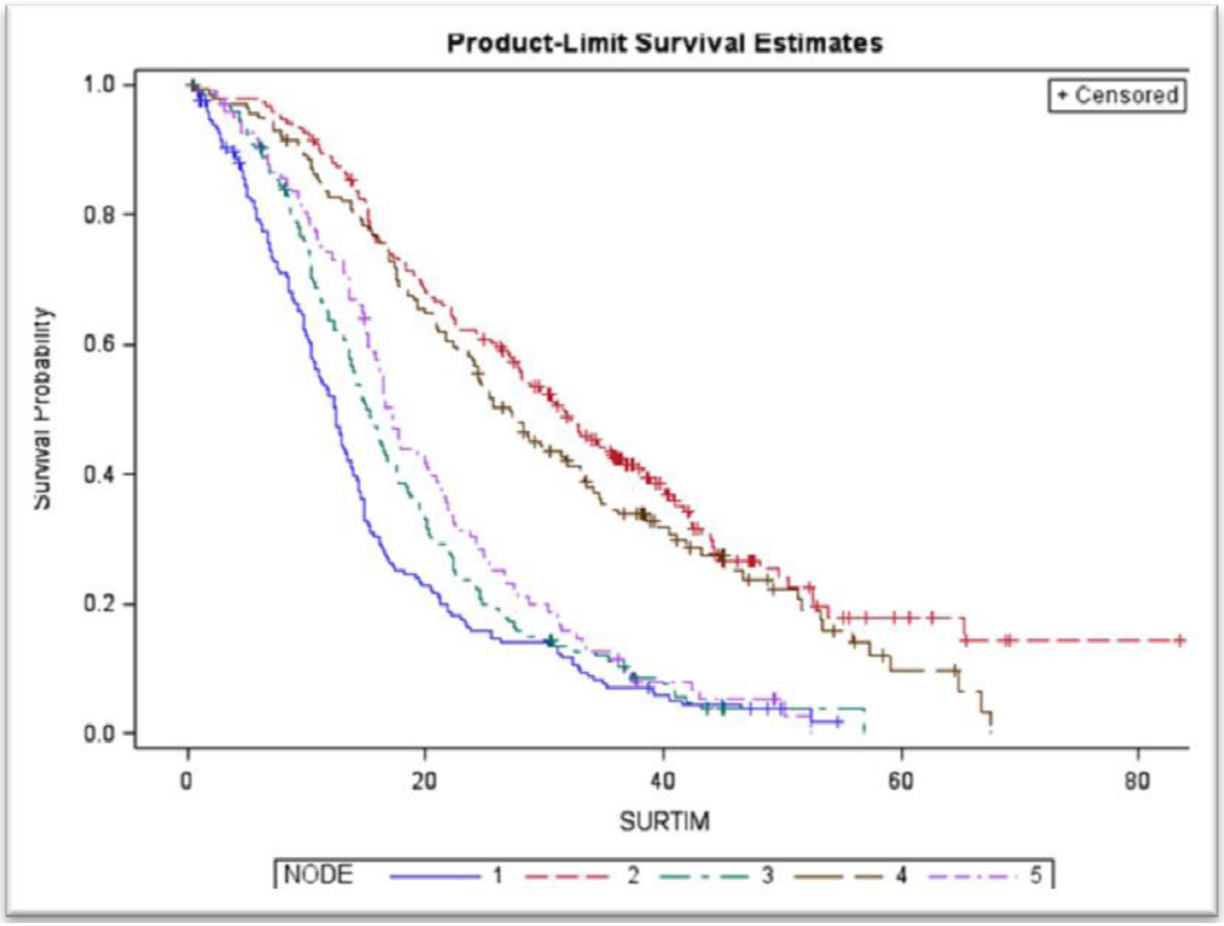


**Figure 3: Kaplan Meier survival curves for each terminal node of the classification and regression tree.**

Kaplan-Meier curves for each terminal node were constructed to demonstrate the survival differences across each identified subset. Node 1 had the worst outcome (median OS of 12.4 months), while Node 2 had the best median OS of 31.6 months (see Table 4).

**Table 1:**

Patient Characteristics

| Variable | N (%) |
|---|---|
| **Total Patients** | 750 |
| **Race** | |
| Black | 100 (13) |
| Other | 15 (2) |
| Unknown | 13 (2) |
| White | 622 (83) |
| **Type of Progression** | |
| Measureable/Evaluable | 606 (81) |
| PSA only | 144 (19) |
| **Bisphosphonate Usage** | |
| No | 293 (39) |
| Yes | 457 (61) |
| **Worst pain score** | |
| < 4 | 446 (59) |
| ≥ 4 | 304 (41) |
| **Extraskeletal metastases** | |
| No | 337 (45) |
| Yes | 413 (55) |
| **Performance Status** | |
| 0 | 333 (44) |
| 1 | 356 (48) |
| 2 | 61 (8) |
| **Gleason Score** | |
| ≤ 6 | 92 (12) |
| 7 | 214 (29) |
| ≥ 8 | 415 (55) |
| Missing | 29 (4) |
| **Treatment arm** | |
| Atrasentan | 372 (50) |
| Placebo | 378 (50) |
| **Age at registration** | |
| Mean (Standard deviation) | 69 (9) |
| Median (Interquartile Range) | 69 (63–76) |
| **Baseline PSA** | |
| Mean (Standard deviation) | 249 (643) |
| Median (Interquartile Range) | 77 (25–208) |

**Table 2.**

Logistic Regression and Receiver Operating Characteristic (ROC) Analysis for Overall Survival

| Bone marker | Area Under the ROC | 95% Confidence Interval | P-value (vs. reference group) |
|---|---|---|---|
| Risk factors (RF), No bone markers * | 0.73 | 0.69, 0.76 | N/A |
| RF + Bone alkaline phosphatase (BAP) | 0.75 | 0.72, 0.79 | 0.0038 |
| RF + C-terminal collagen propeptide (CICP) | 0.76 | 0.72, 0.79 | 0.0025 |
| RF + Pyridinoline (PYD) | 0.73 | 0.69, 0.77 | 0.57 |
| RF + N-telopeptide (N-Tx) | 0.74 | 0.71, 0.78 | 0.07 |
| **RF + (BAP+CICP+ PYD)** | **0.76** | **0.73, 0.80** | **0.0013** |

N= 734 patients (285 alive, 449 dead at 2 years); 16 patients who were lost to follow-up prior to 2 years were excluded from this analysis

* Reference group: model contains all of the risk factors included in Cox model

Abbreviations: N/A = not applicable

**Table 3:**

Impact of Bone Markers on Predicted Probability of Death, Stratified by Survival Status at 24 Months

| **Multivariate Logistic Model, Risk Factors Only*** | | | | | |
|---|---|---|---|---|---|
| | **Model-based Predicted Probability of Death Categories** | | | | |
| | **LOW** | **MED/LOW** | **MED/HIGH** | **HIGH** | **TOTAL** |
| | **< 45 %** | **45–62 %** | **63–79 %** | **>79%** | **N=734** |
| Deceased by 24 months (n, %) | 70<br>15.6 % | 98<br>21.8 % | 143<br>31.9 % | 138<br>30.7 % | 449<br>100 % |
| Alive at 24 months (n, %) | 105<br>36.9 % | 95<br>33.3 % | 67<br>23.5 % | 18<br>6.3 % | 285<br>100 % |
| **When Bone Markers are Added to the Model**** | | | | | |
| | **Model-based Predicted Probability of Death Categories** | | | | |
| | **LOW** | **MED/LOW** | **MED/HIGH** | **HIGH** | |
| | **< 45 %** | **45–62 %** | **63–79 %** | **>79%** | |
| Deceased by 24 months (n, %) | 64<br>14.3 % | 96<br>21.4 % | **110**<br>**24.5 %** | **179**<br>**39.9 %** | 449<br>100% |
| Alive at 24 months (n, %) | **133**<br>**46.7 %** | **69**<br>**24.2 %** | 66<br>23.2 % | 17<br>5.9 % | 285<br>100% |

**Table 4:**

**Prognostic Risk Groups identified by classification and regression tree analysis.**

CART analysis selected CICP, BAP, HGB, and pain score for the final model, and identified five prognostic groups with differential OS outcomes.

| NODE | Description | | | | N | Median OS (months) | HR | 95% CI | | p-value |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | HGB ≤ 11.3 | | | | 177 | 12.4 | REF | REF | REF | REF |
| 2 | HGB > 11.3 | CICP ≤ 6.8 | | | 191 | 31.6 | 0.28 | 0.22 | 0.36 | < 0.001 |
| 3 | HGB > 11.3 | CICP > 6.8 | worst pain ≥ 4 | | 144 | 15.3 | 0.79 | 0.63 | 0.99 | 0.04 |
| 4 | HGB > 11.3 | CICP > 6.8 | worst pain < 4 | BAP ≤ 90.9 | 140 | 27.1 | 0.34 | 0.27 | 0.44 | < 0.001 |
| 5 | HGB > 11.3 | CICP > 6.8 | worst pain < 4 | BAP > 90.9 | 98 | 17.1 | 0.68 | 0.53 | 0.88 | 0.003 |