

## Bisphosphonates and prevention of metastases: the AZURE study

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**Abstract** Most patients present with disease that appears to be confined to the breast. However, a significant proportion of women will go on to develop metastatic breast cancer with bone being the most frequent site of distant relapse. The bone microenvironment provides a fertile soil for circulating metastatic cells as resorbing bone releases growth factors that are thought to attract circulating cancer cells to the bone surface and facilitate their growth and proliferation. These infiltrating tumour cells in turn produce bone cell-activating factors, which lead to increased bone resorption and further release of growth factors in a 'vicious cycle'. The third-generation bisphosphonate zoledronic acid is a potent inhibitor of osteoclast-mediated bone resorption. It also has effects on numerous processes in the metastatic cascade, with *in vitro* and animal studies showing synergistic anti-tumour effects with a range of cytotoxic and endocrine treatments. The addition of zoledronic acid to adjuvant therapy could therefore be a therapeutic strategy of potential importance.

To investigate this, the AZURE trial was designed to determine whether adjuvant zoledronic acid improves the disease-free and bone metastasis-free survival of women with stage II/III breast cancer. Recruitment of 3300 subjects was completed in January 2006. First efficacy data are expected in 2008. There is a potential for synergistic action with chemotherapy to manifest as enhanced toxicity. Reassuringly, initial safety data show that zoledronic acid is well tolerated and can be safely combined with adjuvant chemotherapy without any increase in myelotoxicity or effect on dose intensity.

**Keywords:** Adjuvant; Bisphosphonates; Bone metastases; Breast cancer; Zoledronic acid

### Introduction/background

Bone is the most frequent site of distant relapse, accounting for around 40% of all first recurrence with as many as 70% of breast cancer patients having bone metastasis at *post mortem* [1]. Significant morbidity from bone metastasis may occur due to pathological fracture, hypercalcaemia

of malignancy, pain and spinal cord compression [2]. The mainstay of treatment in advanced cancers consists of external beam radiotherapy plus systemic endocrine and cytotoxic therapies. However, bisphosphonates are the current standard of care in patients with bone metastases, decreasing skeletal complications and reducing bone pain, leading to improved mobility and quality of life [3].

### The process of metastasis to bone

Healthy bone is composed of outer hard, compact bone tissue and inner spongy tissue with a strong honeycomb-like structure. However, bone is not inert and constantly undergoes a complex process

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of remodelling, characterised by two opposing actions: the resorption of old bone by osteoclasts and the formation of new bone by osteoblasts. When healthy, there is a steady-state balance, or 'coupling' of osteoclastic bone resorption and osteoblastic bone formation.

However, this balance is lost when tumour cells enter the bone microenvironment. Osteoclast activity in resorbing bone releases a number of bone-derived growth factors and cytokines that attract circulating cancer cells to the bone surface. From *in vitro* studies, circulating metastatic breast cancer cells have been shown to be very responsive to these factors [4] and to have a high affinity for the bone microenvironment, accounting for the high levels of relapse in the skeleton. These growth factors also facilitate the tumour cells' growth and proliferation [5].

Once incorporated into the bone, the metastatic breast cancer cell interferes with bone homeostasis, releasing factors that enhance osteoclast proliferation and activity [6,7]. The end result is a 'vicious cycle' whereby complex multi-directional interactions among tumour cells, osteoblasts and osteoclasts lead to ever-increasing bone destruction and tumour growth.

## Bisphosphonates

Bisphosphonates are potent inhibitors of osteoclast function and can break this 'vicious cycle'. There are two different classes of bisphosphonates: the relatively weak non-nitrogen-containing compounds (including etidronate and clodronate) and the more potent nitrogen-containing bisphosphonates including pamidronate, alendronate, ibandronate, risedronate and zoledronic acid (in order of increasing potency). All bisphosphonates have been shown to reduce the frequency of skeletal-related events from metastatic bone disease associated with breast cancer, and with many of them important effects on symptoms have been reported with reduced pain and analgesic consumption and an apparent improvement in quality of life [8–10]. Third-generation bisphosphonates, such as zoledronic acid [11] and ibandronate, are more potent than pamidronate and are more convenient due to shorter infusion times.

## Rationale for adjuvant use

There have been comprehensive reviews of the antitumour effects of bisphosphonates [12,13], showing extensive *in vitro* and *in vivo* preclinical evidence that bisphosphonates, particularly the more potent nitrogen-containing bisphosphonates,

have antitumour activity and can reduce skeletal tumour burden. In addition to indirect effects on tumour growth in bone via inhibition of bone resorption and reduced osteoclastogenesis, bisphosphonates have the potential to directly induce apoptosis of tumour cells, inhibit tumour cell adhesion to extracellular bone matrix, reduce the metastatic potential of tumour cells and inhibit angiogenesis. It has also been shown that bisphosphonates antagonise the stimulatory effects of growth factors on human breast cancer cell survival and reduce protective effects against apoptotic cell death. They also have effects on immune function with the stimulation of  $\gamma\delta$ T lymphocytes [14] although the clinical relevance of these actions is uncertain.

There is also increasing evidence that bisphosphonates, particularly third-generation agents, have synergy with commonly used chemotherapeutic agents. It has been shown that ibandronate in combination with taxoids inhibited the adhesion to and invasion of bone by human breast carcinoma cells [15]. Jagdev *et al.* [16] showed that the combination of zoledronic acid plus paclitaxel enhanced apoptosis of MCF-7 breast cancer cells fourfold compared with either agent alone.

Following on from these observations, Neville-Webbe *et al.* found that clinically relevant concentrations of either doxorubicin [17] or paclitaxel [18] and zoledronic acid induced sequence- and schedule-dependent synergistic apoptosis of breast cancer cells, showing greater synergy when doxorubicin was administered before zoledronic acid. Similar results were observed in multiple cancer cell lines (but not 3T3 fibroblasts) and with a variety of anticancer agents. No increase in apoptosis was observed when zoledronic acid was replaced by clodronate.

The literature therefore clearly shows the potential and impressive antineoplastic properties of bisphosphonates, particularly third-generation agents *in vitro*. However, as bisphosphonates are bound so tightly to the bone, how relevant their *in vitro* antitumour effects will be upon circulating metastatic cells needs further elucidation [12]. There are encouraging data from Ottewell *et al.* [19] showing that treatment with doxorubicin followed by zoledronic acid results in significantly reduced breast tumour growth in a murine model, both in bone and at extra-osseous sites.

## Clinical trials to date

Experiments in animals and preliminary clinical observations indicated that early clodronate therapy might reduce the incidence of new bone metastases in breast cancer patients [20]. These observations

formed the basis for three randomised clinical trials with clodronate, which gave conflicting but generally promising results.

Diel and colleagues [20] recruited 302 patients between 1990 and 1995 with primary breast cancer and tumour cells in the bone marrow, a confirmed risk factor for recurrence [21]. Patients were randomly assigned to receive clodronate at a dose of 1600 mg/day orally for 2 years or standard follow-up. In an initial analysis, the incidence rates of both osseous and visceral metastases were shown to be significantly lower in the clodronate group than in the control group ( $P=0.003$  for both osseous and visceral metastases). However, in a re-analysis at a follow-up time of  $103 \pm 12$  months [22], the incidence of osseous and visceral metastases was found to be similar in both groups, although the significant overall survival advantage for the clodronate-treated group ( $P < 0.01$ ) was maintained. Whether the effect on overall survival will also diminish with time will require even longer follow-up.

Over an 11-year period, Powles and colleagues [23] recruited 1069 patients with primary operable stage I–III breast cancer in a randomised, double-blind, placebo-controlled, multi-centre trial evaluating the efficacy and safety of oral clodronate. Patients received oral clodronate (1600 mg/day) or placebo for 2 years, starting within 6 months of primary treatment (surgery, radiotherapy and tamoxifen). An analysis of the results undertaken in 2006 [24] showed that oral clodronate significantly reduced the risk of bone metastases in all patients over the 5-year study period, with the benefits most pronounced in patients with stage II/III disease.

A third trial did not find a clinical benefit for the use of oral clodronate in patients with node-positive breast cancer [25]. In this study, recruiting between 1990 and 1993, 299 women with primary node-positive breast cancer were randomised to oral clodronate 1600 mg daily for 3 years or a control group. All patients received adjuvant chemotherapy or endocrine therapy. After a 10-year follow-up period, [26] the incidence in bone metastases was similar in the clodronate and control groups: 44 (32%) vs. 42 (29%), respectively ( $P=0.35$ ). However, the frequency of non-skeletal recurrences (visceral and local) was significantly higher in the clodronate group, 69 (50%), as compared to the controls, 51 (36%) ( $P=0.005$ ), and the disease-free survival remained significantly lower in the clodronate group (45% vs. 58%,  $P=0.01$ ). When stratified for ER status, disease-free survival in ER-negative patients was highly significant in favour of the controls: 25% vs. 58% ( $P=0.004$ ).

There is no biological rationale to explain these results, and the imbalance in prognostic factors, the

somewhat unconventional use of endocrine treatments due to secondary randomisations and the small size of the trial are more plausible reasons for the outcomes observed.

Based on the conflicting data from these studies, the American Society of Clinical Oncology in their guidance on bisphosphonate use in breast cancer did not recommend the use of adjuvant bisphosphonates for the prevention of bone metastases in patients with breast cancer [27] outside the research setting.

A large randomised controlled trial run under the auspices of the National Surgical Adjuvant Breast and Bowel Project (NSABP-B34) has completed accrual and should provide the definitive answer for the role of clodronate in this setting. A first efficacy analysis is expected in 2008.

As seen from the preclinical evidence, the more potent aminobisphosphonates may have the greatest potential to prevent bone metastases. However, there are currently no informative randomised trials of aminobisphosphonates in the adjuvant setting. Two small trials of pamidronate [28,29] gave apparently encouraging results although methodological inconsistencies make interpretation of results impossible. A large Danish study of more than 1000 patients randomised to oral pamidronate or placebo completed recruitment in the mid-1990s, but to date no results are available. A small ( $n=40$ ) randomised open-label trial of zoledronic acid examined the effect of preventive zoledronic acid treatment on the development of bone metastases in patients with recurrent solid tumours, without bone metastases at the time of randomisation [30]. Patients were followed up until bone metastases were established. The percentage of patients being bone metastases free at 12 months was 60% in the zoledronic acid group and 10% in the control group ( $P < 0.0005$ ), while the percentages at 18 months were 20% and 5%, respectively ( $P=0.0002$ ). They concluded that adjuvant zoledronic acid might be useful for the prevention of bone metastases but acknowledged the need for a larger study.

The South West Oncology Group in the US has set up a large randomised three-arm trial (SWOG 0307/Intergroup) to compare the effects of intravenous (i.v.) zoledronic acid (4 mg via a 15-min i.v. infusion every month for six doses, then every 3 months), oral clodronate (1600 mg/day) and oral ibandronate (50 mg/day) on disease-free survival in 6000 patients with stage I, II or IIIA breast cancer [31]. The trial was initiated at the end of 2005 and is due to end in 2015. The study design assumes that either B34 or AZURE will show an advantage for an adjuvant bisphosphonate but will clarify the choice of agent.

An ongoing study in Germany, BIG 4-04/GBG 32 ICE (Ibandronate with or without Capecitabine in Elderly patients), aims to determine, in the presence of a bisphosphonate, the role of adjuvant chemotherapy with capecitabine in patients aged 65 years and over. A total of 1394 patients will be randomised to receive 50 mg oral ibandronate daily or 6 mg i.v. ibandronate monthly (according to patient choice) with or without capecitabine.

A second German study, GAIN (German Adjuvant Intergroup Node-positive) will recruit 3000 patients and randomise to epirubicin, paclitaxel and cyclophosphamide with or without capecitabine +/- ibandronate.

## The AZURE study

AZURE is a collaborative, multi-centre, open-label, randomised, parallel group trial investigating the adjuvant use of zoledronic acid. There is a 5-year treatment phase and a subsequent 5-year follow-up phase to include 3300 patients. Patients were randomised to no additional treatment vs. zoledronic acid (monthly for 6 months, then every 3 months for 8 doses (approximately 2 years), then every 6 months for 5 doses (approximately 2.5 years)) (Fig. 1). Key entry criteria are summarised in Table 1.

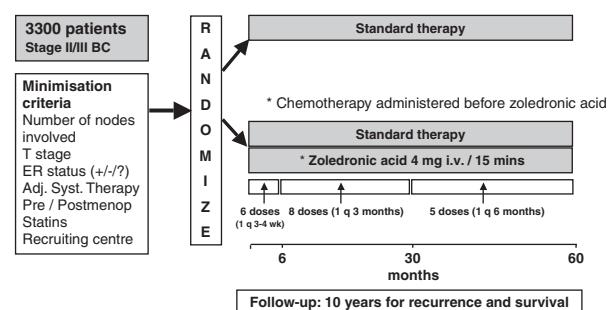
The *primary endpoint* will determine whether adjuvant treatment with 4 mg zoledronic acid plus chemotherapy and/or endocrine therapy is superior to chemotherapy and/or endocrine therapy alone in improving disease-free survival of women with breast cancer at high risk of relapse.

*Secondary endpoints* include time to bone metastases as first recurrence, time to bone metastases *per se*, time to distant metastases, overall survival at the final analysis time point, the incidence of fractures prior to the development of bone metastases,

### Breast cancer: adjuvant zoledronic acid (AZURE®)

<sup>a</sup>adjuvant zoledronic acid to reduce recurrence in patients with high-risk localized BC

#### Study design



**Figure 1.**

Breast cancer: adjuvant zoledronic acid (AZURE®).  
Follow-up after 10 years for recurrence.

**Table 1.** AZURE entry criteria.

### Main eligibility criteria

#### Inclusion criteria

- Female patients with stage II/III primary breast cancer, with T stage  $\geq$  T1
- Patients should be receiving/scheduled to receive chemotherapy and/or endocrine therapy
- Patients receiving *neo-adjuvant* therapy
  - must have tumour size of  $>5$  cm (T3), features of locally advanced disease (T4) or biopsy-proven lymph node involvement (N1)
  - should be scheduled to proceed to definitive surgery and/or radical radiotherapy with curative intent within 6 months of starting *neo-adjuvant* therapy
  - time between commencement of neoadjuvant treatment and planned start date of study drug should be  $\leq 30$  days
- Patients receiving *adjuvant* therapy
  - must have undergone complete primary tumour resection and treatment of the axillary lymph nodes, without any prior neoadjuvant therapy
  - must have evidence of lymph node involvement
  - time between definitive surgery and planned start date of study drug should be  $\leq 60$  days
- Performance status: Karnofsky Index  $\geq 80\%$  or ECOG 0 and 1
- Women of child-bearing potential must be using a reliable and appropriate method of contraception
- Age  $\geq 18$  years
- Patient must have given written informed consent prior to any study-specific procedures.

#### Exclusion criteria

- Metastatic or recurrent breast cancer or a history of breast cancer (aside from DCIS or LCIS) prior to the currently diagnosed case
- History of prior cancers within the preceding 5 years (including previous contralateral breast cancer), aside from non-melanomatous skin cancer or carcinoma in situ of the uterine cervix treated with curative intent
- History of diseases with influence on bone metabolism, such as Paget's disease of bone, primary hyperparathyroidism or osteoporosis requiring treatment at the time of study entry or considered likely to become necessary within the subsequent 6 months
- Severe physical or psychological concomitant diseases that might impair compliance with the provisions of the study protocol
- Prior treatment with bisphosphonates within the past year
- Serum creatinine  $>1.5 \times$  upper limit of normal
- Known hypersensitivity to bisphosphonates
- Pregnancy or breast-feeding
- Use of other investigational drugs in the 30 days prior to study entry. (Patients may be receiving treatments within a clinical trial providing the treatment under test has a licensed indication within your country.)
- Current active dental problems including dental abscess or infection of the jawbone (maxilla or mandible), or a current or prior diagnosis of osteonecrosis of the jaw (ONJ)
- Recent (within 4 weeks of study entry) or planned dental or jaw surgery (e.g. extractions, implants). Recent dental fillings, teeth scaling and polishing or minor gingival surgery do not exclude the patient

skeletal-related events following recurrence in bone (defined as fractures, spinal cord compression, radiation therapy to bone, surgery to bone and hypercalcaemia) and the safety and toxicity of zoledronic acid in this clinical setting.

**Statistical power**

Based on a maximum follow-up time of 6 years and a recruitment time of 3 years, 3282 patients are needed to detect a reduction in the hazard rate of approximately 17% in the zoledronic acid group compared to the control group. This includes a yearly lost-to-follow-up rate of 5%. A 17% hazard rate reduction equates to an absolute difference in disease-free survival of 3.7% (i.e. from 75% in the control group to 78.7% in the zoledronic acid group). This difference can be detected with at least 80% power with 1650 patients per group (a total of 3300 patients). Approximately 940 events are required for this analysis.

**Recruitment**

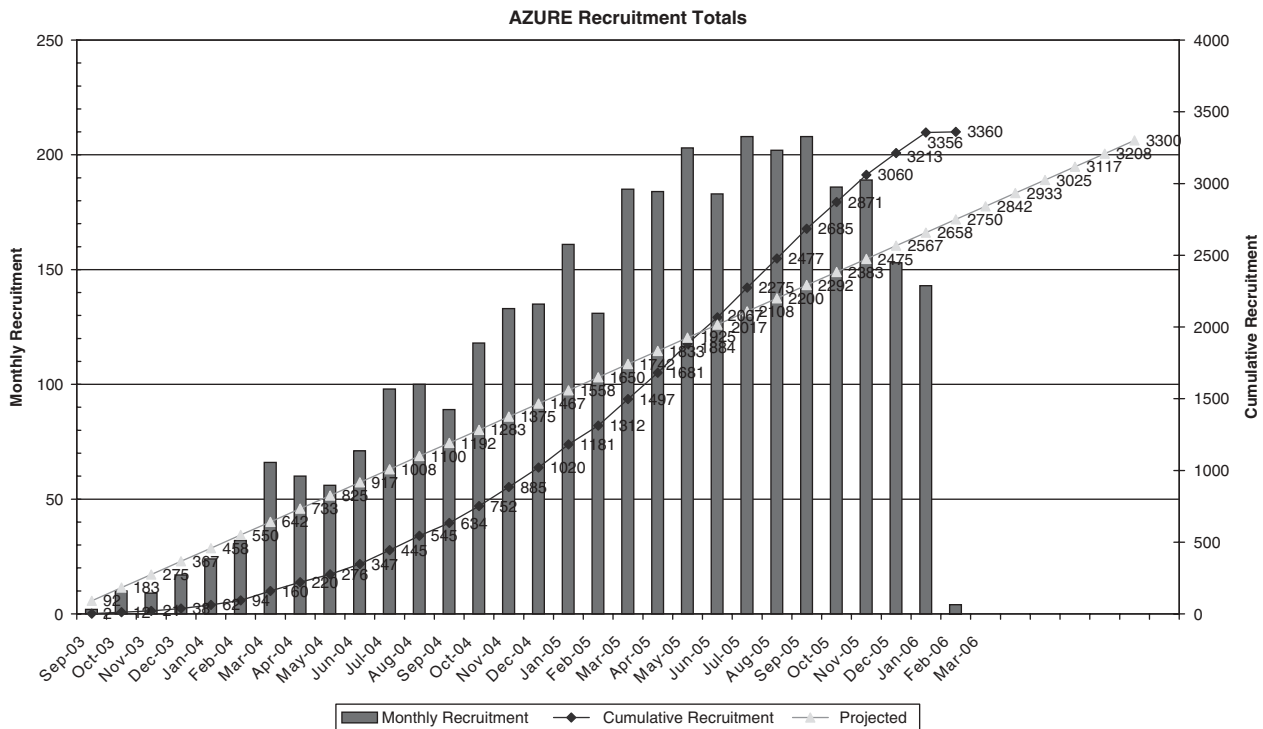
The first patient was enrolled in September 2003 and 176 centres entered patients from the UK, Eire, Spain, Portugal, Australia, Thailand and Taiwan. The study closed to recruitment on 20th January 2006, 8 months ahead of schedule with 3360 women

enrolled. Worldwide recruitment is summarised in Table 2 and Figure 2.

Analysis of the characteristics of the study population shows that both groups are well matched across all criteria (Table 3). A total of 51% of patients have T2 tumour at presentation, with 77% being ER positive. A total of 61% and 34% had 1–3 and ≥4 axillary nodes involved, respectively, and 6% were treated in the neoadjuvant setting; thus pathological nodal status could not be reliably determined. Anthracyclines were administered to 92.6% and 22.7% received taxanes, in large part due to accrual into the TANGO trial concurrent with AZURE. Only 4.6% received endocrine therapy alone. Almost half of the patients (44.6%) were premenopausal at randomisation.

**Table 2.** Worldwide recruitment.

Country	Active centres	Patients recruited
UK	123	2710
Eire	10	247
Australia	29	226
Spain	9	107
Portugal	1	32
Thailand	2	25
Taiwan	2	13
Total	176	3360



**Figure 2.** AZURE: worldwide recruitment.

**Table 3.** Patient characteristics.

Patient characteristics	Allocation		Total Number (%)
	Standard treatment alone	Standard treatment + zoledronic acid	
	Number (%)	Number (%)	
Lymph node involvement			
One to three nodes involved	1015 (60.5)	1017 (60.5)	2032 (60.5)
Four or more nodes involved	560 (33.4)	561 (33.4)	1121 (33.4)
Unknown (neoadjuvant)	100 (6.0)	99 (5.9)	199 (5.9)
Sentinel node positive-axillary RT	3 (0.2)	4 (0.2)	7 (0.2)
Tumour stage			
T1	528 (31.5)	526 (31.3)	1054 (31.4)
T2	857 (51.1)	858 (51.0)	1715 (51.1)
T3	236 (14.1)	235 (14.0)	471 (14.0)
T4	57 (3.4)	59 (3.5)	116 (3.5)
TX	0 (0.0)	3 (0.2)	3 (0.1)
ER status			
ER positive	1283 (76.5)	1289 (76.7)	2572 (76.6)
ER negative	343 (20.4)	342 (20.3)	685 (20.4)
ER unknown	52 (3.1)	50 (3.0)	102 (3.0)
Scheduled for neoadjuvant therapy			
Yes	107 (6.4)	108 (6.4)	215 (6.4)
No	1571 (93.6)	1573 (93.6)	3144 (93.6)
Systemic therapy plan			
Endocrine therapy alone	76 (4.5)	80 (4.8)	156 (4.6)
Chemotherapy alone	379 (22.6)	377 (22.4)	756 (22.5)
Endocrine therapy and chemotherapy	1223 (72.9)	1224 (72.8)	2447 (72.8)
Anthracyclines*			
Yes	1555 (92.7)	1556 (92.6)	3111 (92.6)
No	123 (7.3)	125 (7.4)	248 (7.4)
Taxanes*			
Yes	381 (22.7)	381 (22.7)	762 (22.7)
No	1297 (77.3)	1300 (77.3)	2597 (77.3)
Use of statins			
Yes	92 (5.5)	93 (5.5)	185 (5.5)
No	1586 (94.5)	1588 (94.5)	3174 (94.5)
Menopausal status			
Pre-menopausal	750 (44.7)	748 (44.5)	1498 (44.6)
≤5 years since menopause	250 (14.9)	252 (15.0)	502 (14.9)
>5 years since menopause	512 (30.5)	515 (30.6)	1027 (30.6)
Menstrual status unknown	166 (9.9)	166 (9.9)	332 (9.9)
Total	1678 (100.0)	1681 (100.0)	3359 (100.0)

The protocol for the AZURE study was amended in the early phase of recruitment to instruct administration of zoledronic acid post chemotherapy in order to exploit any clinical advantage of the *in vitro* (and subsequent *in vivo*) findings of sequence dependency previously described.

### Bone marker sub-studies

Sub-studies will use proteomics, tissue micro-array and other modern techniques in an attempt to identify specific prognostic indicators for the development of bone metastases. There will also be analysis of factors that may be able to predict specific benefit from bisphosphonate treatment.

A 20 ml blood sample was taken from all patients who consented, at baseline and again at times of local and distant disease recurrence. To date, 843 baseline patient samples (each sample consisting of 13 aliquots of whole blood, serum and plasma) are currently stored centrally at  $-80^{\circ}\text{C}$ .

To date, in excess of 2000 tumour blocks have been requested from the pathology departments of participating hospitals. These blocks will be returned after extraction of small diameter cores of tissue for the production of tissue micro-arrays.

### Safety issues

The side effects of bisphosphonates are generally mild and well described, with patients experiencing

only mild adverse events (AEs) and occasional instances of decreased renal function [11].

However, when given concurrently with chemotherapy, there is the potential to enhance the toxicities of cytotoxic therapy, particularly if the synergistic effects seen *in vitro* translate to the clinical setting. If this is to be a viable therapeutic option, it has to be shown that the addition of zoledronic acid to adjuvant therapy does not adversely affect chemotherapy delivery or patient safety.

To correspond with the timing of chemotherapy, serious (SAE) and non-serious AE data within 6 months of randomisation were compared. No significant differences were seen in the numbers of patients with any SAE. The incidence of neutropaenic and non-neutropaenic infection cases and Common Toxicity Criteria (CTC) grade 3/4 AE were similar. The frequency of chemotherapy dose reductions and median duration of chemotherapy were similar, confirming that the addition of zoledronic acid has no significant effect on chemotherapy delivery. Further analysis of these data is ongoing as this is the largest safety analysis of zoledronic acid in patients without the confounding influence of metastatic disease and indicates that this can be safely combined with adjuvant chemotherapy.

Retrospective case studies have suggested an association between long-term bisphosphonate therapy and osteonecrosis of the jaw (ONJ) [32]. The typical presentation is a 'non-healing' extraction socket or exposed jawbone with localised swelling and purulent discharge. The incidence of ONJ is not known with any certainty but appears to be low in most unselected series [33]. However, as bisphosphonates are administered to increasing numbers of cancer patients and earlier in the course of their disease, problems may become more prevalent.

In light of these observations, the AZURE protocol and Patient Information Sheet were amended in February 2004 to inform patients of the possible link between bisphosphonate therapy and ONJ. Patients on the treatment arm enrolled prior to this signed updated consent. Expert Panel recommendations were circulated to all Investigators and reporting of any confirmed cases mandated as SAEs from December 2004. A further protocol amendment in July 2005 excluded any patients with current active dental problems from entry into the trial.

Subsequently, two high-profile papers published in December 2005 examined the aetiology of this disease in greater detail [34,35]. The Trial Steering Committee met to discuss the new information and decided against further modifications to the protocol. However, guidelines for the prevention, diagnosis and management of ONJ were produced by the Committee with expert oral surgery input and

distributed to investigators. In addition, all patients received dental hygiene information and a bisphosphonate alert card was issued to patients on the zoledronic acid arm to give to their dentist if attending for treatment.

The International Data Monitoring and Ethics Committee met in October 2005 and 2006 and expressed no concerns regarding patient safety or the conduct of the study in general. Event-driven analysis of efficacy data is anticipated to commence in 2008.

## Conclusion

The use of bisphosphonates is an adjuvant therapeutic strategy worthy of investigation with pre-clinical evidence of direct antitumour effects and synergy with chemotherapy agents. Results of previous small-scale trials have been inconclusive. Two large trials (NSABP-B34 and AZURE) have completed accrual and are anticipated to provide definitive results in the next few years.

The AZURE trial was designed to determine whether zoledronic acid improves the disease-free and bone metastasis-free survival of women with stage II/III breast cancer. The study completed accrual of 3360 women with stage II/III breast cancer in January 2006. Preliminary safety data collected within 6 months of randomisation indicate that zoledronic acid can be safely combined with adjuvant chemotherapy.

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