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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

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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

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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.