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Research Paper

A phase 2 trial exploring the clinical and correlative effects of combining doxycycline with bone-targeted therapy in patients with metastatic breast cancer

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ABSTRACT

Background: Bone-targeting agents (BTAs), such as bisphosphonates and denosumab, have demonstrated no discernable effects on tumour response or disease free/overall survival in patients with bone metastases from breast cancer. Doxycycline is both osteotropic and has anti-cancer effects. When combined with zoledronate in animal models, doxycycline showed significantly increased inhibition of tumour burden and increased bone formation. We evaluated the effects of adding doxycycline to ongoing anti-cancer therapy in patients with metastatic breast cancer.

Methods: Breast cancer patients with bone metastases and ≥ 3 months of BTA use, entered this single-arm study. Patients received doxycycline 100 mg orally, twice a day for 12 weeks. The co-primary endpoints were; effect on validated pain scores (FACT-Bone pain and Brief Pain Inventory) and bone resorption markers (serum C-telopeptide, [sCTx]). All endpoints (pain scores, sCTx, bone-specific alkaline phosphatase, skeletal-related events, toxicity) were evaluated at baseline, 4, 8 and 12 weeks. Bone marrow was sampled at baseline and week 12 for exploratory biomarker analysis.

Results: Out of 37 enroled patients, 27 (73%) completed 12 weeks of therapy. No significant changes were seen in pain scores or bone turnover markers. Failure to complete treatment: drug toxicity (70%) and disease progression (30%). Sixteen (43%) patients had GI adverse events.

Conclusions: Doxycycline 100 mg twice daily for 12 weeks had no significant effects on either bone pain or bone turnover markers. Its toxicity profile in this patient population would make further evaluation challenging.

Translational relevance

This is the largest study to date evaluating the effects of doxycycline in bone-metastatic breast cancer patients. Doxycycline daily for 12 weeks did not appear to significantly enhance palliative benefit nor change bone resorption markers. The toxicity profile of doxycycline in metastatic breast cancer patients will make further evaluation challenging.

1. Background

The biological behaviour of bone metastases causes an uncoupling of the actions of osteoclastic and osteoblastic cells, resulting in increased in bone turnover [1]. In clinical practice the main mechanism of action of bone-targeting agents (BTAs) (e.g. bisphosphonates or denosumab) has been through osteoclast inhibition [1,2] with resulting reduction in skeletal-related events (SREs) [3]. Despite numerous studies reporting direct anti-tumour

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and anti-metastatic activities of bisphosphonates in preclinical models, large randomized placebo-controlled trials in patients with metastatic breast cancer have shown no evidence of improvement in terms of response rate, progression free or overall survival [4,5]. One strategy to enhance the direct anti-tumour activities of BTAs in the metastatic and adjuvant settings might involve the addition of the widely available, safe, and inexpensive drug, doxycycline.

Doxycycline, a tetracycline analogue, is osteotropic with a high affinity for mineralised bone. In experimental systems, it has demonstrated anti-cancer effects including inhibition of matrix metalloproteinases, anti-angiogenesis and cytostatic effects on cancer cells [6]. Preclinical bone metastasis models have shown that doxycycline could directly inhibit tumour growth, induce bone reformation [7] as well as increase inhibition of tumour burden and increase bone formation when combined with zoledronate [8]. In addition to preclinical data, doxycycline has also undergone evaluation as an anti-cancer agent in Phase 1 trials and in breast cancer patients with newly diagnosed bone metastases prior to commencement of bisphosphonate therapy [9]. These studies suggest that the addition of doxycycline to a bisphosphonate regimen in breast cancer patients may work synergistically to enhance the direct anti-tumour effects and potentially result in increased patient benefit.

We initiated a phase II, single-arm study, where we hypothesised that in women with bone metastases from breast cancer, the addition of doxycycline to their standard BTA therapy would result in significant palliative benefits as a result of inhibition of tumour progression and osteolysis. Through the prospective collection of serum, urine and bone marrow samples, putative mechanisms of action would be explored.

2. Materials and methods

2.1. Objectives

This study was designed to evaluate the effect of adding doxycycline 100 mg orally twice a day for 12 weeks to ongoing anti-cancer therapy in women with breast cancer and bone metastases. The primary objective was to explore the potential palliative benefit of doxycycline in this population. Secondary study objectives included: effects on bone turnover markers, potential associations between bone resorption/formation markers, apoptosis and proliferation with palliative or anti-tumour response, and the ability to complete therapy, including toxicity and safety.

2.2. Study population

Patients with metastatic breast cancer with radiologically and/or biopsy confirmed bone metastases who had received ≥ 3 months of BTA therapy (e.g. bisphosphonate or denosumab) were enroled. Patients had to have an ECOG ≤ 2, a life expectancy > 3 months and no changes in systemic anti-cancer therapy for 4 weeks prior to study entry or anticipated changes in the 4 weeks after entering the study. The study was approved by the Ottawa Health Science Network Research Ethics Board and registered with clinicaltrials.gov [10].

2.3. Trial design

All study participants received doxycycline 100 mg orally twice a day for 12 weeks. Participants were provided with a paper diary to record their compliance with doxycycline. Data on self-reported bone pain was measured using 2 validated questionnaires: the Brief Pain Inventory (BPI)-worst pain score [11,12] and Functional Assessment of Cancer Therapy-Bone Pain (FACT-BP) [13,14]. Pain, analgesic use, toxicity, and occurrence of SREs (defined as radiotherapy or surgery to the bone, pathological fractures, spinal cord compression, or hypercalcemia) were assessed at baseline and weeks 4, 8 and 12. Baseline fasting serum c-telopeptide (CTX, a collagen fragment released as a result of tumour-induced bone degradation, and hence a surrogate marker of tumour-induced osteolysis), bone specific alkaline phosphatase (BSAP), parathyroid hormone (PTH) and vitamin D (25-OH-vit D) were also measured. Serum CTx and BSAP were assessed at weeks 4, 8 and 12. At baseline and week 12, bone marrow aspirate and trephine biopsy were performed from the posterior iliac crest. If tumour cells were present in the bone marrow specimen, ER, PR (by immunohistochemistry) and Her2 analysis (by FISH) and a marker of proliferation (Ki67) were measured. Patients could also optionally consent to the collection of plasma, serum and urine samples (baseline, weeks 4, 8 and 12) for future translational research studies.

All patients were advised to take calcium (1200–1500 mg/day) and vitamin D3 (800–1000 IU/day) while on study. Given that this study is pragmatic, all other assessments (e.g. scans, blood work) were at the treating physician's discretion.

2.4. Laboratory analysis

Blood was drawn in the morning following an overnight fast [15,16]. Samples were allowed to clot and were centrifuged at 4 °C for 10 min at 3400 RPM. Urine was collected as a second pass, fasting specimen. Both were frozen at −80 °C until analysis. Serum CTx, BSAP, 25-hydroxyvitamin D and PTH were measured by chemiluminescence immunoassay: CTx using CrossLaps® on an IDS iSYS automated analyzer, BSAP using Ostase®, on the Beckman Coulter Unicel DxI and 25-OH-VitD on the IDS iSYS and PTH on the Beckman Coulter Unicel DxI..

All Immunohistochemistry (IHC) testing was done on the Leica-Bond Platform. IHC for Oestrogen receptor was done using the 6F11 clone (Leica) at 1/150 with Heat Induced Epitope Retrieval (HIER) in Bond epitope retrieval solution-1 (citrate buffer, pH 6.0). IHC for Progesterone receptor was done using the PR clone 16 (Bond ready to use) with HIER in Bond epitope retrieval solution-2 (EDTA, pH 9.0). IHC for Ki67 scoring was done using the MIB-1 clone (DAKO) at 1/75 with HIER in Bond epitope retrieval solution-2. ER and PR positive is defined according to ASCO guidelines as $\geq 1\%$. Ki67 score was expressed as a percentage of cells positive (all cells available counted).

2.5. Statistical analysis

The co-primary endpoints for this study was palliative pain response at 3 months, based on the BPI questionnaire (BPI-worst), analgesic use, FACT-BP score and bone resorption markers. As with previous studies in this patient population, [17,18] complete response for palliative pain response was defined as a pain score of zero at the index site with no concomitant increase in analgesic intake (stable or reducing analgesics in daily oral morphine equivalents). Partial response was defined as either a pain reduction of 2 or more at the index site on 0–10 scale without analgesic increase, or analgesic reduction of 25% or more from baseline without an increase in pain. Pain progression was defined as an increase in pain score of 2 or more points above baseline at the index site with stable analgesic use or an increase of 25% or more in daily oral morphine equivalent compared with baseline, with pain score stable or 1 point above baseline. Pain response was also evaluated using the FACT-BP. Pain decrease was measured as a 10% decline compared to baseline, and pain progression was defined as a 10% increase in the FACT-BP compared to baseline.

The change in CTx was defined as a co-primary endpoint, as decreases in CTx were previously observed to correlate with reduction in bone pain [15,16,19,20]. Change was calculated as (on-study CTx – baseline CTx)/baseline CTx*100%.

Summary statistics were used to describe baseline characteristics and outcomes. Change from baseline for outcomes at different time points was calculated as (on-study value – baseline value) and tested using the Wilcoxon rank sum test or *t*-test as appropriate. Logistic regression analyses were used to evaluate baseline characteristics for potential prognostic ability for completion of study therapy and their prognostic ability of the patients' response on BPI-worst at week 12. Stepwise selection (forward) process was used to construct a multivariable model of prognostic factors. A p-value=0.20 was chosen as the entry criteria for building the multivariable model, which allowed for more factors to enter into the model but could result in some over-fitting of the data, to ensure all potentially prognostic factors were included. The categories of adverse events were chosen on the basis of the anticipated pharmacological profiles of doxycycline and are summarised by AE grade for each patient. Some variables were grouped for statistical power purposes. All tests were two-sided, a p-value of 0.05 or less was considered statistically significant and all analyses performed in SAS version 9.2 (SAS Institute, Cary, NC, USA).

2.6. Sample size

As with previous studies, a two-point reduction in the BPI score from baseline to week 12 was considered a pain response, thus, a two-point reduction was considered to be clinically important. The estimated standard deviation of the change in BPI was 4 (approximately one-quarter of the potential range), therefore, a two-sided, $\alpha=0.05$, one-sample *t*-test would achieve $>80\%$ statistical power, with a total of 34 patients (NCSS-PASS version 8.0.15). Further, this sample size would attain $>90\%$ statistical power to detect an effect size of 0.5 or greater in the change in CTx from baseline and in the change in FACT-BP. Assuming a modest 10% drop out rate from previous trials of this nature [20–22], the targeted sample size was therefore set to 37 patients.

3. Results

3.1. Patient enrolment and baseline characteristics

From April 2013 to May 2015, 55 patients were approached to enter this study, of which 37 patients consented and enroled (Fig. 1). Baseline patient characteristics are shown in Table 1. The median age was 60 (range 42–88), the median duration of bone metastases was 13 months (range 4–94) and median use of prior IV pamidronate was for 10.2 months (range 3.1–67.8) at baseline. At the time of study entry the anti-cancer therapy the patients were receiving were; endocrine therapy (20 patients), chemotherapy (15 patients) and chemotherapy with trastuzumab (2 patients).

No patients were receiving denosumab. Of enroled patients, 20/37 (54.1%) had at least one SRE prior to study entry and 27 (76%, 61.9–89.5%) completed 12 weeks of study therapy. Ten patients did not complete the 12 weeks of study, of these; 7 (70%) withdrew by their own choice due to adverse events and 3 (30%) were withdrawn by their physician due to disease progression.

3.2. Pain reduction

At baseline, patients rated their worst pain in the last 3 days (measured by BPI-worst) as a median score of 2 (range=0–9), with a mean (SD) FACT-BP of 47.2 (12.7). For those patients who had pain scores at baseline and each of various time points, there were no significant changes in either FACT-BP or BPI-worst from baseline to any on-study time point (Table 2). At week 12, the mean (SD) change from baseline in FACT-BP was 1.22 (8.52) and in BPI-worst was −0.16 (2.36), with p-values of 0.48 and 0.74 respectively.

Five patients had a complete pain response (though 4 of these 5 scored a 0 on their BPI-worst at baseline and were taking no analgesics at baseline), 5 patients had a partial pain response, and

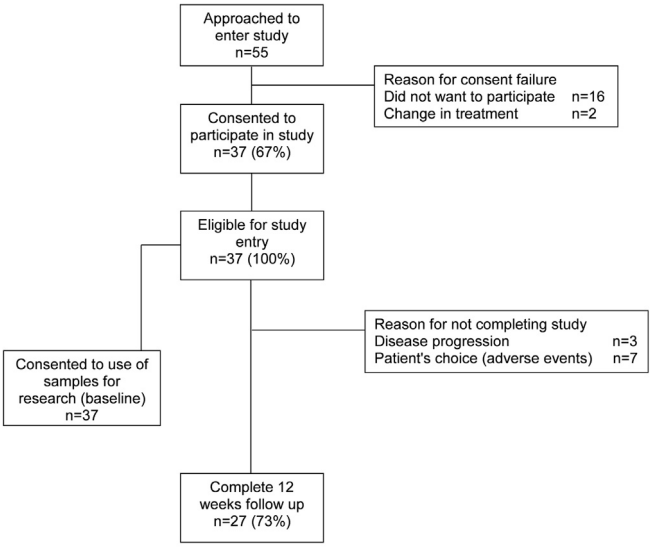

Fig. 1. CONSORT diagram.

Table 1
Baseline characteristics.

		All patients	Missing number
N		37	
Age	Median (range)	60 (42, 88)	0
Duration of bone metastases (*n*=x), months	Median (range)	13 (4, 94)	0
PTH	Median (range)	5 (1.7, 18.3)	0
Vitamin D	Median (range)	91 (48, 185)	0
Months, taking bisphosphonates prior to starting study	Median (range)	10.23 (3.1, 67.8)	0
Baseline CTx	Median (range)	140 (30, 700)	0
Baseline BSAP (*n*=x)	Median (range)	9.2 (4.4, 39.3)	0
FACT-BP subscale	Mean (sd)	46.64 (12.90)	3
BPI-severity (*n*=x)	Median		4
Worst pain in last 3 days	(range)	2 (0, 9)	
Average pain in last 3 days		1 (0, 7)	
Pain right Now		0 (0, 8)	
Pain interfered with activities		3 (0, 23)	
Total number of SRE prior to study entry	0	17 (45.9%)	0
	1	3 (8.1%)	
	2	3 (8.1%)	
	3	3 (8.1%)	
	4	5 (13.5%)	
	≥5	6 (16.2%)	

BPI=Brief pain inventory; BSAP=Bone specific alkaline phosphatase; CTx=serum C-telopeptide.

Table 2
Change of FACT-BP and BPI from baseline.

	Time point	N	Mean (SD) subscale score	Mean (SD) change from baseline	P-value	N (%) who had an improvement in subscale score of >=10% over baseline (FACTBP)	N (%) who had 2 or more increase in BPI-worst	N (%) who had 2 or more decrease in BPI-worst
FACT-BP subscale	1 (Baseline)	35	46.64 (12.90)	–	–	–		
	2	35	47.66 (11.44)	0.14 (5.36)	0.88	7(21.2%)		
	3	31	48.03 (9.56)	0.67 (5.86)	0.54	6 (20.7%)		
	4	27	47.04 (10.06)	1.22 (8.52)	0.48	6 (24.0%)		
BPI-worst	1 (Baseline)	33	2.64 (2.66)	–	–		–	–
	2	34	2.65 (2.44)	0.06 (1.46)	0.81		6 (18.8%)	2 (6.3%)
	3	32	2.63 (2.18)	0.03 (2.64)	0.94		6 (20.7%)	5 (17.2%)
	4	28	2.86 (2.22)	-0.16 (2.36)	0.74		6 (24.0%)	6 (24.0%)
BPI-average	1 (Baseline)	33	1.61 (1.77)	–	–		–	–
	2	34	1.65 (2.09)	0.03 (1.31)	0.89		2 (6.3%)	2 (6.3%)
	3	31	1.55 (1.43)	-0.07 (1.44)	0.80		2 (6.9%)	2 (6.9%)
	4	28	1.75 (1.32)	-0.08 (1.61)	0.81		2 (8.0%)	3 (12.0%)
BPI-now	1 (Baseline)	33	1.33 (2.03)	–	–		–	–
	2	34	1.26 (1.96)	-0.19 (1.57)	0.51		2 (6.3%)	1 (3.1%)
	3	32	1.03 (1.20)	-0.38 (1.80)	0.27		2 (6.9%)	5 (17.2%)
	4	28	1.54 (1.88)	-0.28 (1.81)	0.45		2 (8.0%)	2 (8.0%)
BPI-interference	1 (Baseline)	33	5.58 (5.95)	–	–		–	–
	2	34	5.56 (6.13)	-0.09 (3.79)	0.89		8 (25.0%)	6 (18.8%)
	3	32	5.16 (4.36)	-0.41 (5.32)	0.68		11 (37.9%)	8 (27.6%)
	4	28	6.11 (5.07)	-0.56 (4.92)	0.57		7 (28.0%)	7 (28.0%)

4 had stable pain. Ten of the twelve patients who could not be evaluated had withdrawn from the study, while four patients had missing baseline measures. Considering these 12 patients as 'failures to respond', the overall pain response rate was 10/37 (27%, 95% CI=14–44%). Only 3/37 (8%, 95% CI=2% to 29%) of evaluable patients had a pain response according to FACT-BP.

3.3. CTx and BSAP

Median baseline serum CTx for all patients was 140 ng/L (range 30–700 ng/L, Table 1). No significant changes in CTx were observed from baseline to any time point (see Table 3). By week 12, 29 patients had measured CTx and the median (1st and 3rd interquartile) change was 24 (−50, 73) (p-value=0.23). Similarly, the median (1st and 3rd interquartile) change from baseline in BSAP was an increase of 0.3 (−0.2, 1.3), which was not statistically significant (p=0.18).

3.4. Toxicity

Toxicity was common, with 7 patients coming off study early due to adverse events. Table 4 summarizes the main adverse event data. Out of 37 patients 16 (43%) had GI adverse events (nausea, vomiting and/or heartburn). The median (and range) of time from starting doxycycline to development of GI toxicity was 9 (0–60) days. The most common adverse events were nausea (n=16 or 43.2%), vomiting (n=7 or 18.9%), fatigue (n=6 or 16.2%) and diarrhoea (n=5 or 13.5%). One grade 3 adverse event was experienced by each of 3 different patients (1 nausea, 1 vomiting and 1 diarrhoea).

Four patients had SREs (all palliative radiotherapy for painful bone metastases) during the study.

Table 3
Change of sCTx and BSAP from baseline.

	Time point	N	Median (1st and 3rd interquartile)	Median (1st and 3rd Interquartile) change from baseline	p-value
sCTx	1 (Baseline)	37	140.0 (90.0, 310.0)	–	–
	2	34	130.0 (85.0, 318.5)	9.5 (−39, 46.5)	0.39
	3	32	134.0 (88.8, 283.0)	21.5 (−28.3, 59)	0.18
	4	29	151.0 (81.0, 258.0)	24 (−50, 73)	0.23
BSAP	1 (Baseline)	37	9.2 (7, 11.9)	–	–
	2	33	9.4 (7.2, 12.2)	0.1 (−1.6, 0.6)	0.93
	3	32	8.4 (7.0, 13.1)	−0.1 (−0.9, 1.3)	0.91
	4	29	8.9 (6.8, 11.9)	0.3 (−0.2, 1.3)	0.18

BSAP=Bone specific alkaline phosphatase μg/L; CTx=serum C-telopeptide ng/L.

Table 4
Adverse event data.

Adverse event (AE)[a]	Number (%) of patients having adverse events			
	AE grade1	AE grade2	AE grade ≥ 3	Total
Nausea	1 (2.7%)	14 (37.8%)	1 (2.7%)	16 (43.2%)
Vomiting	0 (0%)	6 (16.2%)	1 (2.7%)	7 (18.9%)
Fatigue	0 (0%)	6 (16.2%)	0 (0%)	6 (16.2%)
Diarrhoea	0 (0%)	4 (10.8%)	1 (2.7%)	5 (13.5%)
Headaches	0 (0%)	3 (8.1%)	0 (0%)	3 (8.1%)
Photosensitivity	0 (0%)	3 (8.1%)	0 (0%)	3 (8.1%)
Rash	0 (0%)	3 (8.1%)	0 (0%)	3 (8.1%)
Heartburn	0 (0%)	2 (5.4%)	0 (0%)	2 (5.4%)
Anorexia	0 (0%)	1 (2.7%)	0 (0%)	1 (2.7%)
Oral Mucositis	0 (0%)	1 (2.7%)	0 (0%)	1 (2.7%)
Malaise	0 (0%)	1 (2.7%)	0 (0%)	1 (2.7%)
Hypercalcaemia	0 (0%)	0 (0%)	0 (0%)	0 (0%)

[a] As defined by the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) Version 4.02.

3.5. Bone marrow specimens

Of 37 patients, 36/37 (97%) completed a baseline biopsy and 25/37 (68%) completed a week 12 bone biopsy. One baseline biopsy was attempted but could not be completed (technical reasons). Twelve patients (32%) did not complete the 12 week biopsy: 8 of whom did not complete the 12 week study, 2 declined, and 2 biopsies were attempted but could not be completed (patient body habitus). Of 36 baseline biopsies, 6 (17%) were positive for metastatic carcinoma and Ki67 results were 42%, 4.1%, 16.9%, 32.6%, 11.4% and 14%. Of 25 week 12 biopsies, 3 were positive for tumour cells and Ki67 results were 23%, 51% and 36.30%. Unfortunately, only one patient had paired results for baseline (Ki67 32.6%) and week 12 (36.30%).

Table 5
Prognostic factors for changes in FACT-BP and BPI-worst scores.

	Outcome = FACT-BP at 12 weeks			Outcome = BPI-Worst at 12 weeks		
	N	Odds ratio (95% CI)	p-value	N	Odds ratio (95% CI)	p-value
Univariable analyses						
Duration of bone mets (log)	36	0.90 (0.34, 2.38)	0.83	37	0.81 (0.31, 2.13)	0.67
PTH (log)	36	0.92 (0.23, 3.72)	0.91	37	1.28 (0.31, 5.33)	0.73
Vitamin D (log)	36	0.30 (0.03, 2.98)	0.31	37	0.95 (0.12, 7.72)	0.96
Month taking bisphosphonates (log)	36	1.51 (0.50, 4.51)	0.47	37	0.98 (0.36, 2.65)	0.96
Baseline CTx (log)	36	0.66 (0.25, 1.74)	0.40	37	0.41 (0.14, 1.17)	0.097
Baseline BSAP (log)	36	0.12 (0.02, 0.82)	0.031	37	0.15 (0.02, 0.93)	0.042
Number of prior SRE of any type	36	1.23 (0.82, 1.83)	0.32	37	1.05 (0.74, 1.48)	0.79
Baseline FACT-BP Subscale	33	0.96 (0.89, 1.04)	0.30	34	0.97 (0.90, 1.04)	0.42
Baseline BPI-severity (worst)	32	1.09 (0.79, 1.52)	0.59	33	1.08 (0.78, 1.50)	0.63
Multivariable analyses						
Number of prior SRE of any type	30	2.35 (1.05, 5.25)	0.037	31	1.44 (0.90, 2.31)	0.131
Baseline BSAP (log)		0.01 (0.00, 0.37)	0.016		0.08 (0.01, 0.93)	0.044
Vitamin D (log)		0.05 (0.00, 2.99)	0.148		–	–

3.6. Correlative analyses

In univariate analyses, only baseline BSAP (log transformed) was significantly associated with completion of therapy (Table 5). Patients with lower baseline BSAP were more likely to complete treatment (Odds Ratio [OR] = 0.12, 95% confidence interval [CI] = 0.02–0.82, p-value = 0.031, on the log scale). Number of prior SRE of any type (OR = 2.35, 95% CI = 1.05–5.25), baseline BSAP (OR = 0.01, 95% CI = 0.00–0.37) and Vitamin D (OR = 0.05, 95% CI = 0.00–2.99) all entered a multivariable regression model with entry criteria of p = 0.15.

Baseline BSAP (log transformed) was univariately prognostic for BPI-worst score at week 12 (Table 5). Lower baseline BSAP were more likely to have response on BPI-worst at week 12 (OR = 0.15, 95% = 0.02–0.93, p = 0.042). It is notable that baseline CTx approached statistical significance (p = 0.097). Lower baseline BSAP (log transformed) (OR = 0.08, 95% CI = 0.01–0.93) and increased number of prior SRE of any type (OR = 1.44, 95% CI = 0.90–2.31) were both prognostic for increased likelihood of response on BPI-worst in the multivariable model.

4. Discussion

Inhibitors of osteoclast function such as bisphosphonates and denosumab are widely used in the care of patients with metastatic bone disease. However, despite preclinical studies showing that bisphosphonates have both direct [23] and indirect anti-tumour [23], clinical studies have not demonstrated any discernable effects of bisphosphonates on either response, progression free or overall survival [24]. We speculated that there are likely other pathways that could be blocked to enhance the efficacy of BTA therapies both in the metastatic and adjuvant settings.

Based on previous preclinical studies suggesting that treatment with the osteotropic antibiotic doxycycline resulted in increased inhibition of tumour burden and increased bone formation when combined with zoledronate [8], clinical studies assessing its efficacy in combination with BTAs were initiated. However, these studies in cancer patients are distinct from the established role of doxycycline (100 mg PO BID) as an antibiotic in the management of rare types of lymphoma [25]. A completed Phase 1 trial of 14 patients with a range of advanced cancers assessed the safety and pharmacology of doxycycline and confirmed drug safety. [26] While no objective responses were observed in this heavily pretreated cancer population, doxycycline concentrations reached steady state levels by day 8. With mean trough levels of >20 μM, the authors felt these concentrations were comparable to those required for in vitro anti-tumour activity [6]. A subsequent study in 12 breast cancer patients with newly diagnosed bone metastases prior to commencement of bisphosphonate therapy used the urinary N-telopeptide (uNTx) bone turnover marker to assess the effects of doxycycline (100 mg PO BID) over a 3 month period [9]. There were no pathological fracture or hypercalcaemia events, serum PTHrP levels (used in this study as a biomarker of tumour growth) declined or remained stable after 12 weeks of therapy in 9/12 patients. Urinary NTx fell in half of the patients and the magnitude of this fall (defined as a 50% relative fall compared to baseline) failed to satisfy the pre-specified outcome measure. Study results suggested that not only does doxycycline (single agent) have positive effects on bone turnover markers but also that this dose appeared to be well tolerated in breast cancer patients with bone metastases. Unfortunately this study is only presented in abstract form and no data is available to evaluate whether these changes in NTx could actually have been due to patients starting anti-cancer therapy [9].

Based on these previous findings and data supporting enhanced anti-tumour activity of doxycycline in combination with zoledronate in preclinical models, our study hypothesised that for women with bone metastases from breast cancer the addition of doxycycline to their standard BTA therapy would result in significant palliative benefits by preventing bone turnover and tumour progression. Unfortunately, despite using similar doses to those used in previous studies, no significant effects on pain, or markers of bone breakdown (i.e. sCTX) or bone formation (i.e. BSAP) were seen. In contrast to the previous study in bisphosphonate-naïve breast cancer patients, our study only enroled patients previously treated with bisphosphonates. The reason we required that patients must have received their prior bisphosphonate therapy for at least 3 months was based on previous studies that have shown that in most patients this is the time required for the maximum fall and stabilisation of bone turnover markers such as CTx and NTx [15,22]. If we had entered patients on study sooner we would not have been aware of whether or not any fall in biomarker levels was due to the bisphosphonate or the doxycycline. Similarly we have also demonstrated previously that a change in anti-cancer agent therapy can also cause a fall in bone biomarkers [16,19]. So again patients could not have a change in anti-cancer therapy for 4 weeks prior to study entry or anticipated changes in the 4 weeks after entering the study. Similarly one would not have anticipated that bone turnover markers would begin to fall after a minimum of 12 weeks of therapy, hence this time point was chosen for the duration of the study as it has been shown to be robust time point in previous studies [21,27]. As such it is possible that previous bisphosphonate treatment alters the

bone microenvironment sufficiently to impair the previously observed anti-tumour effects of doxycycline.

The effects of this treatment on tumour cells was also assessed by evaluation of bone marrow specimens. The proportion of samples that contained identifiable tumour cells was quite low, with tumour cells being seen in only 17% of baseline bone marrow specimens and 12% of the week 12 specimens. Only one patient had tumour cells in paired specimens precluding meaningful proposed analyses pre and post-doxycycline treatment to assess effects on tumour growth.

Toxicity was significant and limiting in this patient population. Drug toxicity was by far the most common reason for patients failing to complete treatment. Out of 37 patients, 16 (43%) had GI adverse events (nausea, vomiting and/or heartburn), and this became limiting for 7 of these patients requiring them to come off study early. Once GI toxicity started it was our clinical impression that it persisted despite lowering the dose of doxycycline. As part of an ad hoc analysis we decided to evaluate the time to development of GI toxicity. The median (and range) of time from starting the doxycycline to development of GI toxicity was 9 (0–60) days, this means that potential future studies evaluating this agent would be significantly limited in this patient population.

An important questions remain as to why the clinical results have not replicated the preclinical data. This can likely be divided into a few key components. First, the fundamental limitations of animal models in bone metastasis research, the second reflecting the limitations of the preclinical research with doxycycline and finally the realities of studies performed in humans. With respect to animal models of bone metastasis behaviour our group and others have reported that preclinical models frequently do not reflect the presence of pre-existing bone metastases and their treatment [28]. For example, the preclinical data for the current study was developed in a breast cancer model of bone metastasis using xenografts used doxycycline releasing pellets implanted subcutaneously in animals 3 days prior to seeding of the bone with breast tumour cells (MDA-MB-231) via intracardiac injections with zoledronic acid injections every 2 days thereafter [8]. The release of doxycycline 3 days prior to seeding of the bone with tumour cells in the preclinical model, may have resulted in modifications of the bone microenvironment that delayed tumour initiation and growth resulting in the observed therapeutic effect at endpoint. This perhaps better reflect effects of this combination treatment on prevention of bone metastasis or inhibition of early disseminated micrometastases as opposed to therapeutic effects on advanced bone metastases as would be the case for the patients enroled in this study.

With respect to the realities of translating preclinical data into studies in humans it is important to remember that animal model are genetically homogeneity whereas patients are heterogeneous with tumours that are also heterogeneous. Because of the well-recognised effects of bisphosphonates on bone turn over markers the clinical study required that patients have received at least three months of bisphosphonate prior to commencing doxycycline. It is possible that pretreatment with bisphosphonates could result in different effects of doxycycline being observed, as many drugs are known to have synergistic effects only if given sequentially instead of concurrently or after the initial drug. As such is remains possible that doxycycline delivered prior to bisphosphonates in the preclinical model sensitised bone metastases to the bisphosphonates, however when given after bisphosphonates, this effect is ameliorated. In addition, in the clinical study, all patients received pamidronate and not zoledronic acid. As zoledronic acid has been shown to be superior to pamidronate in controlling bone turnover in other clinical studies, it is possible that the results are driven by the lack of effect of doxycycline in combination with pamidronate, which was not tested preclinically [22]. Finally, in the clinical trial patients were allowed to be on other concurrent anti-cancer therapies. The preclinical model did not use any combinations with chemotherapy or endocrine therapy and in fact supplemented with oestrogen releasing pellets (even though breast cell line was triple negative). Although some evidence that doxycycline can enhance efficacy of chemotherapies such as cisplatin [29] it is not clear whether or not anti-cancer therapies in the patients could have altered the effects of doxycycline on the bone.

Limitations include the use of surrogate markers of palliative benefit (i.e. pain scores and serum CTx) as co-primary endpoints. Surrogate markers were needed as the sample size is too small for radiological response to be a useful study endpoint. The limitations of pain scores in patients with bone metastases are well recognised [15,19,20,22]. For these reasons many studies of BTAs in cancer patients with bone metastases also use biochemical markers of bone turnover (i.e. uNTX, sCTX). These have been previously correlated with; pain severity, response to treatment, decrease in SREs and survival [30–32]. Similarly, a number of groups are also evaluating the role of bone formation markers such as P1NP (Procollagen type I amino-terminal propeptide) and BSAP as biomarkers of BTA response [33]. Our group has consistently shown that these markers of bone turnover and especially the serum c-telopeptide are rapid and valid surrogates for assessing the palliative benefits caused by changes in BTA therapies [17,18]. However, they remain a surrogate and should only be considered hypothesis generating.

5. Conclusion

This is the largest study to date evaluating the effects of doxycycline in bone-metastatic breast cancer patients. Doxycycline daily for 12 weeks did not appear to significantly enhance palliative benefit nor change bone resorption markers. The toxicity profile of doxycycline in this patient population will make further evaluation challenging.

Conflicts of interest

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