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Study Title: ANZAC: a randomised neoadjuvant biomarker study investigating the anti-tumour activity of the AdditioN of Zoledronic Acid to Chemotherapy in breast cancer

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Background: Pre-clinical studies have demonstrated sequence-dependent synergistic anti-tumour effects of chemotherapy (CT) and zoledronic acid (Z) in bone and soft tissue tumours, including induction of tumour cell apoptosis, reduction in tumour cell proliferation and inhibition of vascularisation. In the treatment of patients with metastatic bone disease, it has been reported that Z induces transient suppression of circulating vascular endothelial growth factor (VEGF), a potent mediator of angiogenesis. We have also recently reported that the addition of Z to neoadjuvant CT appears to improve pathological response in the surgical resection specimen. These data therefore suggest a possible direct anti-tumour effect of zoledronic acid in combination with chemotherapy. The ANZAC study aimed to investigate the short-term anti-tumour effects of neoadjuvant CT +/- Z in patients with invasive breast cancer, evaluating biological end-points including apoptosis, proliferation and angiogenesis.

Methods:
Forty patients were randomised to receive a single 4mg infusion of Z 24 hours after the first cycle of $FE_{100}C$ chemotherapy, as per the most effective schedule demonstrated pre-clinically, or CT alone (CT alone n=20, CT+Z n=20). Randomisation was stratified for tumour (T) stage, ER, HER2 status and menopausal status to minimise biological differences in the two treatment groups that may influence chemosensitivity. All patients had repeat breast core biopsy at Day 5 (D5) +/- Day 21 (D21) using a 14-gauge device. Apoptotic Index (AI) and proliferation were measured on core biopsy specimens using terminal deoxyribonucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay and Ki67 immunohistochemistry (using MM1 monoclonal antibody, cat. No. VP-K452, Vector Labs), counting a total of 2000 and 1000 tumour cells respectively. Serum levels of VEGF165, the most abundant human isoform, were measured in duplicate using quantitative sandwich ELISA (Quantikine human VEGF Immunoassay, R&D Systems) at baseline prior to CT +/- Z, and on days 5 and 21 after surgery. Differences between the groups in change from baseline to subsequent time-points were investigated using the Mann-Whitney U test.

Results:
Baseline clinico-pathological characteristics were well balanced between the two groups. Following the first cycle of chemotherapy all patients had a biopsy on day 5.

Twelve (30%) patients declined to have an additional biopsy on day 21. Seven (17.5%) patients achieved pathological complete response (breast and axilla) (CT alone n=3, CT+Z n=4).

For the different biological end-points, percentage change from baseline to D5 and from baseline to D21 are shown in Table 1. At D5, a greater reduction in serum VEGF occurred in patients treated with CT+Z compared to CT: median percentage change -23.8 (IQR -32.9, -15.8) vs. -8.4% (IQR -27.3, +8.9; p=0.02), but these effects were lost by D21. Cell turnover index fell at D5 in both groups (increased apoptosis and reduced proliferation) but recovered more rapidly with CT+Z than CT alone by D21 to levels above baseline (p=0.006). However these data may be driven by results from 3 patients and smaller number of patients included in the analyses (CT alone n=13, CT+Z n=9).

There were 8 SAEs reported to the Sponsor, MHRA and REC for the study, none of these were SUSARS.

Conclusions:
No definite evidence of a direct anti-tumour effect of the addition of Z to CT was observed, but potentially relevant biological effects were seen in this small study. The clinical relevance of recovery of cell turnover by D21 with CT+Z is unclear. Further prospective studies with more frequent dosing of Z are warranted to exploit any potential anti-angiogenic effect of Z.

Table 1: Median % Change in Factor score or concentration compared to baseline at days 5 and 21 after surgery.

Factor	**Timepoint** B: baseline D5: day 5 D21: day 21	**Median % Change** (IQR)		**P value**
		CT	CT+Z	
Apoptotic Index	B – D5	+81.4 (-18.0, 102.2)	+46.0 (7.84, 188.5)	0.48
	B – D21	- 12.9 (-27.9, 29.5)	- 14.3 (-65.2, -2.2)	0.41
Ki67	B – D5	- 18.4 (-58.9, 20.6)	- 24.2 (-91.0, 17.5)	0.44
	B – D21	- 44.9 (-63.6, -17.1)	+12.7 (-18.9, 47.7)	0.003
Turnover index (KI67/AI)	B – D5	- 35.6 (-56.0, 2.2)	- 68.4 (-94.4, -15.8)	0.11
	B – D21	- 36.7 (-69.1, -0.1)	+72.4 (35.9, 152.9)	0.002
Serum VEGF	B – D5	- 8.4 (-27.3, 8.9)	- 23.8 (-32.9, -15.8)	0.02
	B – D21	+59.3 (19.1, 97.8)	+39.1 (28.7, 102.7)	0.90