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Research Paper

Correlation of baseline biomarkers with clinical outcomes and response to fulvestrant with vandetanib or placebo in patients with bone predominant metastatic breast cancer: An OCOG ZAMBONEY sub-study

Christina L. Addison [a,b,c,*], Gregory R. Pond [d], Brandy Cochrane [d], Huijun Zhao [a], Stephen K. Chia [e], Mark N. Levine [d], Mark Clemons [a,b]

[a] Program for Cancer Therapeutics, Ottawa Hospital Research Institute, Box 926, 501 Smyth Road, Ottawa, ON, Canada K1H 8L6
[b] Department of Medicine, University of Ottawa, Ottawa, ON, Canada
[c] Department of Biochemistry, Microbiology and Immunology University of Ottawa, Ottawa, ON, Canada.
[d] McMaster University and Ontario Clinical Oncology Group, Hamilton, ON, Canada
[e] BC Cancer Agency, Vancouver, BC, Canada

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ABSTRACT

Background: Bone metastases are common in women with breast cancer and often result in skeletal related events (SREs). As the angiogenic factor vascular endothelial growth factor (VEGF) regulates osteoclast activity and is associated with more extensive bone metastases and SRE risk in metastatic breast cancer, we hypothesized that blockade of VEGF signaling could be a therapeutic strategy for inhibiting bone metastases progression and possibly prolonging overall (OS) or progression-free survival (PFS). The Zamboney trial was a randomized placebo-controlled study designed to assess whether patients with bone predominant metastatic breast cancer benefited from addition of the VEGF receptor (VEGFR) targeting agent, vandetanib, to endocrine therapy with fulvestrant. As a companion study, evaluation of biomarkers and their potential association with response to vandetanib or SRE risk was performed.

Methods: Baseline overnight fasted serum from enrolled patients was analyzed for levels of various putative biomarkers including; VEGF-A, soluble (s)VEGFR2, sVEGFR3, transforming growth factor (TGF)-β1 and activinA by ELISA. Spearman correlation coefficients and Wilcoxon rank sum tests were used to investigate potential relationships between biomarker values and baseline clinical parameters. Prognostic and predictive ability of each marker was investigated using Cox proportional hazards regression with adjustments for treatment and baseline strata of serum CTx (<400 versus ≥ 400 ng/L).

Results: Of 129 enrolled patients, serum was available for analysis in 101; 51 in vandetanib and 50 in placebo arm. Mean age amongst consenting patients was 59.8 years. Clinical characteristics were not significantly different between patients with or without serum biomarker data and serum markers were similar for patients by treatment arm. Baseline sVEGFR2 was prognostic for OS (HR=0.77, 95% CI=0.61–0.96, $p=0.020$), and although a modest association was observed, it was not significant for PFS (HR=0.90, 95% CI=0.80–1.01, $p=0.085$) nor time to first SRE (HR=0.82, 95% CI=0.66–1.02, $p=0.079$). When interaction terms were evaluated, sVEGFR2 was not found to be predictive of response to vandetanib, although a modest association remained with respect to PFS (interaction $p=0.085$). No other marker showed any significant prognostic or predictive ability with any measured outcome.

Conclusions: In this clinical trial, sVEGFR2 appeared prognostic for OS, hence validation of sVEGFR2 should be conducted. Moreover, the role of sVEGFR2 in breast cancer bone metastasis progression should be elucidated.

Abbreviations: BP, bisphosphonate; BPI, brief pain inventory; CTx, C-telopeptide; ER, estrogen receptor; FACT-BP, Functional assessment of cancer therapy-bone pain; OS, overall survival; PFS, progression free survival; PR, progesterone receptor; RANKL, Receptor Activator NF-KB ligand; SRE, skeletal related event; TGF-β, transforming growth factor beta; uNTx, urinary N-telopeptide; VEGF, vascular endothelial growth factor; sVEGFR, soluble vascular endothelial growth factor receptor

* Corresponding author at: Program for Cancer Therapeutics, Ottawa Hospital Research Institute, Box 926, 501 Smyth Road, Ottawa, ON, Canada K1H 8L6 .
E-mail address: caddison@ohri.ca (C.L. Addison).

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1. Introduction

Bone is the most common site of metastatic spread of breast cancer, and bone metastases remain incurable [1,2]. Bone metastases are also associated with significant pain, reduced quality of life and skeletal related events (SREs), such as radiotherapy or surgery to the bone, pathological fractures, spinal cord compression, or hypercalcaemia [3,4]. Bone metastases from breast cancer are commonly treated with agents that block bone turnover, such as bisphosphonates or the antibody to the Receptor Activator NF-KB ligand (RANKL), denosumab [5–8]. However, despite statistically significant reductions in SREs with bone-targeted agents, the absolute benefits are modest with no consistent progression free survival (PFS) or overall survival (OS) benefit [5,9,10]. The ability to identify new treatment combinations that prolong survival for patients with bone metastatic breast cancer as well as to identify novel biomarkers of absolute benefits from the use of both bone-targeted therapies and novel anti-cancer agents would be exceedingly valuable.

In the context of breast cancer bone metastases, VEGF can act as an osteolytic factor in the presence of RANKL, further promoting osteoclast maturation and activation [11,12]. Increased VEGF serum levels are associated with more extensive bone metastases and reductions in serum VEGF levels in these patients have been shown to correlate with response to bisphosphonates [13,14]. As such, the Zamboney study was designed to assess whether patients with bone predominant metastatic breast cancer could benefit from the addition of the targeted agent vandetanib to standard endocrine therapy with fulvestrant. Vandetanib (aka ZACTIMA or AZD6474) is a tyrosine kinase inhibitor predominantly targeting the vascular endothelial growth factor receptor (VEGFR)-2, the epidermal growth factor receptor (EGFR), and the REarranged during Transfection (RET) kinase. The primary results are reported elsewhere [15], but briefly, the addition of vandetanib to fulvestrant was not shown to enhance PFS, OS or tumor response as measured by circulating levels of urinary N-telopeptide (uNTx) [15]. Interestingly, a statistically non-significant trend was observed of a differential treatment effect based on baseline uNTx, suggesting the possibility of a predictive effect. Specifically, no difference in OS or PFS was observed between vandetanib and placebo treated patients who had normal baseline NTx, however, amongst patients with abnormal NTx (i.e. >65 nM BCE/mmol Creatine), those who received vandetanib had improved PFS and OS. A test for interaction was statistically significant ($p=0.028$) with PFS, but not for OS ($p=0.25$). Hence, we performed additional biomarker analyzes in order to investigate the possibility of whether particular subgroups of patients derived greater benefit from vandetanib.

To date, no biomarker analyses of response to VEGFR targeting agents in breast cancer patients with bone predominant metastatic disease has occurred. Although the bone turnover markers serum c-telopeptide (sCTx), and urinary N-telopeptide (uNTx) were measured at baseline, and uNTX measured every 8 weeks on patients accrued to the Zamboney study, no statistically significant association of these markers with response to vandetanib were observed in the main Zamboney study. Circulating angiogenic factors such as VEGF, bFGF, SDF-1α or soluble (s)VEGFR2 or sVEGFR3 have been previously suggested to associate with response following administration of the VEGF/VEGFR targeting agents bevacizumab, sunitinib (SU11248), BAY 57-9352, vatalinib (PTK787/ZK222584) and cediranib (AZD2171) [16–22]. Thus, we measured markers of tumor angiogenesis previously suggested to associate with response to VEGF-targeting drugs, namely VEGF, sVEGFR2 or sVEGFR3, and additionally evaluated putative markers of bone metastasis burden, namely transforming growth factor (TGF)-β and its related family member activinA, at baseline in patients enrolled in Zamboney to assess their prognostic or predictive abilities.

2. Materials and methods

2.1. Study population

Post-menopausal women with estrogen receptor (ER)/progesterone receptor (PR) positive breast cancer and radiologically confirmed bone only or bone predominant metastases were eligible for enrolment in Zamboney [15]. Eligible patients providing signed informed consent were randomized 1:1 to receive vandetanib (100 mg/day) or placebo together with fulvestrant (500 mg IM on days 1, 15, 29 and then every 28 days thereafter) following stratification based on baseline fasting levels of serum CTx (<400 ng/L, or ≥ 400 ng/L) measured as described [15]. The primary outcome of the study was significant changes in uNTx levels defined as $\geq 30\%$ reduction in uNTx levels (measured as described [15]) from baseline to any point on study. Other outcomes measured included PFS, defined as the time from randomization until disease progression (as defined by RECIST [23]) or death, OS, calculated from the date of randomization to date of death by any cause, and time to first on-study SRE. As part of the main study consent, patients could also optionally consent to the collection of urine and serum samples for future research. Use of these materials in the current study was approved by the Ottawa Health Science Research Ethics Board.

2.2. Biochemical analysis

For the present analysis, serum obtained at baseline study screening was obtained within 21 days of initiation of study drug. Blood was drawn in the morning following an overnight fast and samples were allowed to clot and subsequently centrifuged at 4°C for 10 min at 3400 RPM. Serum was frozen at -80 °C until analysis. Alternative biomarkers were measured using specific enzyme linked immunosorbant assay (ELISA) kits in baseline serum samples for VEGF-A (Quantikine, R&D Systems, Minneapolis MN, detection limit 9 pg/ml), sVEGFR2 (Quantikine, R&D Systems, Minneapolis MN, detection limit 12 pg/ml), and TGF-β1 (Quantikine, R&D Systems, Minneapolis MN, detection limit 16 pg/ml.) For sVEGFR3 or activinA, human antibody Duosets (R&D Systems, Minneapolis MN) were used to generate sandwich ELISAs according to the manufacturer's directions. The capture antibodies were diluted in phosphate buffered saline (PBS) and used to coat the wells of immunoplates (Cat # 439454, Nalge Nunc International, Rochester NY) overnight at room temperature. Coated plates were washed and blocked with 1% bovine serum albumin (BSA) in PBS prior to addition of serum samples. Biotinylated detection antibodies and horse radish peroxidase (HRP) conjugated streptavidin were subsequently added, and HRP colorimetric substrate (1:1 mixture of H_2O_2 and tetramethylbenzidine) development was assessed by absorbance at 450 nm. The threshold of sensitivity for ELISAs performed in this manner was 20 pg/ml for sVEGFR3 and 250 pg/ml for activinA. For all ELISA analyses, each serum sample was assessed in duplicate, and the concentration of each biomarker protein was determined by comparison to internally generated standard curves using recombinant protein. When levels of measured proteins exceeded that of the standard curve estimate, samples were appropriately diluted until absorbance measures fell within those of the standard curve to reliably estimate the concentration. Patients with levels below the threshold of sensitivity of the assay were assigned a value of 0 pg/ml for statistical analyses. Investigators performing the laboratory analyses were blinded to treatment arm, to the timing of the serum sample, and clinical outcome.

2.3. Statistical analysis

Exploratory statistical analyses were used to compare baseline demographic and disease characteristics between those eligible for biomarker analysis versus ineligible using Fisher's exact tests, χ^2 tests or Wilcoxon rank sum tests as appropriate. Similar analyses were performed to compare baseline biomarker levels between treatment arms amongst women who consented to the biomarker sub-study. The Spearman correlation coefficient was used to evaluate the relationship between biomarker values and selected baseline clinical characteristics and other biomarkers. The concordance correlation coefficient (CCC) was used to measure the reproducibility of biomarker levels. The CCC is a measure which not only measures the degree of association between values, but also whether the measures lie on the line of unity. The prognostic ability of each biomarker on PFS, OS and time-to-first SRE was evaluated using Cox proportional hazards regression. Initially, all biomarkers were assessed in univariable analysis, and then secondarily after adjusting for treatment arm and stratum (serum CTx <400 ng/L versus ≥ 400 ng/L). Lastly the predictive value of each biomarker was evaluated by investigating the potential interaction between each biomarker and treatment. As the estimated hazards ratio of an interaction is difficult to interpret, only the *p*-value is presented for simplicity. All tests were two-sided and a *p*-value of 0.05 or less was considered statistically significant, while a *p*-value of 0.1 to 0.05 was considered as trending towards significance.

3. Results

3.1. Baseline characteristics

Of the 129 women enrolled in Zamboney, 101 had samples available for analysis in the current study. Of these, 51 were in the vandetanib and 50 were in the placebo arm (Table 1). Mean age of consenting patients was 59.8 yrs. Median duration of prior bisphosphonate use at the time of enrolment was 12.9 months for 39 patients (duration unknown for 2 patients), while the remaining patients had no prior bisphosphonate use. Four of 28 (14.3%) patients without biomarker data had NYHA Functional

Table 2
Between treatment comparison of baseline biomarker levels.

	Vandetanib	Placebo	*p*-Value
Randomized	61	68	
Correlative data	51	50	
Mean (SD) VEGF-A pg/ml	365 (278)	308 (270)	0.21
Mean (SD) sVEGFR2 pg/ml	8080 (1837)	8111 (1632)	0.72
Mean (SD) sVEGFR3 pg/ml	32707 (18940)	32449 (14630)	0.82
Mean (SD) TGF-β1 pg/ml	18865 (6004)	18172 (5946)	0.87
Median (range) Activin-A pg/ml	1288 (155–81626)	1351 (155–70330)	0.93

Table 3
Association of biomarkers with clinical variables.

Variable	VEGF-A	sVEGFR2	sVEGFR3	TGF-β	ActivinA
uNTx	0.16	−0.04	−0.13	0.09	0.01
sVEGFR2	0.04	–	–	–	–
sVEGFR3	0.16	0.20	–	–	–
TGF-β	0.19	0.10	0.09	–	–
ActivinA	0.20	0.08	0.06	0.13	–
Months from initial diagnosis	0.09	−0.01	0.02	0.08	0.13
Number of SRE prior to study entry	0.10	−0.15	0.09	−0.08	0.07
Number of SRE on study	0.05	−0.11	−0.01	−0.08	0.00
Age	0.13	−0.32	-0.12	0.28	−0.05
Weight	−0.22	0.02	0.01	−0.06	0.14
BMI	−0.40	0.24	0.11	−0.18	0.18
Time from initial diagnosis to diagnosis of metastatic disease	0.07	0.03	0.14	0.06	0.08
Time from diagnosis of metastatic disease until study enrollment	0.06	−0.17	−0.17	−0.05	0.06
Systolic blood pressure	0.09	0.01	0.00	0.10	0.19
Diastolic blood pressure	0.09	0.23	0.00	0.00	0.18

Table 1
Baseline patient characteristics for those with versus without biomarker data.

Baseline characteristics		ELISA data ($n=101$)	No ELISA Data ($n=28$)	*p*-Value
Eligibility and stratum				
Treatment	Vandetanib	51 (50.5)	10 (35.7)	0.20
	Placebo	50 (49.5)	18 (64.3)	
Measurable disease	Absent	53 (52.5)	15 (53.6)	1.00
	Present	48 (47.5)	13 (46.4)	
Resistance to endocrine therapy	Tamoxifen. or AI for metastatic disease	74 (73.3)	21 (75.0)	0.98
	Tamoxifen or AI in adjuvant	15 (14.9)	4 (14.3)	
	Prior adjuvant endocrine therapy	12 (11.9)	3 (10.7)	
Serum CTx	<400 ng/L	64 (63.4)	18 (64.3)	1.00
	≥ 400 ng/L	37 (36.6)	10 (35.7)	
Demographics				
Months from initial diagnosis	Median (range)	96.3 (8.6–300.1)	81.9 (7.4–256.4)	0.53
ECOG performance status	0	53 (52.5)	16 (57.1)	0.91
	1	44 (43.6)	10 (35.7)	
	2	4 (4.0)	2 (7.1)	
NYHA classification	N (%) Class 1	1 (1.0)	4 (14.3)	0.008
Body-mass index	Mean (SD)	26.8 (5.7)	27.4 (6.0)	0.65
Age	Mean (SD)	59.8 (8.7)	58.4 (10.0)	0.66
Prior treatment				
Prior radiation therapy	N (%) Yes	84 (83.2)	25 (89.3)	0.56
Prior chemotherapy for metastatic disease	N (%) Yes	15 (14.9)	8 (28.6)	0.10

Table 4
Results of prognostic ability of biomarkers on outcomes.

Biomarker		Both treatment arms		Placebo arm only	
		Hazard Ratio (95% CI)	*p*-Value	Hazard ratio (95% CI)	*p*-Value
Progression-free survival					
VEGF-A/100 units	Univariable	0.99 (0.92–1.07)	0.81	0.97 (0.87–1.08)	0.60
		0.98 (0.91–1.05)	0.55	0.96 (0.85–1.07)	0.43
		–	0.74		
sVEGF-R2/1000 units	Univariable	0.91 (0.81–1.02)	0.11	0.81 (0.68–0.98)	0.027
	Adjusted for treatment and stratum	0.90 (0.80–1.01)	0.085	0.79 (0.65–0.95)	0.014
	Interaction with treatment	–	0.085		
sVEGF-R3/10,000 units	Univariable	0.93 (0.82–1.07)	0.31	0.92 (0.75–1.13)	0.43
	Adjusted for treatment and stratum	0.92 (0.80–1.06)	0.24	0.88 (0.71–1.08)	0.22
	Interaction with treatment	–	0.71		
TGF-β1/10,000 units	Univariable	1.00 (0.96–1.03)	0.87	1.01 (0.95–1.06)	0.82
	Adjusted for treatment and stratum	0.99 (0.96–1.03)	0.69	0.99 (0.93–1.05)	0.68
	Interaction with treatment	–	0.78		
Activin-A/1000 units	Univariable	1.00 (0.99–1.02)	0.76	1.00 (0.98–1.03)	0.84
	Adjusted for treatment and stratum	1.01 (0.99–1.02)	0.52	1.01 (0.99–1.03)	0.48
	Interaction with treatment	–	0.96		
Overall survival					
VEGF-A	Univariable	1.04 (0.93–1.17)	0.45	0.99 (0.84–1.17)	0.95
	Adjusted for treatment and stratum	1.03 (0.91–1.16)	0.68	0.99 (0.84–1.17)	0.92
	Interaction with treatment	–	0.72		
sVEGF-R2	Univariable	0.80 (0.64–0.99)	0.044	0.75 (0.56–1.01)	0.060
	Adjusted for treatment and stratum	0.77 (0.61–0.96)	0.020	0.72 (0.52–0.99)	0.043
	Interaction with treatment	–	0.24		
sVEGF-R3	Univariable	0.98 (0.79–1.20)	0.81	1.07 (0.78–1.46)	0.69
	Adjusted for treatment and stratum	0.95 (0.76–1.18)	0.62	1.00 (0.73–1.36)	0.97
	Interaction with treatment	–	0.64		
TGF-β1	Univariable	0.99 (0.94–1.06)	0.86	1.01 (0.93–1.10)	0.86
	Adjusted for treatment and stratum	0.97 (0.91–1.04)	0.43	0.98 (0.90–1.08)	0.72
	Interaction with treatment	–	0.60		
Activin-A	Univariable	0.98 (0.92–1.04)	0.44	0.98 (0.91–1.06)	0.59
	Adjusted for treatment and stratum	0.98 (0.93–1.04)	0.57	0.99 (0.91–1.07)	0.71
	Interaction with treatment	–	0.93		
Time to first skeletal event					
VEGF-A	Univariable	0.99 (0.86–1.13)	0.83	0.99 (0.83–1.17)	0.87
	Adjusted for treatment and stratum	0.97 (0.85–1.12)	0.72	0.98 (0.81–1.20)	0.86
	Interaction with treatment	–	0.78		
sVEGF-R2	Univariable	0.83 (0.67–1.04)	0.10	0.75 (0.55–1.03)	0.072
	Adjusted for treatment and stratum	0.82 (0.66–1.02)	0.079	0.73 (0.52–1.01)	0.057
	Interaction with treatment	–	0.41		
sVEGF-R3	Univariable	0.98 (0.78–1.22)	0.83	1.06 (0.75–1.50)	0.75
	Adjusted for treatment and stratum	0.96 (0.76–1.23)	0.76	1.05 (0.73–1.51)	0.80
	Interaction with treatment	–	0.48		
TGF-β1	Univariable	1.00 (0.94–1.08)	0.93	0.99 (0.90–1.09)	0.85
	Adjusted for treatment and stratum	0.99 (0.92–1.06)	0.80	0.97 (0.87–1.07)	0.51
	Interaction with treatment	–	0.75		
Activin-A	Univariable	0.99 (0.96–1.03)	0.75	1.00 (0.95–1.04)	0.85
	Adjusted for treatment and stratum	1.00 (0.96–1.04)	0.90	1.01 (0.96–1.05)	0.83
	Interaction with treatment	–	0.84		

Class 1 cardiac symptoms, compared with only 1 of 101 (1.0%) patients with biomarker data, a difference which was statistically significant (p-value=0.008). No other statistically significant differences were observed between those who consented and did not consent to the sub-study.

3.2. Biomarker measurements

ELISA measurements for the first and second duplicate samples were assessed for their accuracy in terms of reproducibility. For all markers, the Spearman correlation coefficient and concordance correlation coefficient were extremely high, exceeding 0.93 in all cases (data not shown). This indicates that the samples are highly reproducible, and one does not need to take samples in duplicate. Hence, only the first sample measurement was used as the measure of the biomarker level. Baseline biomarker levels were similar between patients on the vandetanib versus placebo arms with no apparent significant differences (Table 2).

The association of the measured biomarkers with various clinical parameters and with each other at baseline are presented in Table 3. The Spearman r was $\leq|0.40|$ for all measured comparisons, indicating a weak to no association between all biomarkers with each other and clinical characteristics.

3.3. Prognostic and predictive ability of biomarkers

The results evaluating the prognostic ability of each marker on PFS, OS and time to first SRE is presented in Table 4. Baseline sVEGFR2 was borderline significant as a prognostic marker for PFS, OS and time to first skeletal event. Adjusting for treatment and stratum, patients with higher sVEGFR2 trended towards improved PFS (HR=0.90/1000 units, 95% CI=0.80–1.01, p-value=0.085), improved time to first skeletal event (HR=0.82, 95% CI=0.66–1.02, p-value=0.079) and a statistically significantly improved OS (HR=0.77, 95% CI=0.61–0.96, p-value=0.020). Results were relatively similar when looking only at the placebo patients. No

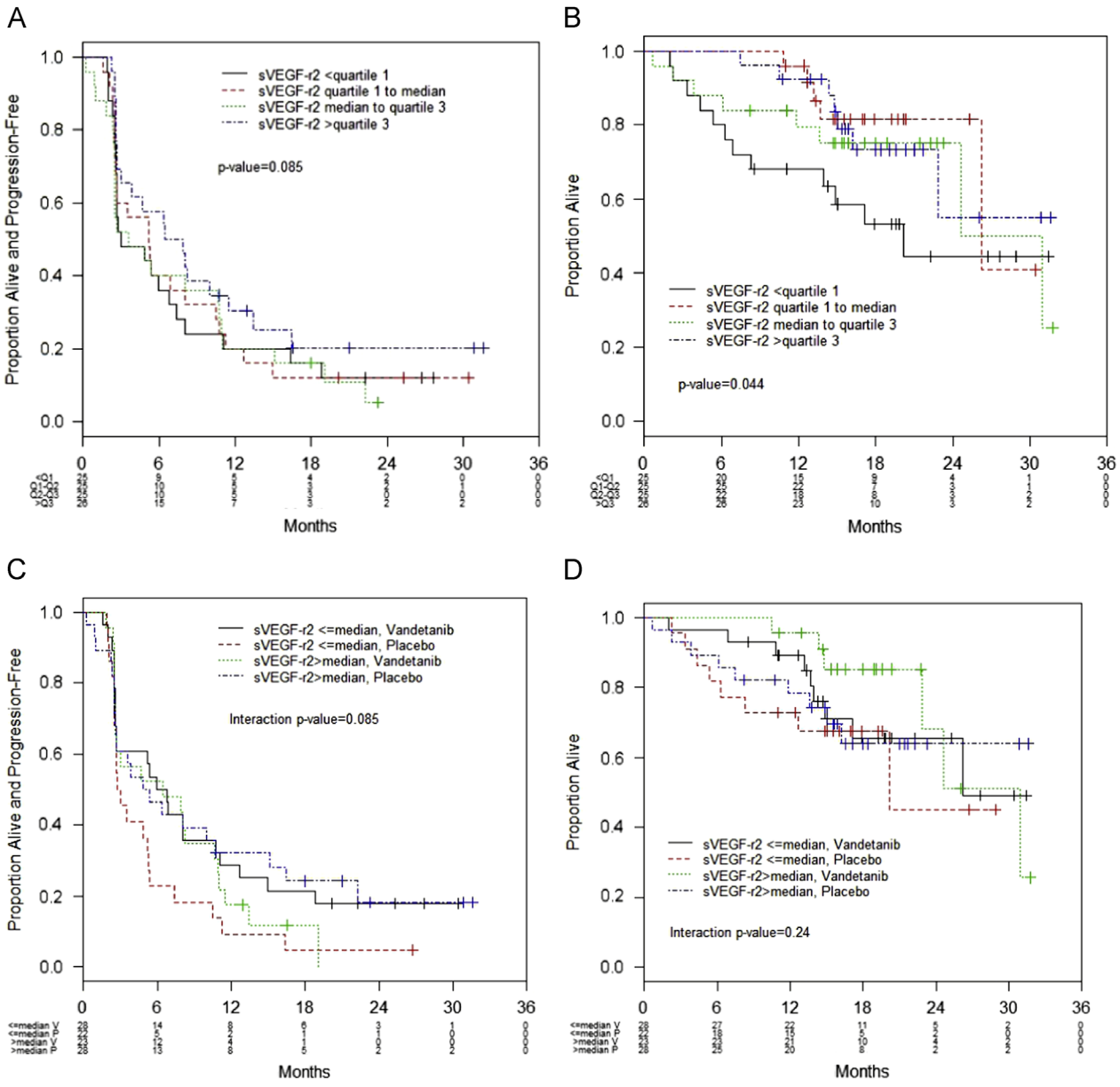

Fig. 1. Biomarker level associated with clinical outcome A) Progression Free Survival by sVEGFR2 Levels, B) Overall Survival by sVEGFR2 Levels, C) Progression Free Survival by Treatment Arm and sVEGFR2 Levels and D) Overall Survival by Treatment Arm and sVEGFR2 Levels

significant interaction effect was observed between sVEGFR2 and treatment for any outcome. These results are presented in Fig. 1 for OS and PFS outcomes based on plotting sVEGFR2 as quartiles. No other biomarker evaluated (VEGF-A, sVEGFR3, TGF-β1 or Activin-A) was significantly (p-value > 0.20 for all comparisons) associated with any outcome (PFS, OS, or time to first SRE).

4. Discussion

The Zamboney study investigated the effect of fulvestrant in combination with vandetanib or placebo in patients with bone predominant metastatic breast cancer.

A subgroup of 101 of patients consented to participate in a correlative marker sub-study, evaluating a number of angiogenic (VEGF-A, sVEGFR2, and sVEGFR3) and bone turnover (TGF-β, activinA) markers. In this subgroup of patients, higher baseline sVEGFR2 was statistically significant as a prognostic marker of improved OS, with trends towards significance as a prognostic marker for improved PFS and time to first SRE. sVEGFR2 has been shown to be prognostic in a number of other cancer types [24,25]. Our findings are similar to those recently reported from the AVADO trial which randomized HER2-negative locally recurrent and metastatic breast cancer patients to receive docetaxel with the anti-angiogenic agent bevacizumab [26]. In this study, higher plasma concentrations of sVEGFR2 were associated with better PFS response (HR=0.46, 95% C.I. (0.28–0.74), $p=0.03$) in bevacizumab treated patients compared to placebo; association of sVEGFR2 with OS was not reported. In contrast, low baseline sVEGFR2 levels were associated with longer PFS for patients on cediranib in hepatocellular carcinoma [24], and with greater benefit from cediranib treatment in colorectal cancer patients [25]. Median biomarker levels used to determine associations in these studies are similar to that used in our study (median sVEGFR2 of 5.9 ng/ml [24] in the hepatocellular carcinoma study and 11.6 ng/ml in the colorectal cancer study [25] compared to 8 ng/ml in our study). However, as neither hepatocellular nor colorectal cancers commonly metastasize to bone while it is the predominant site of

breast cancer metastases, it is perhaps not surprising that different associations are observed and the role of sVEGFR2 in progression of metastatic breast cancer remains unclear. It should also be noted that vandetanib targets alternative kinases compared to cediranib which primarily targets VEGFR1, 2 and 3, which may also contribute to the different observations in sVEGFR2 associations.

The role of sVEGFR2 in specifically regulating bone metastases is not well established. The VEGF ligand plays an important role in establishment of a vascular bed in the bone in addition to promoting survival and differentiation of resident bone cells [27]. Cell surface expression of VEGFR2 has been shown to increase during osteoclast differentiation from mononuclear precursor cells where it is the predominant receptor mediating VEGF signaling in osteoclasts [28,29]. As one of the prime mediators of bone destruction in metastatic breast cancer, it is likely that patients with high circulating sVEGFR2 may have reduced osteoclastogenic activity as a result of binding and sequestration of VEGF ligand from cell surface receptors on osteoclasts. As patients with increased osteolytic activity and bone turnover tend to have worse prognosis [30], higher levels of sVEGFR2 may inhibit VEGF-induced osteoclastogenic activity and osteolysis and hence contribute to better survival outcomes in bone metastatic breast cancer patients.

The finding that baseline VEGF in the Zamboney study was not associated with clinical outcomes are in contrast to other published studies. VEGF was previously shown to be associated with better treatment outcomes in metastatic breast cancer patients treated with bevacizumab [26,31]. This discrepancy may be due to the fact that 100% of patients enrolled in Zamboney had bone predominant metastases, while only ~60–70% of patients enrolled in the AVADO or AVEREL studies had metastases to bone. Alternatively, bevacizumab, which was used in the AVADO and AVERAL studies, is an antibody based therapeutic that binds VEGF ligands directly, while vandetanib is a receptor tyrosine kinase inhibitor that targets the VEGFR2 receptor along with other kinases. Also, as approximately 79% of Zamboney patients were either previously or concurrently treated with bisphosphonates at the time of study entry, this may further confound results, as bisphosphonate administration can result in decreased VEGF levels in patients [32]. The majority of Zamboney patients were also previously treated with either tamoxifen, which causes platelet release of VEGF, or aromatase inhibitors which have no effect on VEGF release [33]. This could also confound the results for VEGF levels obtained in serum at baseline study entry depending on whether patients were given tamoxifen versus aromatase inhibitors. The lack of association of the alternative measured bone biomarkers TGF-β or activinA with clinical outcome is supported by our recent findings in similar patient cohorts in other studies [34,35]. It is important to note that many of the putative predictive and pharmacodynamic markers used to assess response to anti-angiogenic therapies are differentially affected by concurrent treatments, and as such great care should be taken to consider these variables when performing such biomarker measures.

The current sub-study is purely exploratory in nature and the current biomarker hypotheses were not pre-specified, nor was the study powered to detect significant differences in biomarkers or their associations. As such these results should be considered hypothesis generating. Our sample sizes are also relatively small, and due to study design, we were limited to measuring circulating biomarker levels at baseline. Further, over 20% of study patients declined participation in the correlative sub-study. Many studies have suggested that changes in biomarkers from baseline to on treatment are more predictive of treatment response [36–39]. In the current sub-study we were unable to interrogate this question, however given the possible association of sVEGFR2 with clinical outcome in bone metastatic breast cancer patients treated with an anti-angiogenic agent, changes in sVEGFR2 levels from baseline to on treatment should be evaluated in bone metastatic breast cancer patients in the future.

5. Conclusions

In this hypothesis-generating study, sVEGFR2 was identified as a potentially important candidate biomarker for assessing clinical outcome in bone predominant metastatic breast cancer patients. Given the lack of supporting information regarding its role in the bone metastatic microenvironment, studies to determine its effect on bone metastasis progression and to validate its use as a biomarker of clinical response are warranted.

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