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Changes in Bone Turnover Marker Levels and Clinical Outcomes in Patients With Advanced Cancer and Bone Metastases Treated With Bone Antiresorptive Agents

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Douglas Warner and Li Zhu are employees and stockholders of Amgen Inc. Karim Fizazi was a consultant to and/or participated in an advisory board for Astrazeneca, Amgen Inc., Astellas, Bayer, Janssen, Novartis, Orion, Ipsen, Takeda, and Sanofi. Allison Stopeck received research grants from Amgen Inc., Celldex, and Genentech; received honoraria from speakers' bureau from Amgen Inc. and Genomic Health; was a

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Translational Relevance *(136 of 150 words)*

Patients with advanced cancer and bone metastases often have elevated levels of the bone turnover markers urinary N-telopeptide (uNTx) and serum bone-specific alkaline phosphatase (sBSAP). Bone antiresorptive agents such as denosumab and zoledronic acid can reduce uNTx and sBSAP levels. Our study demonstrated that uNTx and sBSAP levels ≥ median levels, compared with < median levels, after 3 months of treatment with denosumab or zoledronic acid were associated with reduced overall survival and increased risk of disease progression and disease progression in bone. These results suggest a potential utility for uNTx and sBSAP as easily measurable, noninvasive, early predictors for response and survival in patients with advanced cancer and bone metastases who are receiving bone antiresorptive agents. Evaluating uNTx and sBSAP levels could complement established prognostic markers based on disease stage factors.

Abstract *(250 of 250 words)*

Purpose: Bone antiresorptive agents can significantly reduce bone turnover markers (BTMs) in patients with advanced cancer. We evaluated association of changes in BTMs with overall survival (OS), disease progression (DP), and disease progression in bone (DPB) in patients with advanced cancer and bone metastases following denosumab or zoledronic acid treatment.

Experimental Design: This is an integrated analysis of patient-level data from three identically designed, blinded, phase III trials with patients randomized to subcutaneous denosumab or intravenous zoledronic acid. Levels of the BTMs urinary N-telopeptide (uNTx) and serum bone-specific alkaline phosphatase (sBSAP) measured at study entry and month 3 were analyzed. OS, DP, and DPB were compared in patients with BTMs ≥ median vs < median based on month 3 assessments.

Results: uNTx levels ≥ the median of 10.0 nmol/mmol at month 3 were associated with significantly reduced OS compared with levels < median (HR for death 1.85, $P<0.0001$). sBSAP levels ≥ median of 12.6 ng/mL were associated with significantly reduced OS compared with levels < median (HR 2.44, $P<0.0001$). uNTx and sBSAP levels ≥ median at month 3 were associated with significantly greater risk of DP (HR 1.31, $P<0.0001$ and HR 1.71, $P<0.0001$, respectively) and DPB (HR 1.11, $P=0.0407$ and HR 1.27, $P<0.0001$, respectively).

Conclusions: BTM levels ≥ median after 3 months of bone antiresorptive treatment were associated with reduced OS and increased risk of DP and DPB. Assessment of

uNTx and sBSAP levels after bone antiresorptive therapy may add to identification of patients at risk for worse clinical outcomes.

Introduction

Bone is a frequent and often the only site of metastasis in patients with advanced solid tumors such as breast cancer, prostate cancer, or lung cancer (1-7), and bone metastases are often associated with significant morbidity and poor prognosis (3, 6, 8). Metastatic bone disease disrupts the homeostasis of osteoclast-mediated bone resorption and osteoblast-mediated bone formation, leading to dysregulation of normal bone remodeling processes (2, 3).

The loss of bone homeostasis compromises the structural integrity of the skeleton and leads to clinical complications including pathological fractures, spinal cord compression, life-threatening hypercalcemia, or the need for radiation or surgery to bone to prevent or treat fractures (2, 5, 8-10). These clinical complications, collectively termed skeletal-related events (SREs) (8, 11-13), often lead to severe pain and a significant decrease in quality of life (14-17).

Osteoblasts produce the cytokine receptor activator of nuclear factor kappa-B ligand (RANKL), which is an essential mediator of osteoclast function, formation, and survival (6, 18, 19). The presence of tumor cells in the bone stimulates osteoblasts to increase RANKL expression (6, 18, 19), which in turn induces osteoclast-mediated bone resorption and bone destruction, leading to SREs (20, 21).

Bone antiresorptive agents such as denosumab and zoledronic acid inhibit osteoclast activity (22-27) and are approved for the prevention of SREs in patients with bone metastases from solid tumors (28, 29). Denosumab has previously been shown to

be more efficacious than zoledronic acid in preventing SREs in this patient population (25-27).

Patients with advanced cancer and bone metastases typically have elevated levels of the bone turnover markers (BTMs) urinary N-telopeptide (uNTx), an indicator of osteoclast activity, and serum bone-specific alkaline phosphatase (sBSAP), an indicator of osteoblast activity (6, 7, 30-32). Both denosumab and zoledronic acid have been shown to significantly reduce uNTx and sBSAP levels (33, 34), and these two BTMs have been investigated as potential prognostic factors for monitoring patients with cancer who are receiving bone antiresorptive agents. A recent study in 1,901 men with castration-resistant prostate cancer demonstrated that decreased baseline levels of uNTx and sBSAP were associated with improved overall survival (OS) (35).

In this retrospective analysis, we used a large integrated dataset from patients who had various advanced solid tumors and bone metastases and had received either denosumab or zoledronic acid to further explore the relationship between changes in BTM (uNTx and sBSAP) levels and clinical outcomes. Here we report the association of changes in uNTx and sBSAP levels with OS, disease progression (DP), and disease progression in the bone (DPB).

Materials and Methods

Patients and Treatments

Details of the three identically designed, blinded, phase III trials comparing denosumab and zoledronic acid in patients with breast cancer (ClinicalTrials.gov: NCT00321464) (27), prostate cancer (ClinicalTrials.gov: NCT00321620) (25), or solid tumors (excluding breast cancer and prostate cancer) or myeloma (ClinicalTrials.gov: NCT00330759) (26) have been previously reported. In those three parent studies, eligible patients ≥18 years old had received either a SC injection of denosumab 120 mg (XGEVA® Amgen Inc., Thousand Oaks, CA, USA) (29) and an IV infusion of placebo every 4 weeks or an IV infusion of zoledronic acid 4 mg (Zometa®, Novartis, East Hanover, NJ, USA) (28) and an SC injection of placebo every 4 weeks (see Supplementary Fig. S1 for study design).

In the three parent studies, creatinine clearance ≥ 30 mL/min and an Eastern Cooperative Oncology Group (ECOG) performance status of ≤ 2 were required at study entry. Daily supplementation with calcium (≥ 500 mg) and vitamin D (≥ 400 U) was strongly recommended. Exclusion criteria included prior treatment with IV bisphosphonates, planned radiation or surgery to bone, or unhealed dental or oral surgery.

Patients who participated in the studies had provided written, informed consent before any study-specific procedure was performed, except for three patients in the zoledronic acid group of the breast cancer study (27), who were excluded from analysis due to lack of proper documentation of informed consent. Study protocols were approved by the relevant institutional review boards and independent ethics committees

for each site, and the studies were conducted in accordance with International Conference on Harmonisation guidelines on Good Clinical Practice and the principles of the Declaration of Helsinki.

Assessments of Outcomes

Levels of uNTx and sBSAP measured at baseline and after 3 months of treatment with either denosumab or zoledronic acid were analyzed. Urine was collected from the second void of the day, before noon. uNTx measurements (corrected for urine creatinine levels) were performed by Amgen Inc. (Thousand Oaks, CA, USA) or PPD Development (Richmond, VA, USA) using an enzyme-linked immunosorbent assay (ELISA; Osteomark, Seattle, WA, USA). sBSAP measurements were performed by the University of Liege (Liege, Belgium) using a chemiluminescent assay (Access Ostase reagents on the Access immunoassay system, Beckman Coulter, Brea, CA, USA). OS, DP, and DPB were compared in patients who had uNTx and sBSAP levels ≥ or < the median levels at month 3. The time point of 3 months after antiresorptive treatment was selected to provide adequate time for response to therapy.

Statistical Analysis

The integrated patient-level dataset from patients with solid tumors enrolled in the three phase III trials (25-27) was used for this analysis. This excludes the multiple myeloma patient population enrolled in the solid tumor and myeloma study (26). In this post-hoc analysis on uNTx and sBSAP levels, respectively, Cox models were used to analyze the association between the level (≥ or < the median levels) at month 3 and OS, DP, and DPB by taking the level category as the independent variable and stratified by study, treatment, and the actual stratification factors based on month 3 assessments. To

determine the impact of risk factors associated with a more advanced disease state, additional analyses that included the covariates of baseline visceral metastases (presence vs absence), baseline number of bone metastases (≤2 vs >2), or baseline ECOG performance status category (0–1 vs ≥2) were performed.

For uNTx and sBSAP, the absolute value of levels at month 3 and percent change from baseline were determined. OS, DP, and DPB were analyzed by month 3 BTM category (≥ or < median) as well as by percent change category (≥ or < median percent change). In addition, clinical outcomes were analyzed by patients' combined category of uNTx and sBSAP levels at month 3: HL (high-low: uNTx ≥ median and sBSAP < median at month 3), LH (low-high: uNTx < median and sBSAP ≥ median at month 3), LL (low-low: uNTx < median and sBSAP < median at month 3), and HH (high-high: uNTx ≥ median and ≥ sBSAP median at month 3).

Results

Patients

A total of 5,543 patients with advanced solid tumors and bone metastases from the three parent phase III studies (25-27) were included in this integrated analysis (denosumab, *n* = 2,775; zoledronic acid, *n* = 2,768). The myeloma patient population (*n* = 180) from the solid tumor and myeloma study (26) was excluded from this analysis. Data on BTM levels were available for most of the patients: uNTx, *n* = 4,299 (breast cancer, *n* = 1,705; prostate cancer, *n* = 1,527; and NSCLC, *n* = 461) and sBSAP, *n* = 4,316 (breast cancer, *n* = 1,708; prostate cancer, *n* = 1,512; and NSCLC, *n* = 480).

Patient demographics and baseline clinical characteristics were generally balanced between the treatment groups (Table 1). Both groups had a median age of 63.0 years. Most (90.0%) patients had an ECOG performance status of 0–1. A total of 2,337 (42.2%) patients had visceral metastases, 4,185 (75.5%) had up to 2 metastatic lesions in the bone. Median levels of uNTx and sBSAP at baseline were 43.7 nmol/mmol and 21.1 ng/mL, respectively.

Changes in BTM Levels at Month 3 of Bone Antiresorptive Treatment

After 3 months of treatment with either denosumab or zoledronic acid, median levels of uNTx for all patients decreased from 43.7 nmol/mmol to 10.0 nmol/mmol (Supplementary Table S1). This decrease was consistent across tumor types, with month 3 median levels of 10.4 nmol/mmol for patients with breast cancer and 9.6 nmol/mmol for patients with prostate cancer or non-small cell lung cancer (NSCLC). Median levels of sBSAP decreased from 21.1 ng/mL to 12.6 ng/mL for all patients

(Supplementary Table S1). By tumor type, median levels of sBSAP at month 3 were higher in patients with prostate cancer (21.4 ng/mL) than in patients with breast cancer or NSCLC (10.9 ng/mL and 10.1 ng/mL, respectively).

Association of BTM Levels With OS, DP, and DPB at Month 3 of Bone Antiresorptive Treatment

In the integrated analysis, patients with uNTx levels ≥ median at month 3 had significantly reduced OS compared with patients with uNTx levels < median (HR 1.85, 95% confidence interval (CI): 1.67–2.04; $P < 0.0001$) (Fig, 1A; Table 2). Similarly, patients with sBSAP levels ≥ median at month 3 had significantly reduced OS compared with those who had sBSAP levels < median (HR 2.44, 95% CI: 2.20–2.71; $P < 0.0001$) (Fig 1B; Table 2).

The risk of DP significantly increased for patients with uNTx and sBSAP levels ≥ median at month 3 (HR 1.31, 95% CI: 1.21–1.41; $P < 0.0001$ and HR 1.71, 95% CI: 1.57–1.85; $P < 0.0001$, respectively) (Fig 2A and B; Table 2). Similarly, the risk of DPB significantly increased for patients with uNTx and sBSAP levels ≥ median at month 3 (HR 1.11, 95% CI: 1.01–1.24; $P = 0.0407$ and HR 1.27, 95% CI: 1.14–1.41; $P < 0.0001$, respectively) (Fig 3A and B; Table 2).

Clinical outcomes were also assessed by tumor type. In patients with breast cancer, uNTx and sBSAP levels ≥ median at month 3 were associated with significantly reduced OS and a significantly increased risk of DP and DPB (Table 2), consistent with results seen for the combined patient population. In patients with prostate cancer, uNTx and sBSAP levels ≥ median at month 3 were associated with a significantly reduced OS

and significantly increased risk of DP; however, nonsignificant changes in the risk of DPB were seen with levels ≥ median at month 3 for uNTx (HR 0.86, 95% CI: 0.73–1.02; $P = 0.0911$) and for sBSAP (HR 1.07, 95% CI: 0.90–1.27; $P = 0.4234$) compared with those with levels < median (Table 2). In patients with NSCLC, uNTx and sBSAP levels ≥ median at month 3 were associated with significantly reduced OS and significantly increased risk of DP. In addition, sBSAP levels ≥ median at month 3 were associated with a significantly increased risk of DPB. Although uNTx levels ≥ median at month 3 showed an association with increased risk of DPB, this association did not reach statistical significance (HR 1.41, 95% CI: 0.97–2.03; $P = 0.0691$).

OS, DP, and DPB Adjusted for Baseline Visceral Metastases, Bone Metastases, or ECOG Performance Status Category

Significant associations of uNTx and sBSAP levels with OS, DP, and DPB were observed even after adjusting for factors associated with advanced cancer such as baseline visceral metastases, baseline multiple metastatic bone lesions, and baseline ECOG performance status category (Supplementary Table S2). After adjusting for baseline visceral metastases, uNTx levels ≥ median, compared with uNTx levels < median, were associated with reduced OS (HR 1.81, 95% CI: 1.64–2.00; $P < 0.0001$), greater risk of DP (HR 1.29, 95% CI: 1.19–1.39; $P < 0.0001$), and also greater risk of DPB (HR 1.11, 95% CI: 1.00–1.23; $P = 0.0469$). Similarly, sBSAP levels ≥ median, compared with sBSAP levels < median, were associated with reduced OS (HR 2.41, 95% CI: 2.17–2.68; $P < 0.0001$), greater risk of DP (HR 1.69, 95% CI: 1.56–1.83; $P < 0.0001$), and also greater risk of DPB (HR 1.26, 95% CI: 1.14–1.41; $P < 0.0001$). Similar results were observed after adjusting for baseline multiple metastatic bone

lesions and baseline ECOG performance status category; i.e., month 3 BTM levels ≥ median, compared with BTM levels < median, were associated with significantly reduced OS and a significantly increased risk of DP and DPB (Supplementary Table S2).

Outcomes by Category (≥ or < Median) of Month 3 BTM Percent Change From Baseline

Overall, sBSAP level is reduced from baseline to month 3, with the median percent change in sBSAP from baseline to month 3 of –35.6%. Patients who achieved a smaller reduction from baseline in sBSAP levels (i.e., percent change from baseline ≥ –35.6%) had reduced OS and an increased risk of DP and DPB. On the other hand, patients who achieved further reduction in sBSAP levels (i.e., percent change from baseline < –35.6%) had improved OS and decreased risk of DP and DPB (Supplementary Fig. 2A-C). An association of outcomes and percentage change in uNTx levels was not observed (data not shown).

Outcomes by uNTx and sBSAP Combined Category (≥ or < Median) at Month 3

Assessment of patients' month 3 combined uNTx and sBSAP levels demonstrated reduced OS for the HH group (uNTx and sBSAP both ≥ median at month 3) and LH group (uNTx < median and sBSAP ≥ median at month 3) compared to the HL (uNTx ≥ median and sBSAP < median at month 3) and LL groups (uNTx and sBSAP both < median at month 3) (Fig. 4A). The risk of DP increased in the HH and LH groups compared to the LL and HL groups (Fig. 4B). A similar trend, though less pronounced, was also observed for DPB (Fig. 4C).

Discussion

In this retrospective study, we analyzed patient-level data from a total of 5,543 patients with advanced solid tumors and bone metastases who had participated in three identically designed phase III trials and had received the bone antiresorptive agents denosumab or zoledronic acid. Overall, our analysis demonstrated that ≥ median levels of the BTMs uNTx and sBSAP after 3 months of bone antiresorptive treatment were associated with significantly reduced OS and significantly increased risk of DP and DPB.

Across tumor types (breast cancer, prostate cancer, and NSCLC), month 3 uNTx and sBSAP levels ≥ median were associated with significantly reduced OS and a significantly increased risk of DP, consistent with data observed for all tumor types combined. However, the pattern of association of BTM levels ≥ median with DPB appeared to vary by tumor type. Month 3 levels of both uNTx and sBSAP ≥ median were associated with a significantly increased risk of DPB in patients with breast cancer but were not associated with an increased risk of DPB in patients with prostate cancer. In patients with NSCLC, month 3 sBSAP levels ≥ median were associated with a significant increase in the risk of DPB, whereas month 3 uNTx levels ≥ median were associated with a nonsignificant increase in the risk of DPB.

Of interest, our study demonstrated an association between clinical outcomes and serum sBSAP percentage change. Patients who achieved a smaller reduction from baseline in sBSAP levels had reduced OS and increased risk of DP and DPB (Supplementary Fig 2A-C). Additional studies and analyses (especially multivariate

analyses that include multiple covariates) are required to further evaluate the correlation of percent changes from baseline in levels of sBSAP and/or uNTx with clinical outcomes.

In a separate analysis, we observed a significant association between clinical outcomes and BTM levels when patients were categorized into HL, LH, LL, and HH subgroups according to their combined uNTx and sBSAP levels at month 3 (Fig. 4 A–C). Patients with high levels of both uNTx and sBSAP at month 3 (≥ median levels) had substantially reduced OS and an increased risk of DP and, to a lesser extent, an increased risk of DPB. A similar negative correlation, was also observed in patients with high sBSAP (≥ median level) but low uNTx (< median level) at month 3.

Previous studies have shown the potential prognostic value of uNTx and sBSAP levels in patients with solid tumors and bone metastases. A recent study in patients with prostate cancer showed low baseline uNTx and sBSAP levels to be prognostic and to be associated with longer OS (35). Low uNTx and sBSAP levels were also shown to be associated with positive clinical outcomes in patients with bone metastases secondary to prostate cancer, NSCLC, or other solid tumors (20, 21, 36, 37), independent of whether patients had received bone antiresorptive agents. Similar to findings from our current study, Coleman et al 2005 (20) reported significantly reduced OS and significantly increased risk of DPB in patients with persistently high uNTx levels (≥100 nmol/mmol creatinine) to moderate uNTx levels (50 to 99 nmol/mmol creatine) vs patients with low uNTx levels (<50 nmol/mol creatinine). Also, that study reported significantly reduced OS and significantly increased risk of DPB in patients with

persistently high serum sBSAP levels (≥146 U/L) vs patients with low sBASP levels (<146 U/L).

Historically, sBSAP has been known to be a potential prognostic marker in bone metastases that are secondary to advanced cancer, whereas uNTx is an emerging marker (20, 38-40). However, to date, neither has been shown to be a definitive prognostic marker in this patient population. As such, an approach that includes assessing levels of both uNTx and sBSAP might provide additional information regarding potential clinical outcomes in patients with advanced cancer and bone metastases.

Tumor growth in the bone is typically associated with increased rates of bone resorption and formation that might be reflected by increased levels of the biochemical markers of bone metabolism such as uNTx and sBSAP (20, 21). Therefore, modalities that reduce bone turnover rates might impact tumor growth and thus limiting DPB and improving survival (20, 21). Denosumab and zoledronic acid are potent bone antiresorptive agents that have been shown to significantly reduce the levels of BTMs such as uNTx and sBSAP (33, 34). As such, decreased levels of uNTx and sBSAP after treatment with denosumab or zoledronic might be an indicator of reduced tumor growth in the bone due to reduced bone turnover rates, whereas high levels of these BTMs might indicate continued tumor growth. However, data from our study do not address the reason for the observed associations between uNTx or sBSAP levels ≥ median at month 3 of antiresorptive therapy and worse clinical outcomes. Possible explanations for this observed association include the possibility that patients responding to therapy targeted at their primary tumors may have lower uNTx and sBSAP levels than those not

responding to therapy, the possibility of involvement of bone antiresorptive agents, or reasons unrelated to the primary tumor or bone antiresorptive agents.

Other baseline variables shown to be associated with improved OS, mostly in patients with prostate cancer, include low alkaline phosphatase levels (35, 40, 41), absence of prior SREs (35, 42, 43), absence of visceral metastases (35, 40, 44), better ECOG performance status (35, 37, 40), low levels of prostate-specific antigen (PSA) (35, 40), and high hemoglobin levels (35, 37, 40, 44). Monitoring PSA levels is limited to the prostate cancer setting, and even within this setting, challenges have been encountered with accurate interpretation of PSA levels following treatment with new therapies that may have novel mechanisms of action. As an example, sipuleucel-T was reported to improve survival with no impact on early PSA levels (45). In other cases, PSA values have been shown to first rise and then decline following effective systemic treatment, thus making timing of sampling an important factor (45, 46). As such, additional variables are needed for predicting clinical outcomes, especially markers that show prognostic value across tumor types.

Several limitations of our study must be noted. This study analyzed data from patients originally recruited for clinical trials in which individuals with poor performance (ECOG performance status >2) or serious medical illnesses were excluded from enrollment, thereby limiting the generalizability of our findings to real world settings. In our study, we defined high uNTx or sBSAP as ≥ median at month 3 of antiresorptive therapy, and these levels were used as cutoffs to determine association with clinical outcomes. However, these cutoff levels may not necessarily reflect definitive categorizations for these biochemical markers, and it is possible that different results

could be obtained by choosing different cutoff levels. In addition, the three parent phase III studies were not prospectively designed to collect all potential covariates for OS, DP, and DPB as the objective of the original studies was to evaluate risk reduction for time to first SRE between denosumab and zoledronic acid. Another limitation is tumor heterogeneity in the analyzed patient population.

While the results of this study do not establish a causal link between decreased levels of sBSAP and uNTx and clinical outcomes, they suggest a potential utility for these BTMs as easily measurable, noninvasive, early predictors for response and survival in patients with advanced cancer and bone metastases who are receiving bone antiresorptive agents such as denosumab or zoledronic acid. In this patient population, changes in BTM levels to higher than or lower than levels at baseline might provide insights into potential clinical outcomes. Taken together, our findings and the findings from earlier studies appear to point to the gross prognostic value of serum sBSAP and uNTx levels, either at baseline or after treatment with bone antiresorptive agents.

In conclusion, patients with BTM levels ≥ median at month 3 of antiresorptive therapy had significantly worse clinical outcomes including OS, DP, and DPB than patients whose BTM levels were < median. Therefore, assessment of BTM levels after antiresorptive therapy may add to the identification of patients most at risk for decreased OS and increased DP and DPB in this patient population.

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Author Contributions

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Development of methodology: Allan Lipton, Douglas Warner, Li Zhu

Acquisition and assembly of data: Li Zhu

Analysis and interpretation of data: All authors

Writing, review, and/or revision of the manuscript: All authors

Administrative, technical, or material support: None

Study supervision: None

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Table 1. Baseline demographic and clinical characteristics[a]

Characteristic	Denosumab 120 mg Q4W (*n* = 2,775)	Zoledronic acid 4 mg Q4W (*n* = 2,768)	All (*N* = 5,543)
Sex, *n* (%)			
Female	1,286 (46.3)	1,310 (47.3)	2,596 (46.8)
Male	1,489 (53.7)	1,458 (52.7)	2,947 (53.2)
Median age (IQR), years	63.0 (54.0–71.0)	63.0 (54.0–72.0)	63.0 (54.0–71.0)
Race, *n* (%)			
White	2,352 (84.8)	2,320 (83.8)	4,672 (84.3)
ECOG PS, *n* (%)			
0–1	2,514 (90.6)	2,472 (89.3)	4,986 (90.0)
≥2	258 (9.3)	288 (10.4)	546 (9.9)
Missing	3 (0.1)	8 (0.3)	11 (0.2)
Primary tumor type, *n* (%)			
Breast cancer	1,026 (37.0)	1,020 (36.8)	2,046 (36.9)
Prostate cancer	950 (34.2)	951 (34.4)	1,901 (34.3)
Non-small cell lung cancer	350 (12.6)	352 (12.7)	702 (12.7)
Other	449 (16.2)	445 (16.1)	894 (16.1)

Median time from diagnosis of cancer to randomization (IQR), months	26.45 (8.18–66.10)	27.04 (8.44–68.07)	26.73 (8.31–67.09)
Median time from diagnosis of bone metastases to randomization (IQR), months	2.22 (1.02–7.20)	2.30 (1.05–7.75)	2.27 (1.02–7.41)
Presence of visceral metastases, *n* (%)	1,185 (42.7)	1,152 (41.6)	2,337 (42.2)
Number of metastatic lesions in bone, *n* (%)			
≤2	2,101 (75.7)	2,084 (75.3)	4,185 (75.5)
>2	674 (24.3)	684 (24.7)	1,358 (24.5)
Bone turnover markers, median (IQR)			
uNTx (nmol/mmol)	44.2 (24.8–82.9)	43.5 (25.1–81.8)	43.7 (25.0–82.4)
sBSAP (ng/mL)	21.1 (14.0–41.5)	21.1 (13.6–41.1)	21.1 (13.8–41.3)

[a]Excludes the myeloma patient population.

ECOG PS, Eastern Cooperative Oncology Group performance status; IQR, interquartile range; Q4W, every 4 weeks.

Table 2. Covariate analysis of OS, DP, and DPB at month 3, overall and by tumor type

Clinical Outcome	*n*[a]	Hazard ratio (95% CI)	*P* value
All tumor types[b]			
uNTx	4,299[d]		
OS[c]		1.85 (1.67–2.04)	<0.0001
DP		1.31 (1.21–1.41)	<0.0001
DPB		1.11 (1.01–1.24)	0.0407
sBSAP	4,316[e]		
OS[c]		2.44 (2.20–2.71)	<0.0001
DP		1.71 (1.57–1.85)	<0.0001
DPB		1.27 (1.14–1.41)	<0.0001
Breast cancer			
uNTx	1,705[f]		
OS[c]		1.54 (1.27–1.87)	<0.0001
DP		1.21 (1.07–1.38)	0.0024
DPB		1.23 (1.05–1.44)	0.0087
sBSAP	1,708[g]		
OS[c]		2.97 (2.42–3.63)	<0.0001
DP		1.67 (1.47–1.89)	<0.0001
DPB		1.56 (1.34–1.82)	<0.0001
Prostate cancer			
uNTx	1,527[h]		
OS[c]		2.12 (1.82–2.48)	<0.0001

DP		1.32 (1.17–1.50)	<0.0001
DPB		0.86 (0.73–1.02)	0.0911
sBSAP	1,512[i]		
OS[c]		2.81 (2.39–3.32)	<0.0001
DP		1.83 (1.61–2.09)	<0.0001
DPB		1.07 (0.90–1.27)	0.4234
Non-small cell lung cancer			
uNTx	461[j]		
OS[c]		1.83 (1.44–2.33)	<0.0001
DP		1.30 (1.03–1.63)	0.0249
DPB		1.41 (0.97–2.03)	0.0691
sBSAP	480[k]		
OS[c]		1.66 (1.31–2.12)	<0.0001
DP		1.37 (1.09–1.71)	0.0061
DPB		1.55 (1.09–2.21)	0.0152

[a]Number of patients included in the analysis.

[b]Excludes the myeloma patient population.

[c]OS is measured by death of all cause. An HR >1 indicates an increased risk of death and decreased OS.

[d]*n* = 2,150 for patients with uNTx levels ≥ median and *n* = 2,149 for patients with uNTx levels < median.

[e]*n* = 2,157 for patients with sBSAP levels ≥ median and *n* = 2,159 for patients with sBSAP levels < median.

[f]*n* = 895 for patients with uNTx levels ≥ median and *n* = 810 for patients with uNTx levels < median.

[g]*n* = 855 for patients with sBSAP levels ≥ median and *n* = 853 for patients with sBSAP levels < median.

[h]*n* = 764 for patients with uNTx levels ≥ median and *n* = 763 for patients with uNTx levels < median.

[i]*n* = 758 for patients with sBSAP levels ≥ median and *n* = 754 for patients with sBSAP levels < median.

[j]*n* = 231 for patients with uNTx levels ≥ median and *n* = 230 for patients with uNTx levels < median.

[k]*n* = 239 for patients with sBSAP levels ≥ median and *n* = 241 for patients with sBSAP levels < median.

Figure Legends

Fig 1. OS stratified by uNTx (A) and sBSAP (B) levels at month 3.[a]

[a]Excludes the myeloma patient population.

Fig 2. DP stratified by uNTx (A) and sBSAP (B) levels at month 3.[a]

[a]Excludes the myeloma patient population.

Fig 3. DPB stratified by uNTx (A) and sBSAP (B) levels at month 3.[a]

[a]Excludes the myeloma patient population.

Fig 4. OS (A), DP (B), and DPB (C) stratified by uNTx and sBSAP combined category (≥ or < median) at month 3[a,b]

[a]Excludes the myeloma patient population.

[b]Combined category of uNTx and sBSAP levels at month 3: HL (high-low: NTX ≥ median and sBSAP < median at month 3), LH (low-high: uNTx < median and sBSAP ≥ median at month 3), LL (low-low: uNTx < median and sBSAP < median at month 3), and HH (high-high: uNTx ≥ median and ≥ sBSAP median at month 3).

Figures

Fig 1. OS stratified by uNTx (A) and sBSAP (B) levels at month 3[a]

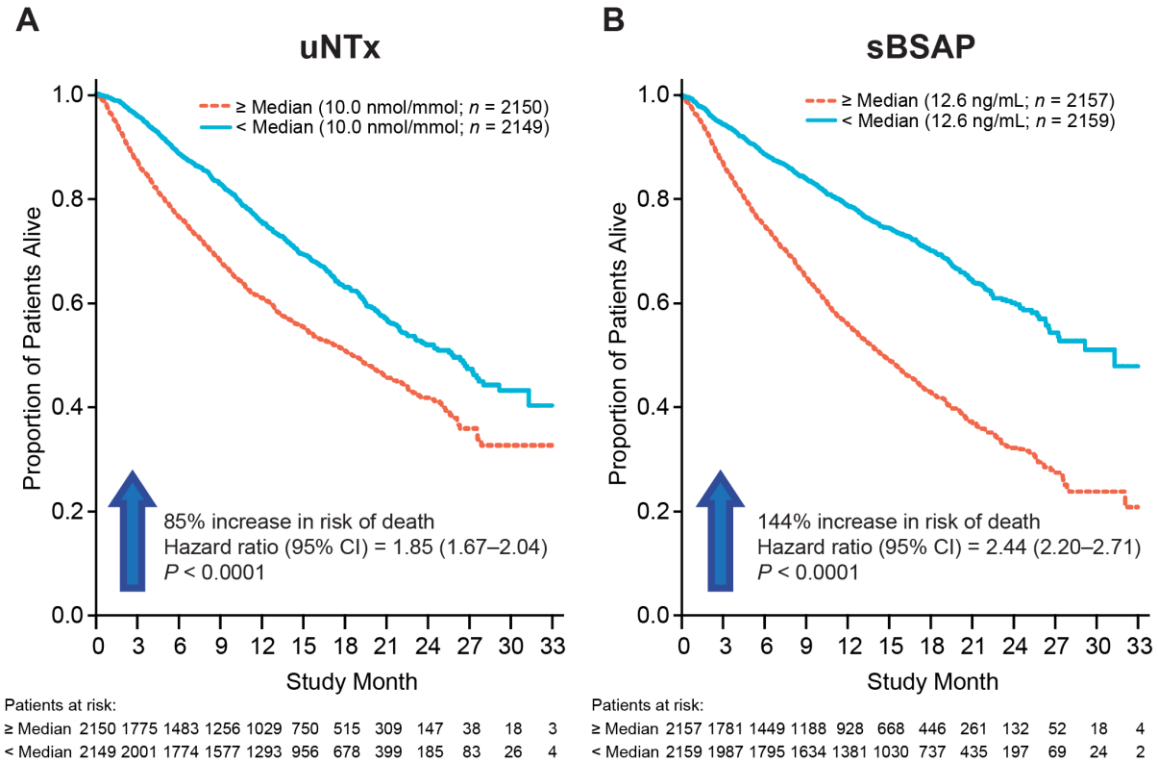


Fig 2. DP stratified by uNTx (A) and sBSAP (B) levels at month 3[a]

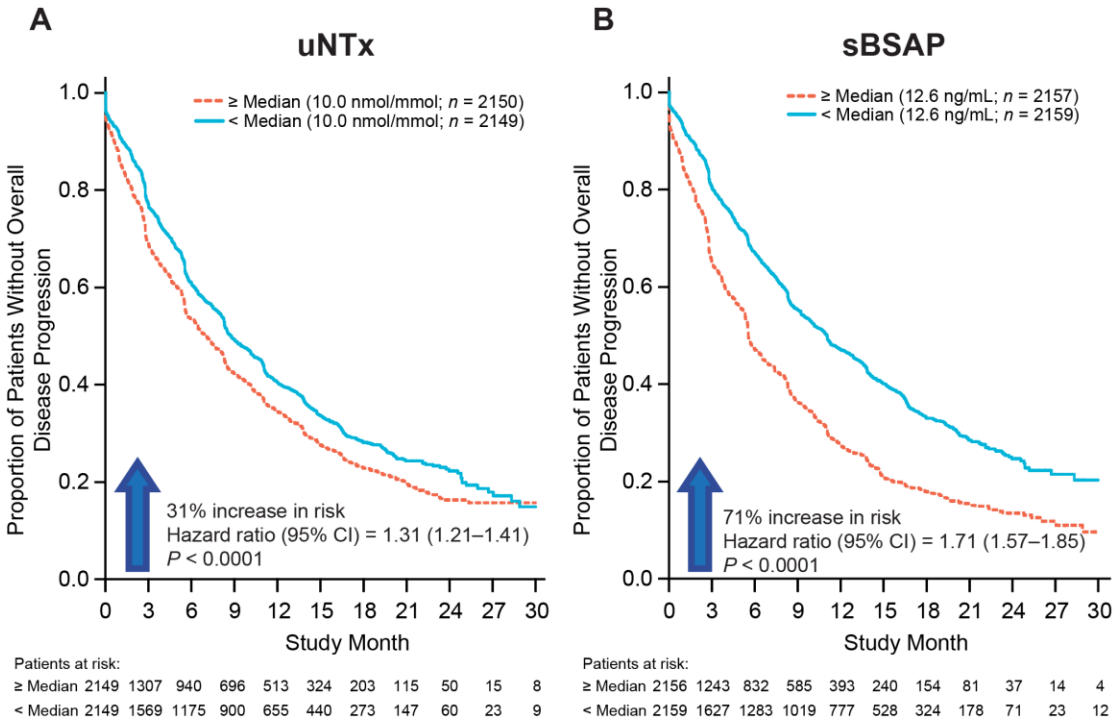


Fig 3. DPB stratified by uNTx (A) and sBSAP (B) levels at month 3[a]

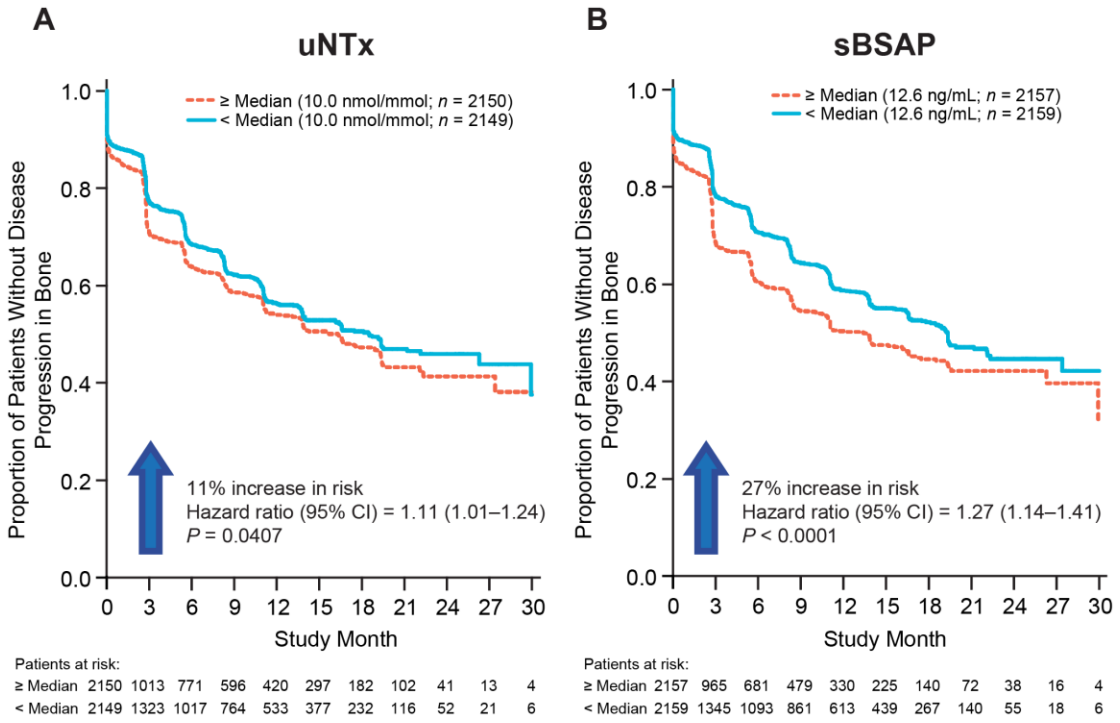
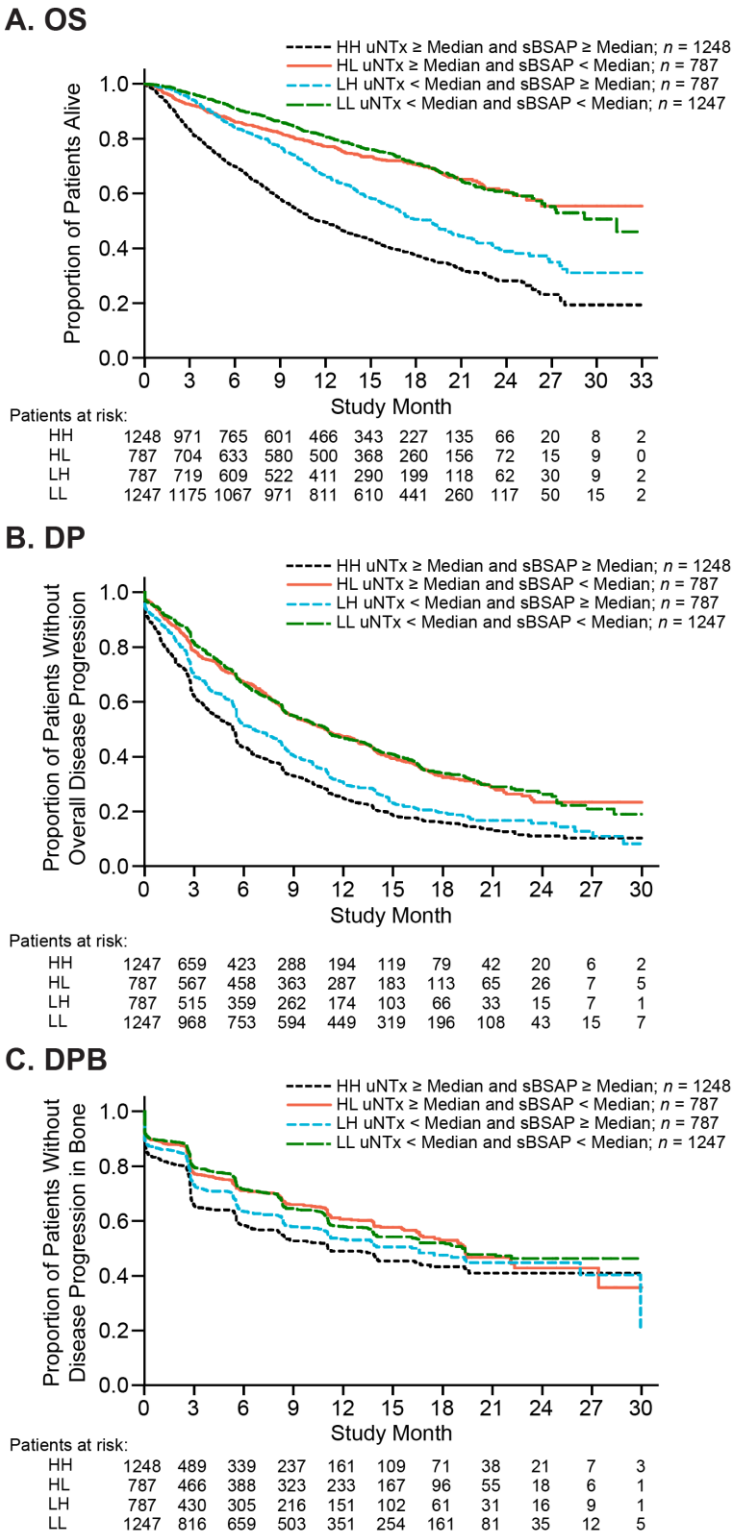


Fig 4. OS (A), DP (B), and DPB (C) stratified by uNTx and sBSAP combined category (≥ or < median) at month 3[a,b]



Supplementary Material (Online Only)

Legend to Supplementary Figure S1. Combined patient population from the three phase III studies and treatments (25-27).

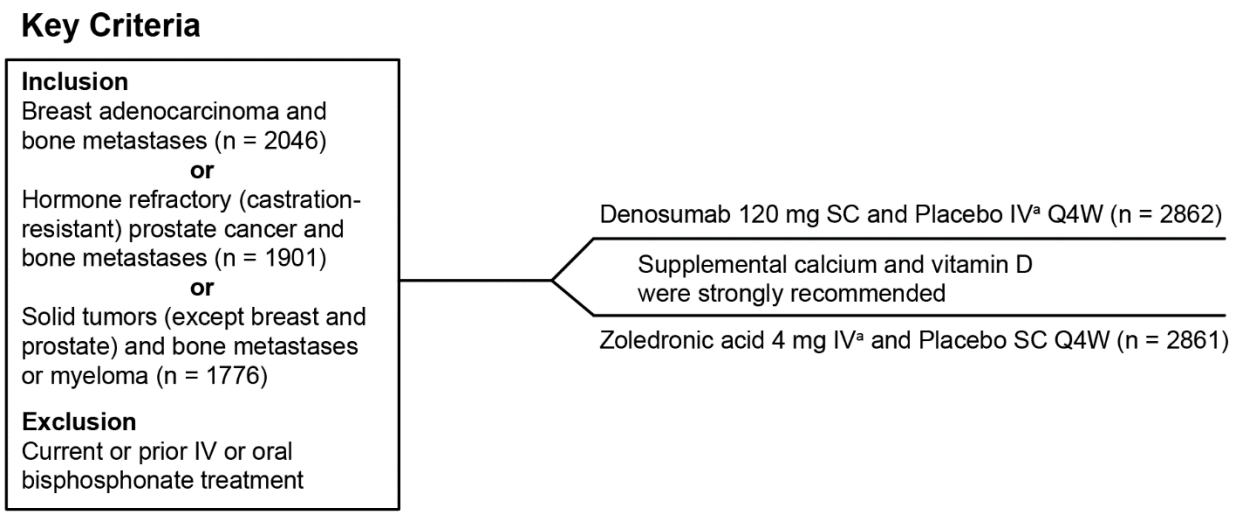
[a]IV product dose adjusted for baseline creatinine clearance and subsequent dose intervals determined by serum creatinine, per prescribing information for zoledronic acid (28).

Q4W, every 4 weeks

Legend to Supplementary Figure S2. OS (A), DP (B), and DPB (C) stratified by category (≥ or < median) of month 3 sBSAP percent change from baseline.[a]

[a]Excludes the myeloma patient population.

Supplementary Fig S1. Combined patient population from the three phase III studies and treatments (25-27).



Supplementary Table S1. BTM levels at baseline and at month 3 after bone antiresorptive treatment

		Median BTM levels		
	At baseline		At month 3	
Tumor type	*n*[a]	Median (IQR)	*n*[b]	Median (IQR)
uNTx (nmol/mmol)				
All tumor types[c]	4,951	43.7 (25.0–82.4)	4,299	10.0 (6.3–19.9)
Breast cancer	1,797	42.6 (24.9–74.6)	1,705	10.4 (6.6–20.8)
Prostate cancer	1,802	51.9 (27.9–112.0)	1,527	9.6 (6.0–20.8)
Non-small cell lung cancer	583	36.6 (21.4–65.1)	461	9.6 (5.5–17.7)
sBSAP (ng/mL)				
All tumor types[c]	5,080	21.1 (13.8–41.3)	4,316	12.6 (8.7–25.5)
Breast cancer	1,814	20.0 (13.9–30.9)	1,708	10.9 (8.3–17.5)
Prostate cancer	1,846	32.9 (17.4–86.5)	1,512	21.4 (10.8–69.0)
Non-small cell lung cancer	622	15.0 (11.1–23.7)	484	10.1 (7.7–15.4)

[a]Number of patients with uNTx or sBSAP measurement at baseline.

[b]Number of patients with uNTx or sBSAP measurement at month 3.

[c]Excludes the myeloma patient population.

IQR, interquartile range.

Supplementary Table S2. Covariate analysis of OS, DP, and DPB at month 3 adjusted for baseline visceral metastases, bone metastases, or ECOG category[a]

Clinical outcomes	*n*[b]	Hazard ratio (95% CI)	*P* value
Baseline visceral metastasis (presence vs absence)			
uNTx	4,299[d]		
OS[c]		1.81 (1.64–2.00)	<0.0001
DP		1.29 (1.19–1.39)	<0.0001
DPB		1.11 (1.00–1.23)	0.0469
sBSAP	4,316[e]		
OS[c]		2.41 (2.17–2.68)	<0.0001
DP		1.69 (1.56–1.83)	<0.0001
DPB		1.26 (1.14–1.41)	<0.0001
Baseline number of bone metastases (≤2 vs >2)			
uNTx	4,299[d]		
OS[c]		1.83 (1.66–2.02)	<0.0001
DP		1.30 (1.20–1.40)	<0.0001
DPB		1.14 (1.03–1.27)	0.0105
sBSAP	4,316[e]		
OS[c]		2.44 (2.20–2.72)	<0.0001
DP		1.72 (1.58–1.86)	<0.0001
DPB		1.38 (1.24–1.54)	<0.0001

Baseline ECOG PS category (0–1 vs ≥2)			
uNTx	4,299[d]		
OS[c]		1.80 (1.63–1.99)	<0.0001
DP		1.29 (1.19–1.39)	<0.0001
DPB		1.11 (1.00–1.24)	0.0415
sBSAP	4,316[e]		
OS[c]		2.39 (2.16–2.66)	<0.0001
DP		1.69 (1.56–1.83)	<0.0001
DPB		1.27 (1.14–1.41)	<0.0001

[a]Excludes the myeloma patient population.

[b]Number of patients included in the analysis.

[c]OS is measured by death of all cause. An HR >1 indicates an increased risk of death and decreased OS.

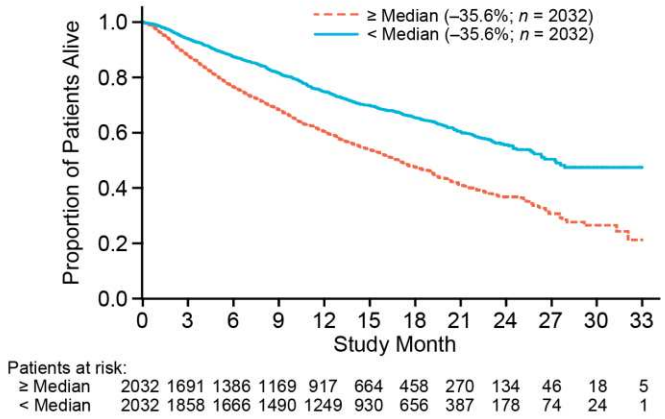
[d]*n* = 2,150 for patients with uNTx levels ≥ median and *n* = 2,149 for patients with uNTx levels < median.

[e]*n* = 2,157 for patients with sBSAP levels ≥ median and *n* = 2,159 for patients with sBSAP levels < median.

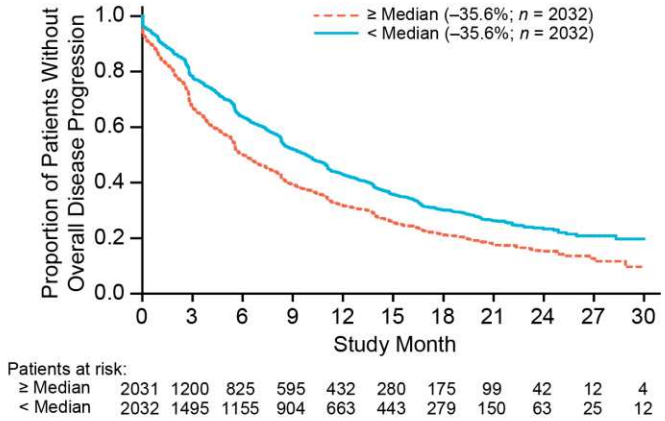
ECOG PS, Eastern Cooperative Oncology Group performance status.

Supplementary Fig S2. OS (A), DP (B), and DPB (C) stratified by category (≥ or < median) of month 3 sBSAP percent change from baseline.[a]

A. OS



B. DP



C. DPB