Adjuvant zoledronic acid in patients with early breast cancer: final efficacy analysis of the AZURE (BIG 01/04) randomised open-label phase 3 trial

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Summary

Background The role of adjuvant bisphosphonates in early breast cancer is uncertain. We therefore did a large randomised trial to investigate the effect of the adjuvant use of zoledronic acid on disease-free survival (DFS) in high-risk patients with early breast cancer.

Methods In the AZURE trial, an open-label, international, multicentre, randomised, controlled, parallel-group phase 3 trial, women (age ≥18 years) with stage II or III breast cancer were randomly assigned (1:1) by a central automated 24-h computer-generated telephone minimisation system (balanced for number of involved axillary lymph nodes, tumour stage, oestrogen receptor status, type and timing of systemic therapy, menopausal status, statin use, and treatment centre) to receive standard adjuvant systemic treatment alone (control group) or with 4 mg intravenous zoledronic acid every 3–4 weeks for six doses, then every 3 months for eight doses, followed by every 6 months for five doses, for a total of 5 years of treatment. The primary endpoint was disease-free survival (DFS). Secondary endpoints were invasive DFS (IDFS), overall survival, time to bone metastases, time to distant recurrence, and subgroup analyses of variables included in the randomisation. All patients have completed study treatment. Results from the intention-to-treat final analysis of this fully recruited study are presented after a median follow-up of 84 months (IQR 66–93). This final efficacy analysis was planned to take place after 940 DFS events. This trial is registered with ClinicalTrials.gov, NCT00072020.

Findings 3360 women were recruited from 174 centres in seven countries between Sept 4, 2003, and Feb 16, 2006. The number of DFS events did not differ between groups: 493 in the control group and 473 in the zoledronic acid group (adjusted hazard ratio [HR] 0·94, 95% CI 0·82–1·06; p=0·30). IDFS (HR 0·93, 95% CI 0·82–1·05; p=0·22), overall survival (0·93, 0·81–1·08; p=0·37), and distant recurrences (0·93, 0·81–1·07; p=0·29) were much the same in both groups. Zoledronic acid reduced the development of bone metastases, both as a first event (HR 0·78, 95% CI 0·63–0·96; p=0·020) and at any time during follow-up (0·81, 0·68–0·97; p=0·022). The effects of zoledronic acid on DFS were not affected by oestrogen-receptor status. However, zoledronic acid improved IDFS in those who were over 5 years since menopause at trial entry (n=1041; HR 0·77, 95% CI 0·63–0·96) but not in all other (premenopause, perimenopause, and unknown status) menopausal groups (n=2318; HR 1·03, 95% CI 0·89–1·20). 33 cases of suspected osteonecrosis of the jaw have been reported, with 26 confirmed on central review, all in the zoledronic acid group (1·7%, 95% CI 1·0–2·4).

Interpretation These results suggest no overall benefit from the addition of zoledronic acid to standard adjuvant treatments for early breast cancer. However, zoledronic acid does reduce the development of bone metastases and, for women with established menopause, improved disease outcomes.

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Introduction

The bone-marrow microenvironment has a major effect on metastasis from breast cancer. Circulating tumour cells are attracted to bone surfaces within the bone marrow and bind to the osteoblastic niche by displacing haemopoietic stem cells.[1] Here, tumour cells can remain quiescent for years and can evade the effects of adjuvant systemic treatments. Subsequently, these cells can exit this dormant state and start to proliferate.[2] These, now active, micrometastatic foci can either activate osteoclasts to resorb bone and establish bone metastases or leave the bone microenvironment and potentially initiate metastases at other organ sites.[3] Thus, targeting bone-cell function to manipulate this microenvironment provides a potential additional approach to present standard adjuvant treatments for breast cancer.[4]

We designed the AZURE (does Adjuvant Zoledronate redUce REcurrence in early breast cancer?) randomised trial to investigate the adjuvant use of zoledronic acid in patients with early breast cancer. An interim analysis of this trial led to the release and publication of results that showed no benefit of adjuvant zoledronic acid in the study population as a whole.[5] However, in a prespecified subgroup analysis, we identified significant improvements in disease outcomes in women who had passed through the menopause at the time of study entry. Findings from

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See Online for appendix

several other studies have shown similar benefit in women with low levels of reproductive hormones resulting from either the effects of natural menopause[6,7] or ovarian-suppression treatment.[8] Here, in the planned final analysis, we report the mature results of the AZURE trial after a median of 84 months (IQR 66–93) of follow-up.

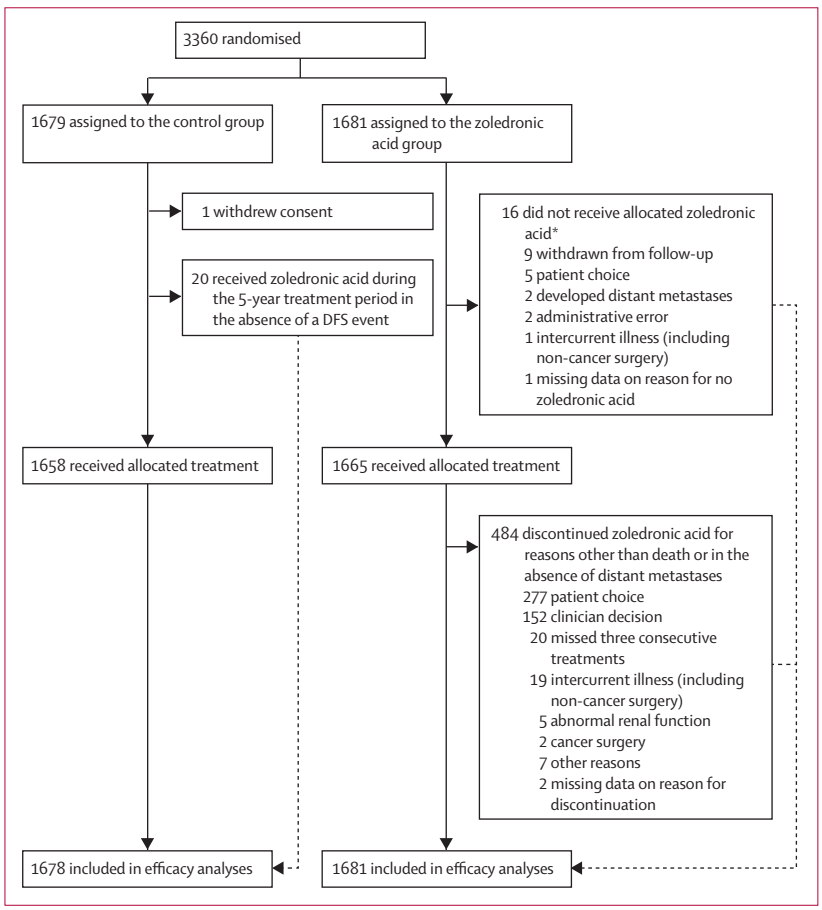

Figure 1: **Trial profile**
DFS=disease-free survival. *More than one reason was given for some patients.

	Control group (n=1678)	Zoledronic acid group (n=1681)
Age (years)	51·3 (10·0)	51·6 (9·9)
Lymph node involvement		
0	32 (2%)	30 (2%)
1–3	1033 (62%)	1042 (62%)
≥4	607 (36%)	604 (36%)
Unknown	6 (<1%)	5 (<1%)
Tumour stage		
T1	523 (31%)	542 (32%)
T2	867 (52%)	850 (51%)
T3	228 (14%)	228 (14%)
T4	59 (4%)	58 (3%)
TX	1 (<1%)	3 (<1%)
Oestrogen receptor status		
Positive	1315 (78%)	1319 (78%)
Negative	356 (21%)	349 (21%)
Unknown	7 (<1%)	13 (1%)

(Table 1 continues on next page)

Methods

Study design and patients

The AZURE trial is a prospective, open-label, international, multicentre, randomised, controlled, parallel-group phase 3 trial. Eligibility has been reported previously;[5] in summary, women with histologically confirmed stage II or III invasive breast cancer of any subtype with either pathologically involved axillary lymph node metastasis or a T3 or T4 primary tumour were eligible for inclusion. Previous complete resection of the primary tumour was necessary or had to be planned if patients were treated with neoadjuvant chemotherapy. Other inclusion criteria were age at least 18 years, Karnofsky performance status index of at least 80, and neither pregnant nor breastfeeding.

Patients were not eligible if there was clinical or imaging evidence of distant metastases before study entry, a history of cancer within the preceding 5 years, present or recent (previous year) use of bisphosphonates, pre-existing bone disease likely to need bone-targeted treatment, or a serum creatinine concentration over 1·5 times the upper limit of normal. Haematological, renal, and hepatic function tests were done before randomisation. Staging imaging tests were done in accordance with institutional protocols. After reports of osteonecrosis of the jaw in patients with advanced cancer during treatment with bisphosphonates,[9,10] on the basis of advice from the trial steering committee, the protocol was amended on July 7, 2005, to exclude patients with clinically significant active dental problems or those planned for jaw surgery.

Ethical approval was obtained for each of the 174 participating centres. All patients gave written informed consent before enrolment.

Randomisation and masking

Patients were randomly assigned (1:1) by the treating clinical team by a central automated 24-h computer-generated telephone minimisation system, at the Clinical Trials Research Unit, University of Leeds (Leeds, UK), to either standard treatment alone (control) or standard treatment with zoledronic acid (zoledronic acid monohydrate, Novartis Pharmaceuticals, Summit, NJ, USA). To reduce possible imbalances in tumour and treatment characteristics, a minimisation process was used that took into account the number of involved axillary lymph nodes (none, 1–3, ≥4, or unknown), clinical tumour stage (T1, T2, T3, or T4), oestrogen receptor status (positive, negative, or unknown), type of systemic therapy (chemotherapy with or without endocrine treatment, endocrine treatment alone, taxanes [yes or no], or anthracyclines [yes or no]), timing of systemic treatment (adjuvant or neoadjuvant),

menopausal status (premenopausal, within 5 years of last menstruation, >5 years since last menstruation, or unknown), statin use (yes or no), and treating centre. Centre and consultant authorisation codes, provided by the Clinical Trials Research Unit, were needed to access the randomisation system, and the use of an automated telephone randomisation system ensured the concealment of the next treatment assignment. The study was open label in nature.

Procedures

After diagnosis of invasive breast cancer, patients received appropriate standard surgery to the breast and axilla and adjuvant treatments including endocrine treatment for oestrogen-receptor-positive disease, adjuvant chemotherapy, locoregional radiotherapy, and, after June, 2005, trastuzumab for HER2+ disease, in accordance with standard protocols and each participating centre. Only investigational drugs were prohibited. Patients randomly assigned to zoledronic acid received 4 mg intravenous zoledronic acid every 3–4 weeks for six doses, then every 3 months for eight doses, followed by every 6 months for five doses, for a total of 5 years of treatment. The dose of zoledronic acid was adjusted in accordance with the product licence for renal function abnormalities. Calcium and vitamin D oral supplements were recommended for all trial participants during the first 6 months on study and were continued thereafter at the discretion of the treating physician.

The follow-up schedule was similar for both control and zoledronic acid groups and included clinical assessment, adverse-event monitoring, and measurement of haematological, renal, and hepatic function. Patients were clinically assessed before each dose of zoledronic acid or at the same timepoints for patients assigned to the control group. Visits between scheduled timepoints were made as clinically indicated. Routine follow-up imaging was not mandated; investigations for possible recurrence were done when deemed appropriate by the treating physician. The date of recurrence was defined as the date on which relapse was first suspected, to reduce the risk of ascertainment bias.[11] When possible (93% of recurrence events); recurrences were independently validated by either on-site or telephone-based monitoring.

Patients stopped zoledronic acid on completion of 5 years of treatment or after distant recurrence, unacceptable toxicity, three consecutively missed treatments, patient request, or physician recommendation. Continuation of study drug was recommended after locoregional recurrence, and was at the physician's discretion after a new primary cancer. Patients were followed up on an annual basis after completion of the 5-year treatment phase (zoledronic acid or control) for disease recurrence, death, skeletal-related events, and osteonecrosis of the jaw as a potential late adverse event of note.

Baseline serum samples were taken at study entry for analysis of bone biomarkers (n-telopeptide of type I procollagen [PINP] and c-telopeptide of type I collagen [CTX]) and reproductive hormones (oestradiol, follicle-stimulating hormone [FSH], and inhibin A). Samples were collected at selected centres within the UK and stored at –20°C or –80°C, depending on local facilities. Samples were transferred to Weston Park Hospital, Sheffield, on a regular basis where they were stored at –80°C until central batch analysis.

PINP and CTX concentrations were measured using Cobas e411 automated immunoassays (Roche Diagnostics, Mannheim, Germany). The threshold for high PINP was set at 70 ng/mL on the basis of previous reports of a

	Control group (n=1678)	Zoledronic acid group (n=1681)
(Continued from previous page)		
Progesterone receptor status		
Positive	698 (42%)	725 (43%)
Negative	424 (25%)	382 (23%)
Unknown	548 (33%)	571 (34%)
Missing	8 (<1%)	3 (<1%)
HER2 status		
Positive	223 (13%)	192 (11%)
Negative	603 (36%)	648 (39%)
Unknown or missing	852 (51%)	841 (50%)
Received trastuzumab	242 (14%)	204 (12%)
Histological grade		
1	140 (8%)	145 (9%)
2	708 (42%)	731 (43%)
3	787 (47%)	765 (46%)
Not specified	36 (2%)	31 (2%)
Missing	7 (<1%)	9 (1%)
Intended for neoadjuvant treatment		
Yes	107 (6%)	105 (6%)
No	1571 (94%)	1576 (94%)
Intended systemic treatment plan		
Endocrine treatment alone	76 (5%)	76 (5%)
Chemotherapy alone	358 (21%)	361 (21%)
Endocrine treatment and chemotherapy	1244 (74%)	1244 (74%)
Intended use of anthracyclines		
Yes	1564 (93%)	1568 (93%)
No	114 (7%)	113 (7%)
Intended use of taxanes		
Yes	385 (23%)	390 (23%)
No	1293 (77%)	1291 (77%)
Intended use of statins		
Yes	100 (6%)	97 (6%)
No	1578 (94%)	1584 (94%)
Menopausal status		
Premenopausal	753 (45%)	751 (45%)
≤5 years since menopause	243 (14%)	247 (15%)
>5 years since menopause	522 (31%)	519 (31%)
Menstrual status unknown	160 (10%)	164 (10%)

Data are mean (SD) or number (%). Some percentages do not total 100 because of rounding.

Table 1: **Demographics and baseline characteristics**

For the **AZURE trial protocol** see http://ctru.leeds.ac.uk/Azure

possible predictive role of PINP in the development of bone metastases[12] and advice from Roche Diagnostics. For CTX, the upper limit of normal for premenopausal women (0·299 ng/mL) was used. Both PINP and CTX were analysed at above or below these threshold values and as continuous variables.

Oestradiol and FSH measurements were done on an automated Roche 602 Elecsys electrochemiluminescence immunoassay (Roche Diagnostics), and inhibin A on an automated ACCESS chemiluminescence immunoassay system (Beckman Coulter, High Wycombe, UK). The lower limits of detection for the assays were 1 pg/mL for inhibin, 18·4 pmol/L for oestradiol, and 0·1 IU/L for FSH. Reference ranges for premenopausal and post-menopausal women were assay specific, stipulated by the manufacturer, and validated in house. Patients who had already started endocrine treatment at study entry or those receiving hormone-replacement therapy or tibolone at diagnosis were excluded. To classify a patient as postmenopausal, all three of FSH greater than 26 IU/L, oestradiol less than 50 pmol/L, and inhibin A less than 3·6 pg/mL were necessary.

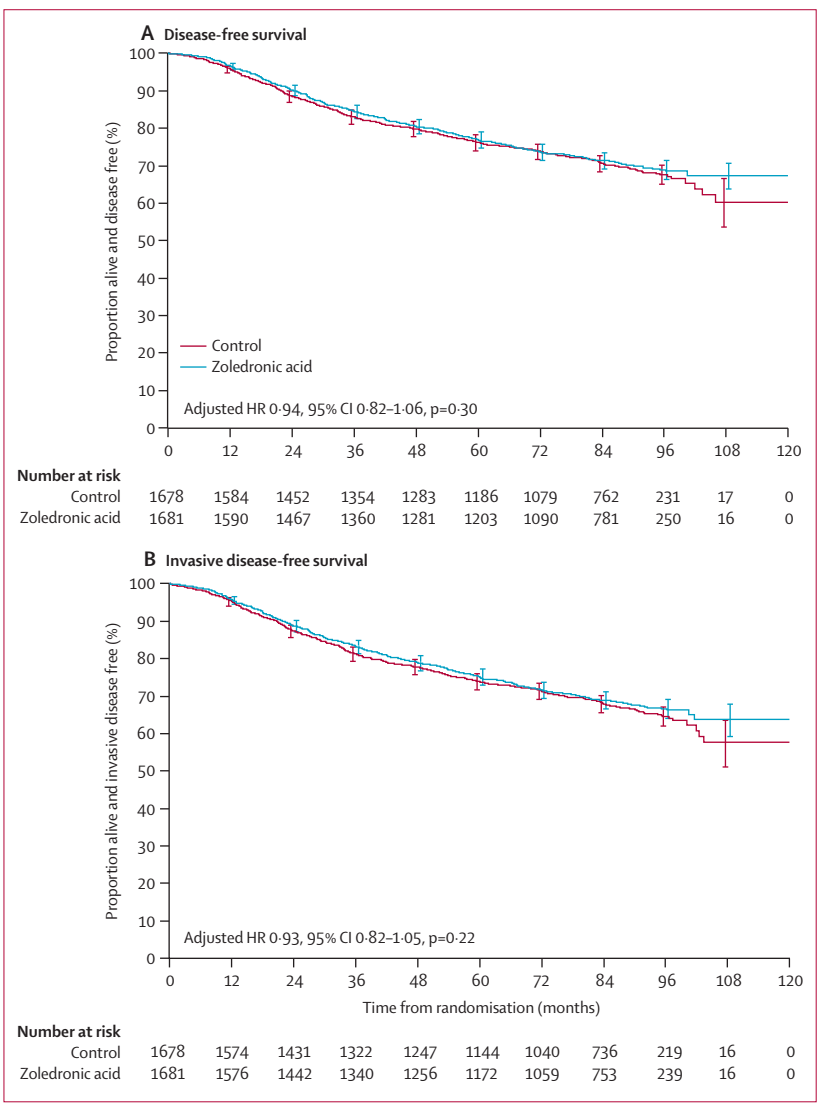

Figure 2: **Kaplan-Meier curves of (A) disease-free survival and (B) invasive disease-free survival**
Analyses were by intention to treat. HR=hazard ratio.

Outcomes

The primary endpoint of the study was disease-free survival (DFS), defined as distant recurrence, any invasive locoregional recurrence except for ipsilateral operable relapse within a conserved breast, and death without recurrence. After a protocol amendment on Aug 28, 2008, on the advice of the trial steering committee, invasive DFS (IDFS),[13] defined as death from any cause, invasive ipsilateral breast tumour recurrence, local or regional invasive recurrence, distant recurrence, invasive contralateral breast cancer, or second primary invasive cancer (non-breast but excluding basal-cell or squamous skin cancers), was added as a key secondary endpoint because this is included in the international standard definition for recurrence that emerged since the trial began. Secondary endpoints were overall survival, time to bone metastases, distant recurrences, subgroup analyses of variables included in the randomisation, and adverse events deemed potentially related to either the study drug or cancer treatments. We also did exploratory post-hoc analyses to investigate treatment effects on sites of first recurrence.

Statistical analysis

This final analysis was planned after 940 DFS events to provide 80% power to detect a 17% reduction in the hazard ratio (HR) for DFS at a 5% (two-sided) level of significance, approximating to a 3·7% absolute benefit, with a null hypothesis of no difference between the treatment groups with respect to DFS, and an alternative hypothesis of a difference, with the expectation of superior DFS in the zoledronic acid group. Assumptions included a 3-year recruitment period of 3300 patients, 75% DFS (control arm) at 3 years, and 5% annual loss to follow-up. A planned interim analysis after 470 events with a two-sided alpha spend of 0·005 was done in 2008, but no efficacy data were released. Because the event rate was less than predicted (combined arms DFS of 85% at 3 years), two independent statisticians who were not involved in the first interim analysis defined revised stopping boundaries for a second interim analysis to assess both efficacy and a lower threshold of benefit. At this analysis, done with 752 events, the predefined lower threshold of efficacy boundary was crossed and the independent data monitoring committee recommended the release of results. These results have been published previously.[5]

We used Kaplan-Meier survival curves to investigate DFS, IDFS, and overall survival, and cumulative incidence function curves for the development of bone metastases, with differences between treatment arms compared with the log-rank test and Cox's proportional hazards model to

adjust for the minimisation factors (excluding centre). Hypothesis testing for the final analysis was at a 3·5% significance level (two sided) after 0·5% and 1% were spent on the first and second interim analyses, respectively. Subgroup analyses were planned for the baseline criteria used in the minimisation process (except treating centre), with menopausal status and oestrogen receptor status the criteria expected from a biological standpoint to be of most interest. For the subgroup analyses, we used the Cox model to adjust for statistically significant factors in the corresponding overall analysis (oestrogen receptor status, lymph node involvement, neoadjuvant treatment, and tumour stage for both IDFS and overall survival). We used a treatment-interaction term to test for heterogeneity between subgroups. Analyses were done in SAS version 9.2.

Efficacy endpoints were analysed according to the intention-to-treat population, consisting of all patients in the trial, grouped according to randomised treatment. Safety endpoints were analysed according to the safety population, consisting of all patients who received at least one dose of study drug and had at least one safety assessment after baseline, grouped according to treatment received during the 5-year treatment period. These endpoints have been reported previously[14,15] and, other than an updated assessment of osteonecrosis of the jaw and serious adverse events (SAEs), are not included in this report.

This trial is registered with ClinicalTrials.gov, NCT00072020.

Role of the funding source

Novartis was given an opportunity to comment on the manuscript but all decisions on publication rested with the authors and the trial steering committee. Novartis approved the study design, received regular updates on the progress of the study, had a non-voting member on the trial steering committee, and had the opportunity to comment on the manuscript. Novartis was not involved in data collection, analysis, or interpretation. RC, DC, RBe, SH, and HM had access to the raw data and supervised all analyses. RC had full access to all the data in the study and had final responsibility for the decision to submit for publication.

	Control group (n=537 events)	Zoledronic acid group (n=512 events)
Ipsilateral breast	22 (4%)	32 (6%)
Other locoregional recurrence	108 (20%)	98 (19%)
Distant recurrence	348 (65%)	326 (64%)
Bone as first distant recurrence	189 (54%)	150 (46%)
Contralateral breast	20 (4%)	16 (3%)
Death as first event	33 (6%)	29 (6%)
New primary	50 (9%)	44 (9%)

Data are number of events (%). Categories are not mutually exclusive.

Table 2: **Type of invasive disease-free survival**

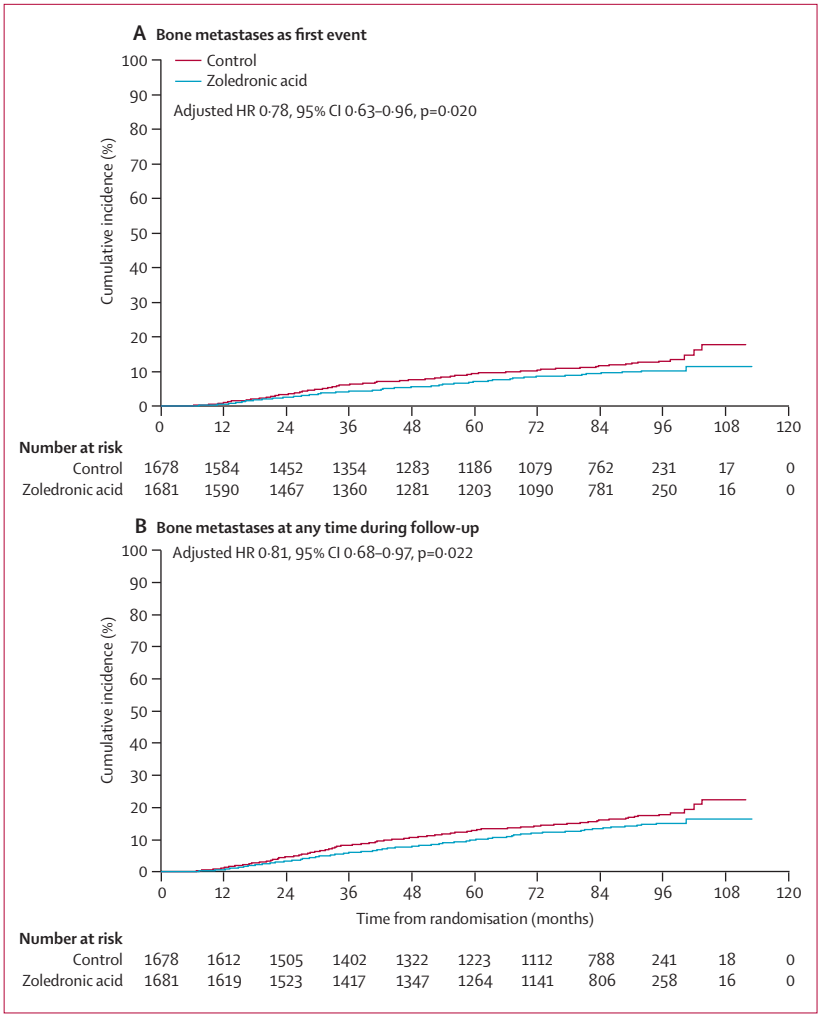

Figure 3: **Cumulative incidence of bone metastases (A) as a first event and (B) at any time during follow-up**
Analyses were by intention to treat. HR=hazard ratio.

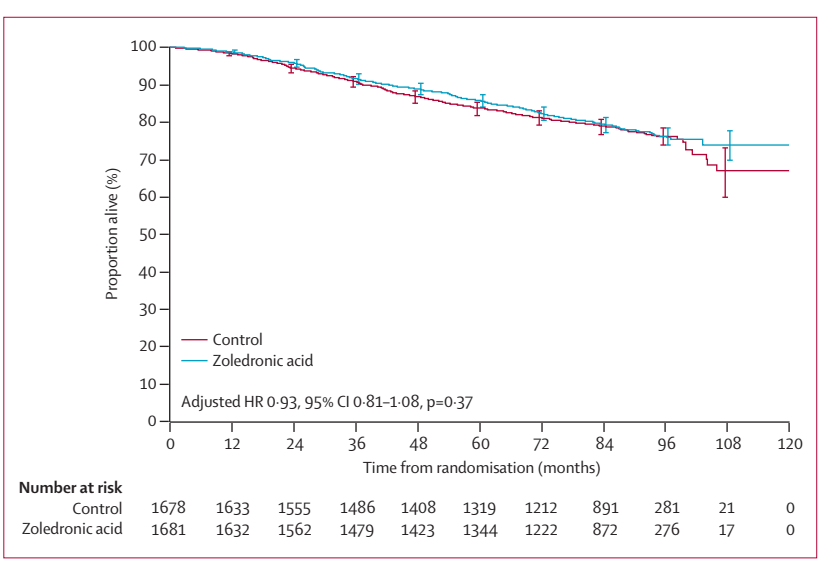

Figure 4: **Kaplan-Meier curve of overall survival**

Results

3360 women were recruited from 174 centres in seven countries (2710 from the UK) between Sept 4, 2003, and Feb 16, 2006. 1679 women were randomly assigned to the control group and 1681 to the zoledronic acid group (figure 1). One patient in the control group withdrew consent 4 days after random allocation, before any follow-up data collection, and so was excluded from all analyses. The tumour and treatment characteristics were well balanced (table 1). 3207 (95%) of 3359 patients received neoadjuvant or adjuvant chemotherapy. 222 control group patients (13%) received a bisphosphonate (typically low-dose oral treatment for bone loss or osteoporosis) before or in the absence of a DFS event.

At the database cutoff on April 30, 2013, median follow-up was 84·0 months (IQR 63·3–92·2) for the control group and 84·0 months (69·7–93·2) for the zoledronic acid group. The median time since last follow-up for all surviving patients was 8·7 months (IQR 5·0–15·3) for the control group and 8·8 months (5·0–14·9) for the zoledronic acid group (appendix). 401 (30%) of 1316 patients in the control group and 416 (31%) of 1335 in the zoledronic acid group who were still alive at data cutoff had their last follow-up visit more than 12 months before data cutoff. The median number of zoledronic acid infusions received in patients allocated to zoledronic acid was 18 (IQR 11–19). There were 185 dose reductions of zoledronic acid (range 1–17) in 56 (3%) of 1665 patients who received allocated zoledronic acid.

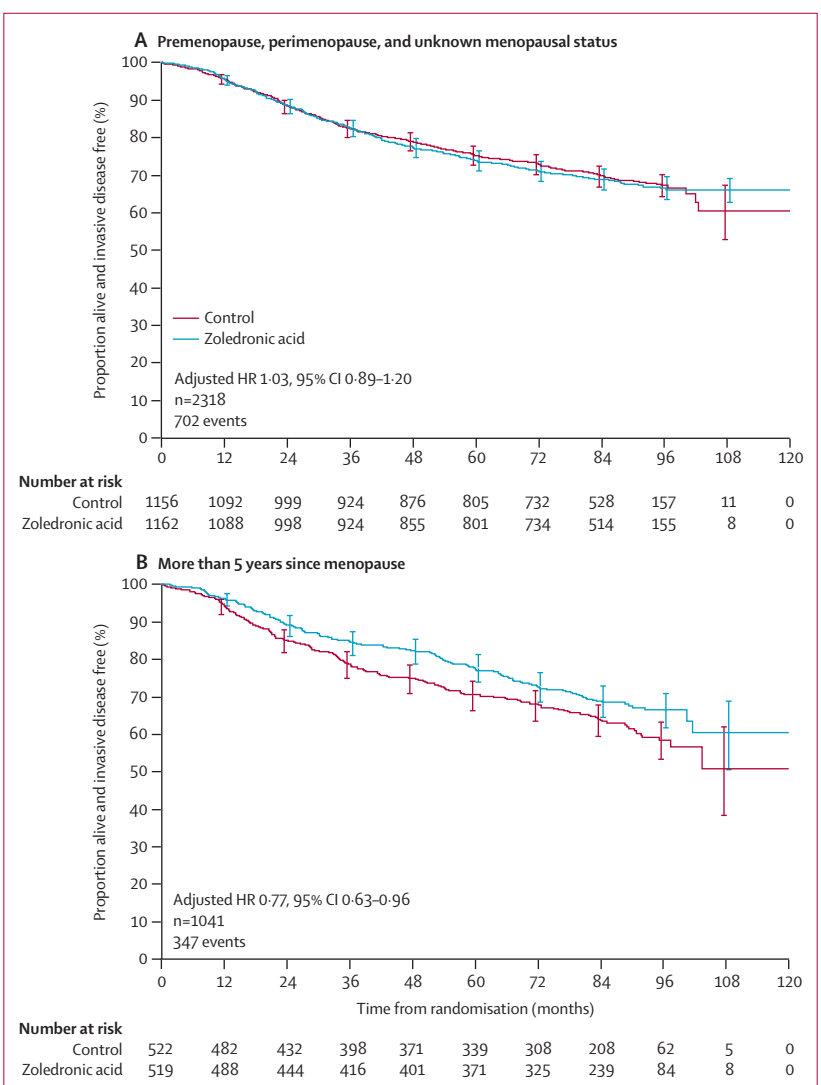

Figure 5: **Kaplan-Meier curve of invasive disease-free survival by menopausal status**
(A) Premenopause, perimenopause, and unknown menopausal status and (B) more than 5 years since menopause. Test for heterogeneity by menopausal status χ^2_1 4·71; p=0·03.

The number of DFS events did not differ between groups: 493 in the control group and 473 in the zoledronic acid group (adjusted HR 0·94, 95% CI 0·82–1·06; p=0·30; figure 2A). The findings were similar for IDFS: 537 events in the control group and 512 in the zoledronic acid group (adjusted HR 0·93, 95% CI 0·82–1·05; p=0·22; figure 2B). The distribution of events comprising IDFS are shown in table 2. 674 (64%) of 1049 events were distant recurrences. Significant reductions in the incidence of bone metastases as either the first recurrence (adjusted HR 0·78, 95% CI 0·63–0·96; p=0·020) or at any time (0·81, 0·68–0·97; p=0·022) occurred in the zoledronic acid group (figure 3). The number of distant DFS events was similar in the two treatment groups: 413 in the control group and 390 in the zoledronic acid group (adjusted HR 0·93, 95% CI 0·81–1·07; p=0·29; appendix). Up to data cutoff, 708 deaths have occurred (figure 4): 362 in the control group and 346 in the zoledronic acid group (adjusted HR for survival 0·93, 95% CI 0·81–1·08; p=0·37). Eight deaths were related to cancer treatment, but there were no deaths attributed to zoledronic acid.

We undertook preplanned subgroup analyses looking at the minimisation factors used in randomisation. In the appendix, a forest plot of randomised treatment allocation HRs summarises all of the planned subgroup analyses for IDFS. We noted significant heterogeneity of treatment effect by menopausal status (DFS χ^2_1 4·42; p=0·04; IDFS χ^2_1 4·71; p=0·03). In the 1041 women for whom at least 5 years had passed since menopause at study entry, zoledronic acid improved IDFS (figure 5). This improvement did not occur in the other menopausal groups (figure 5). No other potential treatment modifiers were identified. We deemed the discordance in outcome between T3 and T4 tumours to be a chance finding in small subgroups and with no biological basis for plausibility.

The effect of menopausal status on zoledronic acid treatment effects was driven by marked differences in extraskeletal IDFS events (figure 6). In women for whom at least 5 years had passed since menopause at trial entry, a reduction in risk of extraskeletal recurrence was noted with zoledronic acid compared with the suggestion of an increased risk for women who were premenopausal at study entry (χ^2_3 heterogeneity 10·04; p=0·018); there was

no difference in risk for perimenopausal women (figure 6A). By contrast, the effect of zoledronic acid on bone IDFS events was not affected by menopausal status (χ^2_3 for heterogeneity 0·12; p=0·99; figure 6B), with similar findings across menopausal groups. There was evidence of benefit in distant DFS for postmenopausal women (adjusted HR 0·75, 95% CI 0·58–0·97), but not for the other menopausal groups (1·04, 0·88–1·23), and there was significant heterogeneity between menopausal groups (χ^2_3 for heterogeneity 4·44; p=0·04).

The HRs for overall survival were 0·81 (95% CI 0·63–1·04) for women who were more than 5 years since menopause and 1·04 (0·86–1·25) for those for whom fewer than 5 years had passed since menopause (χ^2_1 for heterogeneity 2·47; p=0·12; appendix).

Zoledronic acid protected against fractures, with 140 (8·3%) of 1678 control and 104 (6·2%) of 1681 zoledronic acid patients reporting a fracture (HR 0·69, 95% CI 0·53–0·90; p=0·005). The proportions of women who had fractures at 5 years were 5·9% (95% CI 4·8–7·1) for the control group and 3·8% (2·9–4·7) for the zoledronic acid group. There was a marked reduction in reported fractures at the time of, or after, a DFS event, compared with those who had fractures before a DFS event: 49 (9·9%) of 493 control and 18 (3·8%) of 473 zoledronic acid patients experienced one or more fractures after a DFS event. The corresponding proportions of patients who had fractures at 2 years after a DFS event were 9·8% (95% CI 7·1–12·6) and 2·8% (1·2–4·4). Bone metastases were reported before, or coincident with, fracture in 45 of 49 and 15 of 18 of these patients, respectively.

The heterogeneity of treatment effect by menopause (appendix) seemed to be similar in patients with oestrogen-receptor-negative tumours (>5 years since menopause: HR 0·69, 95% CI 0·47–1·00; not >5 years since menopause: 1·16, 0·88–1·53) compared with those with oestrogen-receptor-positive tumours (>5 years since menopause: HR 0·82, 95% CI 0·63–1·07; not >5 years since menopause: 0·98, 0·82–1·17).

In an exploratory post-hoc analysis, we assessed age as an alternative treatment modifier to menopause (appendix). In women younger than 40 years, the HR for IDFS was 1·47 (95% CI 1·08–2·01), whereas in those aged 60–69 years, it was 0·76 (0·58–0·99). In those aged 40–59 years, outcome seemed to be related to menopausal status rather than age, with women aged 40–49 years for whom at least 5 years had passed since menopause seeming to derive more benefit than those aged 50–59 years who were not in established menopause at study entry (appendix).

Oestradiol, FSH, and inhibin A measurements were available for 804 patients: 400 in the control group and 404 in the zoledronic acid group. Both the patient characteristics and disease outcomes of this biomarker subset of patients were similar to the whole study population (appendix).

High serum PINP at baseline (n=238) seemed to be associated with an increased risk of bone metastases when compared with patients with low baseline PINP (n=629; HR 1·58, 95% CI 1·00–2·50; p=0·06) but not for distant recurrence overall (HR 0·99, 95% CI 0·72–1·37; p=0·96). PINP levels did not predict benefit from zoledronic acid on bone recurrence or any other component of invasive relapse (data not shown). Serum CTX, whether categorised as high (n=262) versus low (n=601) or as a continuous variable, was neither prognostic for bone metastases or distant recurrence nor able to predict benefit from zoledronic acid (data not shown).

We used the reproductive hormone levels assessed in the biomarker subset to reclassify menopause into two groups—postmenopausal (n=301) and not postmenopausal (n=505)—and did exploratory post-hoc analyses to assess study treatment effects on DFS. The HR for DFS was 0·78 (95% CI 0·51–1·20) for the postmenopausal patients and 1·00 (0·70–1·43) for those who were not postmenopausal.

Adverse events were similar in the two groups and have been reported previously.[14,15] SAEs are shown in the appendix. 735 SAEs were reported by 509 (31%) of 1667 patients receiving standard treatment and 856 SAEs were reported by 580 (34%) of 1685 patients receiving standard treatment plus zoledronic acid. Other than osteonecrosis of the jaw, there were no differences between the treatment groups in individual SAEs, with most related to adjuvant chemotherapy. 33 cases of suspected osteonecrosis of the jaw have been reported, with 26 confirmed on central review, all in the zoledronic acid group (1·7%, 95% CI 1·0–2·4), three of which

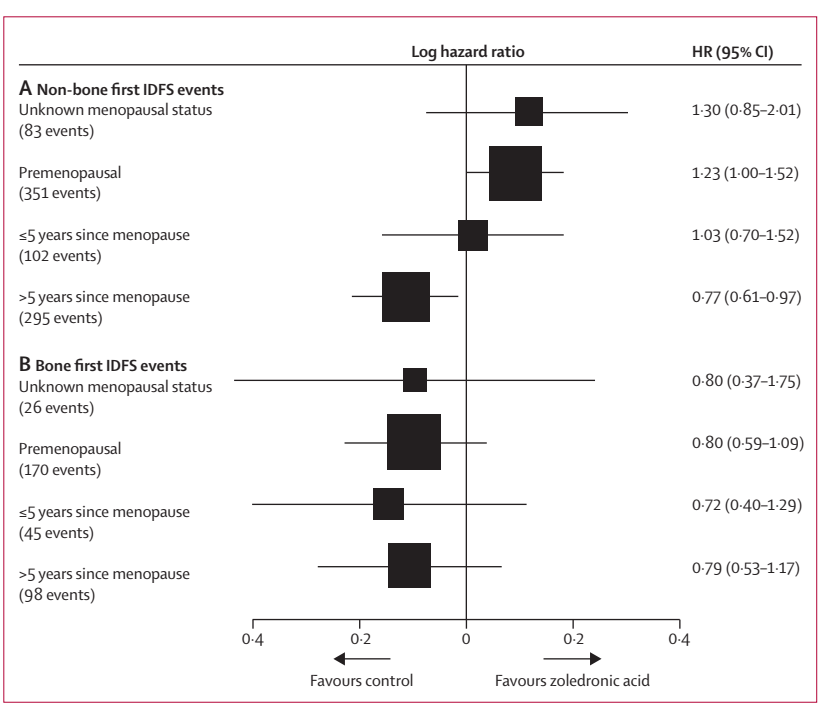

Figure 6: **Forest plot of invasive disease-free survival by menopausal group**
(A) Non-bone first IDFS events (χ^2_3 heterogeneity 10·04; p=0·018) and (B) and bone first IDFS events (χ^2_3 for heterogeneity 0·12; p=0·99). IDFS=invasive disease-free survival.

occurred after relapse in bone and zoledronic acid use in the metastatic setting. The only other risk factor for osteonecrosis of the jaw was dental extraction.

Discussion

In this final analysis of the AZURE trial, no improvement in the primary endpoint, DFS, was noted for the study population as a whole. DFS, IDFS, and overall survival outcomes were similar in both treatment groups. However, significant improvements in IDFS were seen in women who were postmenopausal at study entry, irrespective of the oestrogen-receptor status of the primary tumour (panel).

The role of adjuvant bisphosphonates in early breast cancer is unclear. After variable results with oral clodronic acid[17–19] and pamidronic acid,[20] provocative results from the Austrian Breast Cancer Study Group 12 trial (ABCSG-12)[8,21] changed the landscape for this treatment approach, focusing more on the host (bone-marrow micro-environment) than the cancer. In ABCSG-12, 1803 pre-menopausal women with oestrogen-receptor-positive breast cancer with good prognosis treated with goserelin to induce menopause and either tamoxifen or anastrazole were randomly assigned to 6-monthly zoledronic acid for 3 years or no additional treatment. Zoledronic acid reduced the risk of disease recurrence by about a third,[8,21] with benefits in both DFS and overall survival maintained at a final analysis with a median follow-up of 84 months.[22]

Therefore, the absence of activity in the first findings from the AZURE trial were somewhat of a surprise.[5] These findings seemed to contradict those from ABCSG-12. However, a planned subgroup analysis suggested significant benefit in women for whom at least 5 years had passed since menopause at study entry. Although this group of women were older than the ABCSG-12 population, they were probably similar in terms of reproductive hormone levels because of the use of ovarian-suppression treatment in all patients in ABCSG-12.

In this final analysis, menopause remained a substantial treatment modifier, with fewer recurrences in women with established menopause (>5 years since last menstruation) who were treated with zoledronic acid. Tumour characteristics and—other than use of aromatase inhibitors—the adjuvant treatments, including the use of chemotherapy, were similar across menopausal groups (appendix). A similar reduction in the HR for DFS was noted in the subset of patients with menopause identified by biochemical criteria compared with those in the clinically prespecified postmenopausal subgroup. The benefits in postmenopausal women seemed to be independent of oestrogen-receptor status of the primary tumour, with the reduction in HR in postmenopausal patients with oestrogen-receptor-negative tumours at least as large as that reported in patients with oestrogen-receptor-positive tumours. Any possible relation between treatment effects and HER2 status or use of trastuzumab is difficult to assess. Only a few patients had HER2 status available because recruitment largely predated the use of adjuvant trastuzumab.

The Early Breast Cancer Trials Collaborative Group is undertaking a formal individual patient meta-analysis of randomised trials of adjuvant bisphosphonates for early breast cancer.[16] Preliminary results in nearly 18 000 women with early breast cancer suggest a slight benefit for adjuvant bisphosphonates with a reduction in the risks of bone recurrence (HR 0·77, SE 0·06; two-sided p=0·0009) and breast cancer death (HR 0·90, SE 0·05; two-sided p=0·03). However, this reduction was due entirely to the benefit noted in the subset of more than 11 000 postmenopausal women who had a reduction in risks of bone recurrence (HR 0·66, SE 0·08; two-sided p<0·0001) and breast cancer death (HR 0·83, SE 0·06; two-sided p=0·004). As in our study, no reductions were noted in breast cancer recurrence or mortality in women who were not postmenopausal.

We thought the benefits reported in postmenopausal women with zoledronic acid might be related to the higher rate of bone turnover that occurs at menopause. However, within the limitations of an exploratory analysis of a subset of patients with biomarker measurements, we were unable to detect any associations between the bone turnover markers PINP or CTX and effects of zoledronic acid on recurrence either in bone or at extraskeletal distant sites.

In comparison with our first report,[5] with longer follow-up and more events, we show that zoledronic acid reduced bone metastases as either a first or subsequent site of recurrence. This endpoint is perhaps the most likely to be affected by a bone-targeted treatment with its powerful inhibitory effects on osteoclast function and is consistent with findings from several previous trials.[7,17,18,21]

The marked treatment heterogeneity by menopausal status on recurrence outside bone is provocative, with an

Panel: Research in context

Systematic review

We searched PubMed and ClinicalTrials.gov in January, 2014, using the terms "adjuvant", "bisphosphonate" and "breast cancer" to find relevant trials. The identified trials are the subject of an ongoing individual patient meta-analysis by the Early Breast Cancer Clinical Trials Group.[16]

Interpretation

In this large randomised trial in a broad population of patients with early breast cancer, adjuvant zoledronic acid had no effect on disease-free survival, invasive disease-free survival, or overall survival, but it did reduce the development of bone metastases. Zoledronic acid is not recommended for routine use in an unselected population. Zoledronic acid improved invasive disease free-survival in women with established menopause but not in women with residual ovarian function. Osteonecrosis of the jaw was the most important adverse event but was uncommon and largely confined to patients who needed dental extractions.

apparent increase in extraskeletal metastases in premenopausal women, a neutral effect in perimenopausal women, and benefit in postmenopausal women. In the presence of oestrogen, inhibin, follistatin, and other hormones that characterise the premenopausal state, zoledronic acid had a deleterious effect outside bone and thus no net benefit despite the reduction in bone metastases. Conversely, in postmenopausal women, when activin regulation of transforming growth factor signalling becomes dominant,[23] the presence of zoledronic acid seems to not only prevent bone metastases, but also the presumed onward dissemination to other sites of metastasis, resulting in overall benefit.

Zoledronic acid was well tolerated. A full description of the adverse-event profile[14] and the effects on dental health and dental quality of life are published elsewhere.[15] The incidence of osteonecrosis of the jaw in this study seems to be higher than reported in trials that have assessed less frequent dosing of zoledronic acid[6,8,21] or daily oral bisphosphonates,[7] but comparative trials are needed to address this reliably. However, for postmenopausal women with stage II or III breast cancer, the benefits in IDFS, with an absolute benefit at 5 years of around 5% and an osteonecrosis of the jaw rate of 1–2%, suggest a favourable risk-to-benefit ratio.

There are some limitations to this study. The open-label design could result in bias. However, the follow-up protocol was the same and the data flow was similar in both treatment groups. We made every effort to monitor and validate all recurrence endpoints. 712 (93%) of 766 recurrences were independently validated by either on-site or telephone-based monitoring. There was no discernable difference in follow-up investigations such as bone scans (data not shown) between treatment groups. The finding that benefits are restricted to a subgroup of patients, although preplanned, is in itself only hypothesis generating. However, in view of other adjuvant bisphosphonate trial results and the ongoing meta-analysis from the Early Breast Cancer Trialists' Collaborative Group,[16] we believe the benefits reported in postmenopausal women are probably real and clinically relevant.

In conclusion, these data do not support use of adjuvant zoledronic acid in unselected patients with early breast cancer. However, our results, supported by other trial data, including a recently presented meta-analysis,[16] make a case for considering zoledronic acid in the adjuvant treatment programme for postmenopausal women with early breast cancer.

Contributors

RC, DC, DD, and RBe developed and wrote the study concept and protocol. RC, DC, DD, RBe, CW, ER, RBu, and HM undertook the study. RC, DC, RBe, VL, SH, and HM collected, analysed, and interpreted the data. RBe, MK, and MG coordinated academic participation in Australasia, Ireland, and Spain and Portugal, respectively. RC, RBe, MK, MG, RG, PB-L, and DR were major recruiters. RC wrote the first draft of the manuscript. All authors were involved in revision and approval of the manuscript.

Declaration of interests

RC has received research funding and expert testimony from Novartis and honoraria from Amgen, Bayer, and Celgene. DC has received consultancy fees, honoraria, and travel support from Novartis. RBe has received consultancy and expert testimony fees from Novartis. PB-L has received honoraria and travel support from Novartis. All other authors declare no competing interests.

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