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

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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 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.

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## 1. 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.

## 2. 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 75 mg/m$^2$ intravenously over 1 h 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 min. Serum was separated from cells within 45–60 min of venipuncture by centrifugation at 3000 × for 10 min. 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.

## 3. 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. $\geq 4$), 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%