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Research Paper

Benefits and risks of adjuvant treatment with zoledronic acid in stage II/III breast cancer. 10 years follow-up of the AZURE randomized clinical trial (BIG 01/04)

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ABSTRACT

Adjuvant bisphosphonates improve disease outcomes in postmenopausal early breast cancer (EBC) but the long-term effects are poorly described. The AZURE trial (ISRCTN79831382) was designed to determine whether adjuvant zoledronic acid (ZOL) improves disease outcomes in EBC. Previous analyses showed no effect on overall outcomes but identified benefits in postmenopausal women. Here we present the long-term risks and benefits of adjuvant ZOL with 10-years follow-up.

Patients and methods: 3360 patients with stage II/III breast cancer were included in an academic, international, phase III, randomized, open label trial. Patients were followed up on a regular schedule until 10 years. Patients were randomized on a 1:1 basis to standard adjuvant systemic therapy +/− intravenous ZOL 4 mg every 3–4 weeks x6, and then at reduced frequency to complete 5 years treatment. The primary outcome was disease free survival (DFS). Secondary outcomes included invasive DFS (IDFS), overall survival (OS), sites of recurrence, skeletal morbidity and treatment outcomes according to primary tumor amplification of the transcription factor, MAF. Pre-planned subgroup analyses focused on interactions between menopausal status and treatment effects.

Results: With a median follow up of 117 months [IQR 70.4–120.4), DFS and IDFS were similar in both arms (HR_{DFS} = 0.94, 95%CI = 0.84–1.06, p = 0.340; HR_{IDFS} = 0.91, 95%CI = 0.82–1.02, p = 0.116). However, outcomes remain improved with ZOL in postmenopausal women (HR_{DFS} = 0.82, 95%CI = 0.67–1.00; HR_{IDFS} = 0.78, 95%CI = 0.64–0.94). In the 79% of tested women with a MAF FISH negative tumor, ZOL improved IDFS (HR_{IDFS} = 0.75, 95%CI = 0.58–0.97) and OS HR_{OS} = 0.69, 95%CI = 0.50–0.94), irrespective of menopause. ZOL did not improve disease outcomes in MAF FISH + tumors. Bone metastases as a first DFS recurrence (B_{DFS}) were reduced with ZOL ($HR_{B\text{-}DFS}$ = 0.76, 95%CI = 0.63–0.92, p = 0.005). ZOL reduced skeletal morbidity with fewer fractures and skeletal events after disease recurrence. 30 cases of osteonecrosis of the jaw in the ZOL arm (1.8%) have occurred.

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Conclusions: Disease benefits with adjuvant ZOL in postmenopausal early breast cancer persist at 10 years of follow-up. The biomarker MAF identified a patient subgroup that derived benefit from ZOL irrespective of menopausal status.

Introduction

Despite improvements in both early diagnosis and treatment, breast cancer remains the leading cause of cancer death in women in the developed world [1]. Late recurrence of the disease is frequent, especially to bone, with disseminated malignant cells seemingly able to evade adjuvant treatments and remain dormant, before re-activating and causing disease relapse many years after diagnosis [2]. Therapeutic strategies to improve disease outcomes include the use of bisphosphonates to affect the bone microenvironment [3].

The AZURE trial is an academic study, designed to test whether treatment with the bisphosphonate, zoledronic acid (ZOL), could improve disease outcomes in patients with stage II/III breast cancer. Previous analyses after 752 [4] and 966 [5] disease free survival (DFS) events showed that, despite a reduction in the risk of developing bone metastases with ZOL, there was no effect on overall breast cancer recurrence. However, preplanned subgroup analyses identified benefit in women who were in established menopause at the time of study entry. This observation was subsequently confirmed by the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) meta-analysis of adjuvant bisphosphonates [6], resulting in recommendations both in Europe [7] and North America [8] to consider use of adjuvant bisphosphonates in postmenopausal women with early breast cancer.

Here, we present the 10 year follow-up results from the AZURE trial and describe the effects of ZOL on disease relapse, skeletal morbidity, site(s) of recurrence, overall survival and toxicity. We also explore the interactive effects of menopausal status, age at diagnosis and the predictive biomarker MAF with ZOL on disease outcomes.

Methods

AZURE (BIG01/04) is an academic, prospective, randomized controlled phase III, open label multi-national and multi-center trial (ISRCTN79831382). The study design and patient eligibility have been described previously [4,5], but, in summary, patients had histologically confirmed invasive breast cancer of any biological subtype with either pathologically confirmed axillary lymph node metastases or a T3/T4 primary tumor. Patients were not eligible if there was clinical or imaging evidence of distant metastases prior to study entry, current or recent (previous year) use of bisphosphonates or pre-existing bone disease likely to require bone-targeted treatment. All patients gave written informed consent.

Between September 2003 and February 2006, 3360 patients from 174 centers were randomized on a 1:1 basis using a central automated 24-hour computer-generated telephone randomization system to receive (neo)adjuvant systemic therapy with (ZOL) or without (CONTROL) zoledronic acid. A minimization method was used to ensure balance in key prognostic and treatment variables across the two groups [4].

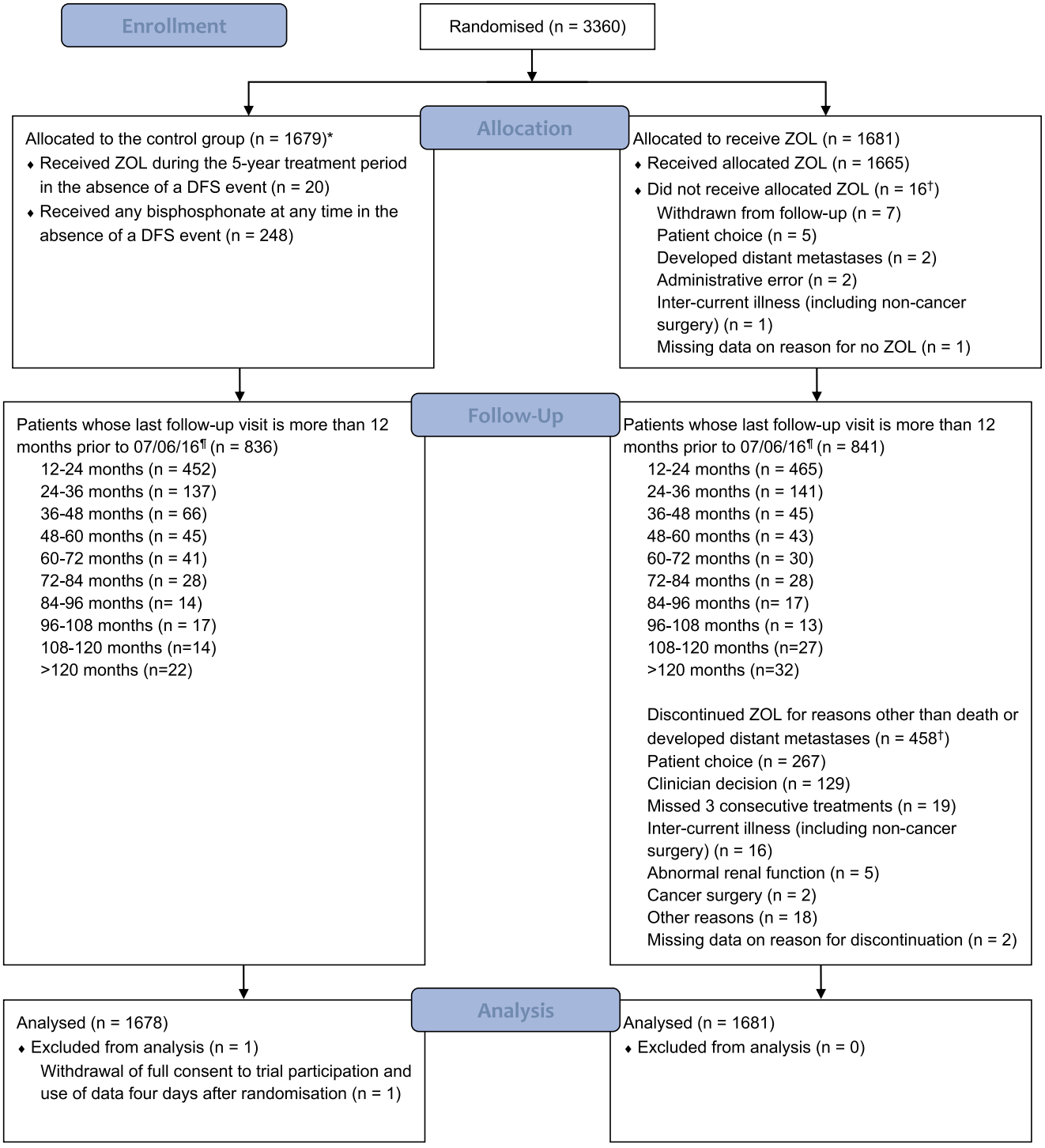
ZOL 4 mg was administered by intravenous (iv) infusion every 3–4 weeks for 6 doses, then 3 monthly x 8 and 6 monthly x 5 to complete 5 years treatment. Novartis Pharmaceuticals provided study-specific supplies of ZOL. Daily oral supplements containing calcium (400–1000 mg) and vitamin D (200–500 IU) were recommended to all trial subjects during the first six months, and continued thereafter at the discretion of the treating physician.

Surgical management, the use and delivery of loco-regional external beam radiotherapy, timing of adjuvant treatments in relation to surgery and selection of chemotherapy and endocrine treatments were all decided in accordance with standard protocols at each participating institution. Trastuzumab was allowed in patients with HER2 + tumors after regulatory approval for adjuvant use in 2006.

Table 1
Baseline patient characteristics.

	Allocation			
	Standard treatment alone		Standard treatment + ZOL	
	Number	Percent	Number	Percent
Lymph node involvement	32	1.9	30	1.8
• 0 nodes involved				
• One–three nodes involved	1033	61.6	1042	62.0
• = > four nodes involved	607	36.2	604	35.9
• Unknown involvement	6	0.4	5	0.3
Tumour stage	523	31.2	542	32.2
• T1				
• T2	867	51.7	850	50.6
• T3	228	13.6	228	13.6
• T4	59	3.5	58	3.5
• TX	1	0.1	3	0.2
ER status	1315	78.4	1318	78.4
• ER positive				
• ER negative	356	21.2	350	20.8
• ER unknown	7	0.4	13	0.8
PR status	699	41.7	725	43.1
• Positive				
• Negative	424	25.3	382	22.7
• Unknown/missing	555	33.1	574	34.1
HER2 status	223	13.3	192	11.4
• Positive				
• Negative	604	36.0	648	38.5
• Unknown/missing/not measured	851	50.7	841	50.0
Histological grade	141	8.4	146	8.7
• 1				
• 2	708	42.2	731	43.5
• 3	787	46.9	765	45.5
• Not specified/missing	42	2.5	39	2.3
Intended for neo-adjuvant therapy	107	6.4	105	6.2
• Yes				
• No	1571	93.6	1576	93.8
Intended systemic therapy plan	76	4.5	76	4.5
• Endocrine therapy alone				
• Chemotherapy alone	358	21.3	361	21.5
• Endocrine therapy and chemotherapy	1244	74.1	1244	74.0
Intended use of anthracyclines*	1564	93.2	1568	93.3
• Yes				
• No	114	6.8	113	6.7
Intended use of taxanes*	385	22.9	390	23.2
• Yes				
• No	1293	77.1	1291	76.8
Intended use of statins	100	6.0	97	5.8
• Yes				
• No	1578	94.0	1584	94.2
Menopausal status	753	44.9	751	44.7
• Pre-menopausal				
• Less than or equal to 5 years since menopause	243	14.5	247	14.7
• More than 5 years since menopause	522	31.1	519	30.9
• Menstrual status unknown	160	9.5	164	9.8
TOTAL	1678	100.0	1681	100.0

* Patients intended to receive endocrine therapy alone are included as `no' to the `anthracyclines' and `taxanes' questions



** One patient randomised to the control group withdrew full consent to trial participation and use of data four days after randomisation; this patient is therefore not included in any summary tables or analyses.*

† More than one reason may be given per patient

¶ Patients not known to have died and whose last follow-up information is more than 12 months prior to the database lock on 7th June 2016 (all patients at the time of the database lock were either off study or completed the 10-year follow-up visit schedule)

Fig. 1. CONSORT diagram.

Patients in both treatment arms were followed up on the same schedule to fit in with administration of ZOL (where allocated) during the first 5 years on trial. Thereafter, patients were reviewed annually until 10 years. Follow-up investigations were as clinically indicated with no protocol mandated imaging to identify sub-clinical metastatic disease. Dates of recurrence were backdated to the first clinical suspicion of relapse rather than the date confirmed.

The primary endpoint of the study was DFS, defined specifically for

Table 2
Recurrence events.

A – Disease free survival (DFS) as defined specifically for AZURE trial (see methods)

1st DFS event	Standard treatment [n = 1678] number of events (%)	Standard treatment + ZOL [n = 1681] number of events (%)	Total [n = 3359] number of events (%)
Loco-regional recurrence (excluding within conserved breast)	106 (6.3%)	110 (6.5%)	216 (6.4%)
Distant recurrence*	431 (25.7%)	403 (24.0%)	834 (24.8%)
• Bone	244 (14.5%)	188 (11.2%)	432 (12.9%)
• Bone marrow	2	0	2
• Viscera	272	286	558
• CNS	35	36	71
• Soft tissue and other	53	59	112
Death without recurrence	63 (3.8%)	62 (3.7%)	125 (3.7%)
Total DFS events	575 (34.3%)	555 (33.0%)	1130 (33.6%)

B - Invasive disease free survival (IDFS) as defined by STEEP guidelines [9]

1st IDFS event	Standard treatment [n = 1678] number of patients with event (%)	Standard treatment + ZOL [n = 1681] number of patients with event (%)	Total (n = 3359) number of patients with event (%)
Loco-regional recurrence	127 (7.6%)	136 (8.1%)	263 (7.8%)
Distant recurrence	405 (24.1%)	378 (22.5%)	783 (23.3%)
Contralateral breast cancer	35 (2.1%)	22 (1.3%)	57 (1.7%)
Second non -breast Malignancy	73 (4.4%)	59 (3.5%)	132 (3.9%)
Death without recurrence	36 (2.1%)	38 (2.3%)	74 (2.2%)
Total IDFS events	644 (38.4%)	606 (36.0%)	1250 (37.2%)

The distant recurrence categories are not mutually exclusive hence the numbers (and percentages where presented) in each category do not equal the total number of patients with distant recurrences (as patients' first DFS event). If a patient has more than one site of 'viscera' or 'soft tissue and other' distant recurrence reported on the same day as their 1st DFS event, all sites will be reported and the patient will appear more than once for each distant recurrence; percentages are therefore not presented for these categories.

this study as any recurrence of breast cancer (except for ipsilateral operable invasive or in-situ relapse within a conserved breast) and death from any cause without recurrence. Secondary endpoints included invasive DFS (IDFS), defined according to STEEP guidelines [9]; time to bone metastasis at any time (TTBM) and as first DFS recurrence (B-DFS); overall survival (OS); skeletal morbidity including fractures before and after a DFS recurrence event, hypercalcaemia and skeletal related events (SREs) associated with bone metastases (need for radiotherapy, orthopaedic surgery and spinal cord compression). Pre-specified subgroup analyses included menopausal status of the patient prospectively defined at presentation and prior to randomization as established (> 5years since menses), perimenopausal (within 5 years of menses), premenopausal (regular menses) or unknown. Exploratory analyses investigating treatment effects on site(s) of first recurrence, breast cancer specific survival and relationships between patient age at randomization and outcomes were also performed.

Following the discovery that amplification of the v-maf avian musculoaponeurotic fibrosarcomaoncogene homolog (MAF) gene predicted for benefits and harms of adjuvant ZOL in an earlier analysis of the AZURE trial [10], the long-term relationships between MAF amplification and treatment effects were evaluated. Primary tumor tissue blocks were collected from 1769/2710 (65%) patients treated at participating sites in the UK. Site participation in the collection of tumor blocks was encouraged but not mandatory and, for logistical reasons, restricted to UK sites. The MAF biomarker analysis was completed on TMAs from these primary tumors.

All tumor blocks were sent to Sheffield for tissue microarray (TMA) construction where the location of invasive tumor within the tissue blocks was indicated by a single breast pathologist as a guide to the technician extracting the tissue cores for construction of the TMAs. Quadruplicate cores of 1 mm in diameter of each of the tumor tissue sample were arranged in 4 sets of 13 TMAs (150 samples each). All analyses are restricted to study participants who gave specific patient consent for the use of tissue samples.

The methodology for determining MAF status using a FISH based assay has been described previously [10]. The MAF FISH test was performed on primary tumors preserved in tissue microarray format by an independent laboratory (Targos Molecular Pathology, Kassel, Germany, blinded to patient details and treatment allocation. A pre-specified cut-off for MAF positivity of ≥ 2.5 copies was defined prior to any analysis. Prediction of treatment benefit was focused primarily on IDFS, rather than the AZURE specific definition of DFS, and OS to enable future validation in other studies.

DFS, IDFS and OS were investigated using Kaplan–Meier survival curves; time to bone metastases endpoints were calculated using cumulative incidence functions (CIFs). Deaths without diagnosis of bone metastases for TTBM and deaths without metastases and non-bone first DFS recurrences for B-DFS were considered competing-risk events. Differences between treatment arms were compared using the log-rank test and Cox's proportional hazards model to adjust for minimization factors (excluding center).

Osteonecrosis of the jaw (ONJ) rates were calculated using CIFs, where deaths without diagnosis of ONJ were considered as competing-risk events.

Subgroup analyses were performed using Cox's proportional hazards model to adjust for statistically significant factors in the overall analyses for DFS, IDFS, B-DFS and OS (lymph node involvement and tumor stage for all plus: ER status for DFS, IDFS and OS; menopausal status for OS; neoadjuvant therapy for IDFS). Predictive analyses assessing the interaction of MAF status with zoledronic acid were performed using a Cox's proportional hazards model adjusted for minimization factors previously shown to impact on prognosis (node status, grade, T stage).

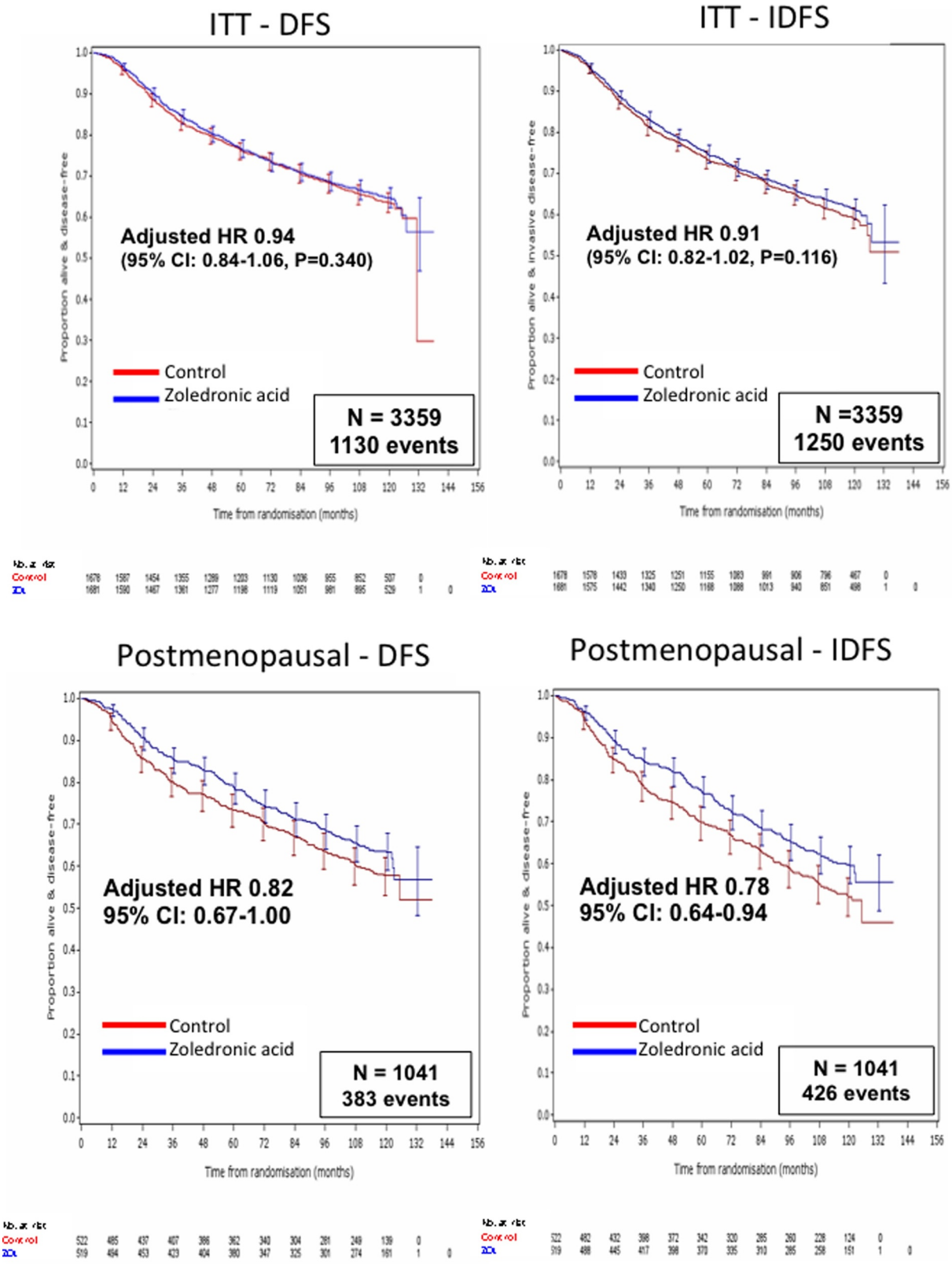


Fig. 2. Disease free (DFS) and invasive disease free survival (IDFS) by treatment allocation (Top panels - ITT analysis; Bottom panels - women who were in established menopause (> 5 years since last menses) at study entry).

The assumption of proportional hazards for each treatment arm was assessed by plotting the hazards over time (i.e. the log cumulative hazard plot from SAS's LIFETEST procedure). A piecewise hazards approach [11] was utilized to investigate the effect of ZOL in years 0–5 and 5–10 for IDFS in postmenopausal patients.

For the predictive MAF biomarker analyses, the response to zoledronic acid treatment was tested comparing control and ZOL groups. A predictive analysis, assessing the interaction of MAF status with

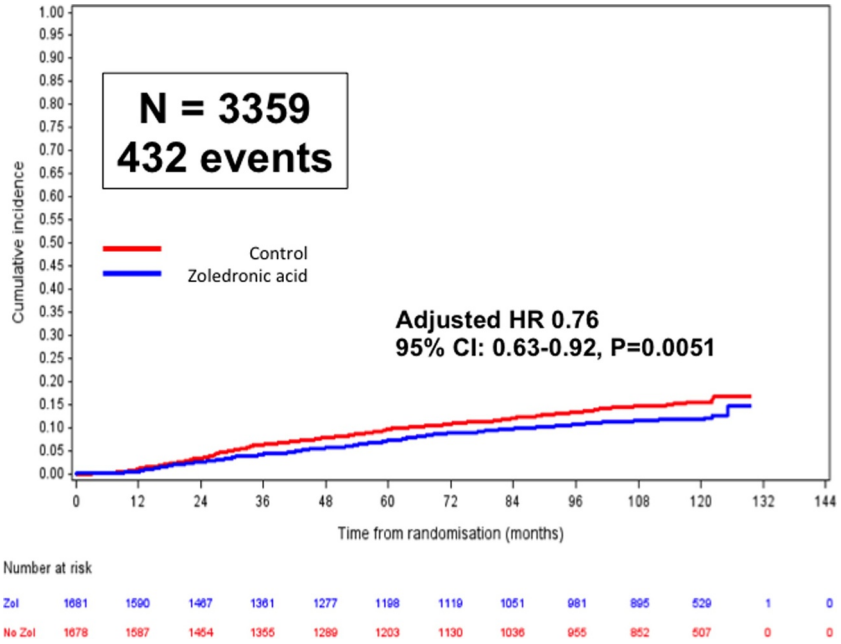


Fig. 3. Cumulative incidence of bone metastases as first DFS recurrence.

Table 3
Number of patients with each site of bone recurrence (as their first DFS recurrence).

	Standard treatment alone [n = 1678] number of patients with event (%)	Standard treatment + ZOL [n = 1681] number of patients with event (%)	Total [n = 3359] number of patients with event (%)
Site(s) of bone recurrence (as their first DFS recurrence)*			
Hip (femur)	44 (2.6%)	25 (1.5%)	69 (2.1%)
Pelvis (not neck of femur)	60 (3.6%)	49 (2.9%)	109 (3.2%)
Spine (lumbar, thoracic, cervical, coccyx)	153 (9.1%)	119 (7.1%)	272 (8.1%)
Leg (below knee)	1 (0.1%)	1 (0.1%)	2 (0.1%)
Arm	21 (1.3%)	7 (0.4%)	28 (0.8%)
Rib	52 (3.1%)	48 (2.9%)	100 (3.0%)
Scapula	14 (0.8%)	9 (0.5%)	23 (0.7%)
Sternum	27 (1.6%)	20 (1.2%)	47 (1.4%)
Clavicle	3 (0.2%)	4 (0.2%)	7 (0.2%)
Skull and facial bones	30 (1.8%)	15 (0.9%)	45 (1.3%)
Mandible	0 (0%)	2 (0.1%)	2 (0.1%)
Others	16 (1.0%)	16 (1.0%)	32 (1.0%)
Bone marrow – no focal bone lesion	2 (0.1%)	6 (0.4%)	8 (0.2%)
Unknown	2 (0.1%)	2 (0.1%)	4 (0.1%)
Total	244 (14.5%)	188 (11.2%)	432 (12.9%)

* The categories of sites of bone recurrences are not mutually exclusive hence the numbers (and percentages) in each category do not equal the total number of patients with bone recurrences. If a patient has more than one site of bone recurrence reported on the same day as their first recurrence, all of the sites will be reported and the patient will appear more than once in the corresponding column

treatment allocation, was performed using a Cox proportional hazards model. Only minimization factors identified as statistically significant in the prognostic analysis were included in the model to reduce potential overfitting. In addition, predictive analyses were carried out for patients who were unequivocally post-menopausal (> 5 years since last menses) at trial entry separately to patients who were not post-menopausal (pre-menopausal, ≤ 5 years since menopause and menopausal status unknown), given the significant heterogeneity of treatment effect between established post-menopausal patients and all other patients observed in the AZURE trial as a whole. The SAP for these biomarker tests was defined before any data analysis was performed with interactions between MAF + ve and effects of ZOL on disease outcomes by menopausal status pre-specified.

All analyses presented are adjusted analyses. Hazard ratios with 95% confidence intervals (CI) are presented. Hypothesis testing was two-sided with significance at 5%. No adjustments were made for multiplicity. P values are restricted to the ITT analyses (main study and biomarker subset) and, due to previous analyses [4,5] should be considered descriptive rather than inferential. Analyses were carried out in SAS version 9.4 (SAS Institute Inc., Cary, NC).

Results

Baseline patient demographics, disease characteristics and systemic anticancer treatments were balanced across the two randomized study groups (Table 1). 3207 patients (95%) received chemotherapy (207

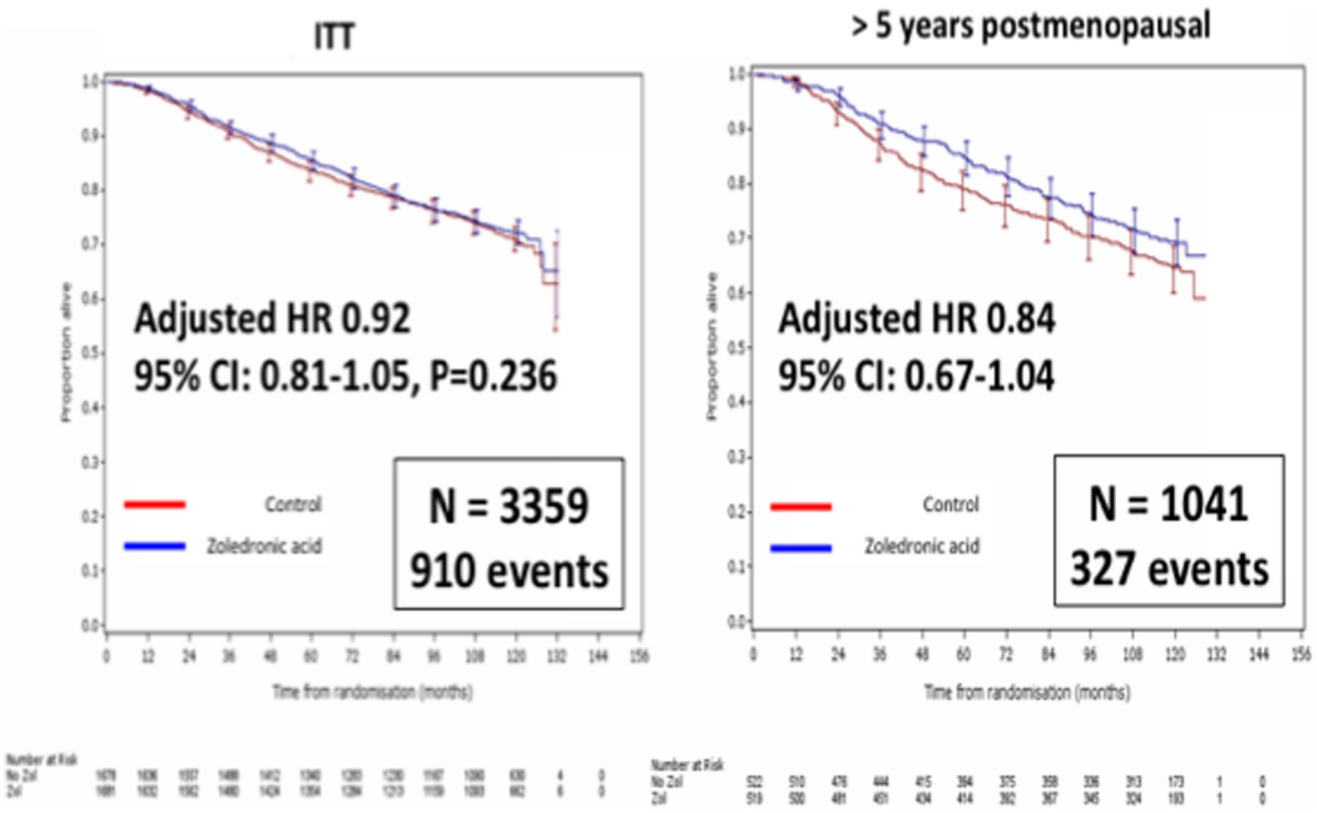


Fig. 4. Overall survival in the ITT analysis (A: left) and > 5years postmenopausal subgroup (B: right).

neoadjuvant chemotherapy), 2488 patients both chemotherapy and endocrine treatment(s) and 152 patients endocrine treatment(s) alone (CONSORT DIAGRAM, Fig. 1).

With a median follow up of 117 months (interquartile range (IQR) 70.4–120.4 months) at the data-lock on 7th June 2016, there have been 1130 DFS, 1250 IDFS, 910 OS events and 781 breast cancer deaths [Table 2]. Follow-up was almost identical for ZOL (median 118, IQR 71.4–120.5 months) and CONTROL patients (median 116.3, IQR 69.2–120.4 months). Four ZOL patients were not evaluable for DFS or IDFS (3 metastatic disease at randomization, one neoadjuvant patient not proceeding to definitive surgery) and one CONTROL patient was not evaluable for IDFS (another primary tumor at randomization); all were censored at time zero in the relevant analysis.

The number of DFS (ZOL = 555, CONTROL = 575) and IDFS (ZOL = 606, CONTROL = 644) events were similar in both arms (HR_{DFS} = 0.94, 95%CI:0.84–1.06, P = 0.340; HR_{IDFS} = 0.91, 95%CI:0.82–1.02, P = 0.116) [Fig. 2A and B]. As with earlier analyses [4,5], ZOL improved DFS and IDFS in patients who were > 5years postmenopausal (ZOL = 177, CONTROL = 206 DFS events) at the time of randomization (HR_{DFS} = 0.82, 95%CI:0.67–1.00; HR_{IDFS} = 0.78, 95%CI:0.64–0.94) [Fig. 2C and D]. The absolute difference in IDFS at 10 years was 7.8% (95%CI:1.4% to 14.2% [ZOL = 59.9%, CONTROL = 52.1%]). The benefits from treatment appear in the first five years (during treatment) following which the IDFS curves run parallel. The HRs were 0.68 (95%CI:0.53–0.87, P = 0.002) and 0.89 (95%CI:0.65–1.21, P = 0.456) in years 0–5 and 5–10 respectively. The absolute difference in IDFS between treatments averaged from five years onwards is consistent at about 6.5%.

Women who were not post-menopausal at study entry did not gain benefit for either DFS (ZOL = 378, CONTROL = 369 events; HR_{DFS} = 1.03, 95%CI:0.89–1.19) or IDFS (ZOL = 413, CONTROL = 411 events; HR_{IDFS} = 1.01, 95%CI:0.88–1.16). The test for interaction (TFI) by menopause on DFS was of borderline significance (Chi^2 = 3.25, P = 0.07).

Bone metastases as a first DFS recurrence (B-DFS) were reduced with ZOL (ZOL = 188, CONTROL = 244 events; $HR_{B\text{-}DFS}$ = 0.76, 95%CI:0.63–0.92, P = 0.005) (Fig. 3). The absolute difference in B-DFS at 10 years was 3.6% (95%CI:1.1% to 6.0% [ZOL = 11.9%, CONTROL = 15.5%]). Treatment effects on this endpoint were similar in postmenopausal ($HR_{B\text{-}DFS}$ = 0.76, 95%CI:0.53–1.07) and non-postmenopausal women ($HR_{B\text{-}DFS}$ = 0.77, 95%CI:0.61–0.96); $TFI_{B\text{-}DFS}$ by menopause Chi^2 = 0.005, P = 0.94. Specific sites of bone metastases as first DFS recurrence by treatment allocation are shown in Table 3.

Extra-skeletal distant recurrence DFS (E-dDFS) events (ZOL = 279, CONTROL = 264) were similar in the two treatment groups. However heterogeneity of treatment effects on E-dDFS by menopause was seen. In postmenopausal women the HR for E-dDFS was 0.77 (95%CI:0.57–1.04), whereas it was 1.22 (95%CI:0.99–1.50) in non-postmenopausal women (TFI Chi^2 = 6.11, P = 0.01).

Overall survival was similar in the two arms in the ITT analysis (HR_{OS} = 0.92 95%CI:0.81–1.05, P = 0.24) [Fig. 4A]. However in the postmenopausal cohort, improvements in OS (HR_{OS} = 0.84, 95%CI:0.67–1.04) [Fig. 4B] and breast cancer mortality (HR = 0.80, 95%CI:0.62–1.03) that approached statistical significance were seen. The absolute difference in OS at 10 years was 4.4% (95%CI: − 1.5% to 10.4% [ZOL = 69.0%, CONTROL = 64.6%]).

The MAF FISH test was assessable in 865 (26% of the entire AZURE cohort) patients. These patients had similar tumor and treatment characteristics (Table 4) and 10-year IDFS and OS outcomes to the entire AZURE cohort. Six hundred and eighty (79%) were MAF negative with ≤ 2.5 copies per cell (ZOL 321, CONTROL 359). ZOL significantly improved 10 year IDFS (HR_{IDFS} = 0.75, 95%CI:0.58–0.97, P = 0.026),

Table 4
Characteristics of MAF tested sub-set and overall AZURE trial population.

Variable	FISH evaluable result (n = 865)	Tumour provided (n = 1739)	AZURE population (n = 3359)
Menopausal status			
Non post-menopausal	590 (68.2%)	1192 (68.5%)	2318 (69.0%)
Post-menopausal	275 (31.8%)	547 (31.5%)	1041 (31.0%)
Age			
< 40	87 (10.1%)	198 (11.4%)	384 (11.4%)
40–49	299 (34.6%)	571 (32.8%)	1108 (33.0%)
50–59	281 (32.5%)	580 (33.4%)	1126 (33.5%)
60–69	162 (18.7%)	332 (19.1%)	628 (18.7%)
70+	36 (4.2%)	58 (3.3%)	113 (3.4%)
Lymph node involvement			
0	2 (0.2%)	17 (1.0%)	62 (1.8%)
1–3	563 (65.1%)	1122 (64.5%)	2075 (61.8%)
≥4	300 (34.7%)	598 (34.4%)	1211 (36.1%)
Unknown	0 (0%)	2 (0.1%)	11 (0.3%)
Tumour stage			
T1	274 (31.7%)	577 (33.2%)	1065 (31.7%)
T2	475 (54.9%)	901 (51.8%)	1717 (51.1%)
T3	99 (11.4%)	214 (12.3%)	456 (13.6%)
T4	17 (2.0%)	47 (2.7%)	117 (3.5%)
TX	0 (0.0%)	0 (0.0%)	4 (0.1%)
ER status			
ER positive	689 (79.7%)	1388 (79.8%)	2634 (78.4%)
ER negative	171 (19.8%)	341 (19.6%)	705 (21.0%)
ER unknown	5 (0.6%)	10 (0.6%)	20 (0.6%)
Systemic therapy plan			
Endocrine therapy (ET)	46 (5.3%)	89 (5.1%)	152 (4.5%)
Chemotherapy (CT)	166 (19.2%)	339 (19.5%)	719 (21.4%)
ET and CT	653 (75.5%)	1311 (75.4%)	2488 (74.1%)
Anthracyclines			
Yes	794 (91.8%)	1604 (92.2%)	3132 (93.2%)
No	71 (8.2%)	135 (7.8%)	227 (6.8%)
Taxanes			
Yes	126 (14.6%)	267 (15.4%)	775 (23.1%)
No	739 (85.4%)	1472 (84.6%)	2584 (76.9%)
HER2 status			
Positive	93 (10.8%)	186 (10.7%)	415 (12.4%)
Negative	250 (28.9%)	503 (28.9%)	1251 (37.2%)
Unknown / Not measured	522 (60.3%)	1050 (60.4%)	1693 (50.4%)
Histological grade			
1	61 (7.1%)	147 (8.5%)	285 (8.5%)
2	333 (38.5%)	748 (43.0%)	1439 (42.8%)
3	467 (54.0%)	820 (47.2%)	1552 (46.2%)
Not specified	4 (0.5%)	24 (1.4%)	83 (2.5%)
PR status			
Positive	308 (35.6%)	633 (36.4%)	1423 (42.4%)
Negative	159 (18.4%)	350 (20.1%)	806 (24.0%)
Unknown	398 (46.0%)	756 (43.5%)	1130 (33.6%)

bone IDFS (HR_{BDFS} = 0.65, 95%CI:0.45–0.94, P = 0.022) and OS (HR_{OS} = 0.69, 95%CI:0.50–0.94, P = 0.019) in women with MAF negative tumors, irrespective of menopause (Fig. 5). On the other hand, women with MAF positive tumors did not benefit from ZOL (HR_{IDFS} = 1.54, 95%CI:0.96–2.47, P = 0.074) and OS (HR_{OS} = 1.40, 95%CI:0.83–2.33, P = 0.204). Indeed, non-postmenopausal women with MAF positive tumors had much worse outcomes (HR_{IDFS} = 2.31, 95%CI:1.18–4.52, P = 0.015; HR_{OS} = 2.28, 95%CI:1.07–4.82, P = 0.032) driven largely by an increase in extra-skeletal recurrences in this patient sub-group ($HR_{E\text{-}DFS}$ = 4.57, 95%CI:1.66–12.57, P = 0.003).

Exploratory analyses of study treatment effects on DFS by age identified significant heterogeneity (Chi^2(trend) = 7.70, P = 0.0055). Patients who were < age 40 at diagnosis had a worse outcome with zoledronic acid (HR_{DFS} = 1.69; 95%CI:1.24–2.29) [Fig. 6A]. This was due in part to more B-DFS events ($HR_{B\text{-}DFS}$ = 1.59; 95%CI:1.01–2.49) [Fig. 6B] but mainly reflected the markedly higher rates of DFS events outside bone (E-DFS) ($HR_{E\text{-}DFS}$ = 2.06; 95%CI:1.43–2.97) in these young women treated with ZOL [Fig. 6C]. The increase in relapse associated with zoledronic acid treatment in women aged < 40 was associated also with worse survival outcomes (HR_{OS} = 1.56, 95%CI:1.09–2.22) at 10 years (Fig. 7).

Treatment with ZOL reduced skeletal morbidity. 470 SREs occurred in 284/1681 (16.9%) of patients on ZOL compared with 700 SREs in 378/1678 (22.5%) of CONTROL patients (Table 5). Unlike in our earlier report on fractures [12], ZOL now not only reduced fracture incidence after a recurrence event (ZOL = 52, CONTROL = 79; P = < 0.05) but also had an effect on fractures occurring in the absence of disease recurrence (ZOL = 143, CONTROL = 193; P = < 0.05)).

Further follow up has not identified any new safety concerns since the previous safety publication [13]. 30 confirmed cases of osteonecrosis of the jaw (ONJ), all in the zoledronic acid arm, have occurred (Fig. 8) with 4 occurring after a relapse in bone and use of a bisphosphonate in the metastatic setting and only 4 further cases have been reported since the previous detailed analysis of ONJ reported earlier [14]. The cumulative incidence of ONJ is 1.8% (95%CI:1.2%−2.5%). There have been no reports of atypical femoral fracture.

Discussion

AZURE is one of the largest phase III studies of adjuvant bisphosphonates, and the first to report results with 10 years of follow-up. For the entire ITT population, although zoledronic acid reduced bone metastases as a first DFS recurrence, this did not translate into significant effects on DFS, IDFS or OS. However, treatment benefits in women who were in established menopause at the time of randomization persist out to ten years with a clinically and statistically significant 6.5% absolute IDFS benefit and a trend for improved breast cancer specific mortality. Our findings remain consistent with those reported in the EBCTCG meta-analysis [6].

In women who were not in established menopause at the time of randomization, although there was a reduction in the risk of bone metastases as a first DFS recurrence, this did not translate into benefits in DFS, IDFS or OS. Indeed in the youngest patients included in this study (aged < 40), zoledronic acid treatment was associated with an increase in relapse, especially at extra-skeletal sites, and worse overall survival, suggesting that inhibition of bone resorption in young women may promote displacement and/or increase dissemination of viable tumor cells to other organs.

MAF is a transcription factor of the AP-1 family encoded within 16q. MAF regulates the expression of a set of genes that collectively support several steps of breast cancer cell metastasis and progression [15] epithelial-mesenchymal transition [16], macrophage function and a number of interactions within the bone marrow niche including cell adhesion [17]. Evaluation of the biomarker MAF using a FISH assay to prospectively define MAF amplification appeared to identify the population of women likely to benefit from adjuvant ZOL that avoids the imprecise assessment of menopause and impacts of treatment on ovarian function. The majority of women (79%) was deemed to be MAF negative and, in this biomarker defined population, ZOL was associated with relative risk reductions at 10 years of 35% for B_{DFS}, 25% for IDFS and 31% for OS irrespective of the postmenopausal status of the patient at study entry. The predictive power of the MAF test has increased with further follow-up since our original observations [10]. Women with a MAF positive tumor do not benefit from ZOL and, in those who were not postmenopausal at study entry, MAF positivity was associated with a 4–5 fold risk increase in disease recurrence outside bone and worse

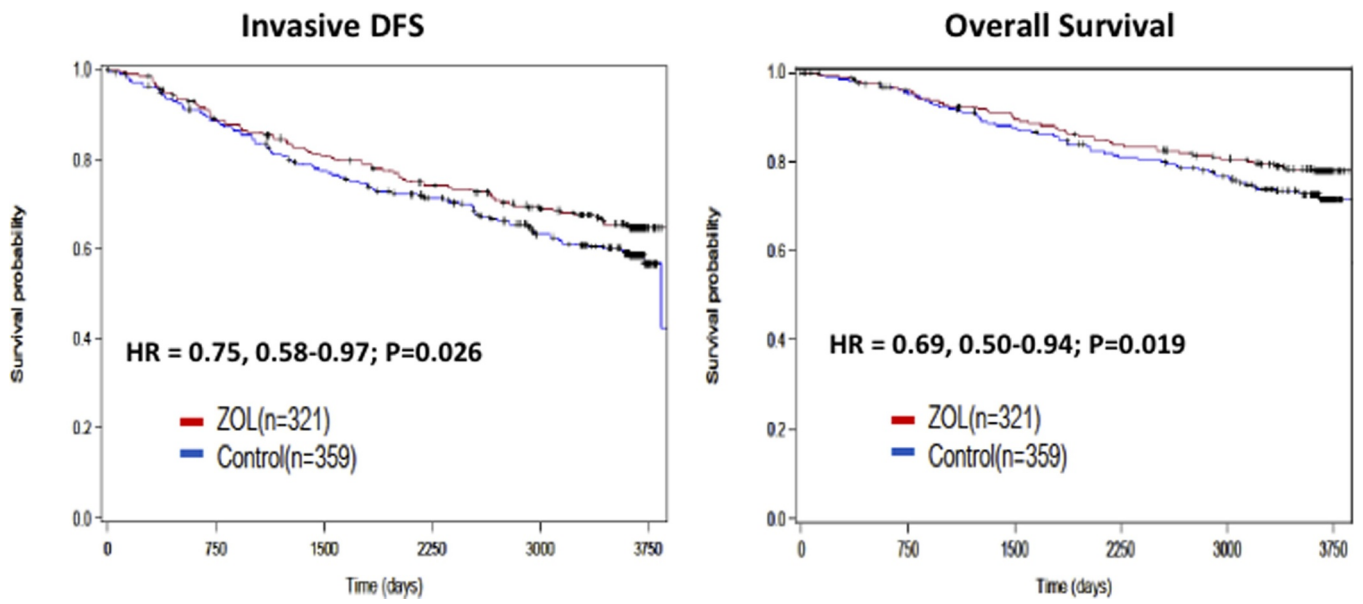


Fig. 5. Invasive disease free (IDFS) and overall survival (OS) by treatment allocation in the 79% of patients tested who did not over-express (copy number < 2.5) the MAF gene.

survival with ZOL use.

The mechanisms underpinning the inter-relationships between MAF status and treatment effects are unclear. MAF over-expression appears to influence trafficking of disseminated tumor cells away from the bone microenvironment where bisphosphonates can influence the tumor and stromal interactions, perhaps through additional inhibitory effects on adhesion molecules and/or macrophage function, that may be influenced by bisphosphonates, and encourage onward metastasis within extra-skeletal sites. We are currently investigating the impact of MAF status in preclinical models of bone metastasis and intend to try and validate the predictive ability of MAF using the primary tumors and trial data from the NSABP-B34 study of adjuvant oral clodronate. If our findings in AZURE are confirmed, MAF could be used in the future to select patients for adjuvant bisphosphonate treatment

Although adverse breast cancer mortality in young women was not identified in the EBCTCG meta-analysis, there was significant heterogeneity of treatment effect in breast cancer mortality across age groups (Chi^2 for trend = 3.9, $P = 0.05$) [6] and worse survival was reported in one of the early clodronate trials that recruited a large proportion of younger women [18]. Our results serve as a timely reminder that manipulating the host environment, either intentionally as a cancer treatment strategy or as a bystander effect of other therapeutic interventions, has the potential to cause harm as well as good.

Of note, women aged 40–50, many of whom would likely have experienced chemotherapy-induced amenorrhea (data not collected), did not appear to gain benefit. Zoledronic acid was administered for five years and, due to the very long retention of the drug in the skeleton [19] with continuing effects on bone metabolism expected throughout much if not all of the additional 5 years of follow-up, manipulation of the bone environment did not appear to modify the late emergence of tumor cells from the dormant state. The benefits observed from adjuvant bisphosphonates in postmenopausal women would seem to argue for effects on early events in the metastatic process, perhaps including seeding of tumor cells to osteoblastic and other cellular niches in the bone marrow and/or an influence on tumor dormancy within a postmenopausal bone micro-environment, rather than on later steps such as emergence from dormancy and onward dissemination.

Adjuvant zoledronic acid was associated with a reduction in skeletal morbidity with fewer fractures during follow-up and a reduction in SREs related to metastatic bone disease. Improvements in the structural integrity of bone achieved with ZOL appear to persist beyond treatment cessation with a carry-over effect that reduces the morbidity associated with metastatic disease even though most patients (data not shown) received bone targeted treatment after a bone relapse.

Since the first report of the AZURE trial [4] and the suggestion that the benefit of adjuvant bisphosphonates might be restricted to women with low levels of reproductive hormones, as in ABCSG-12 [20] and the pre-defined subgroup of women in AZURE with established menopause (defined as > 5 years since last menses), there have been many attempts to try and validate this hypothesis. NSABP B34 identified possible benefits from adjuvant oral clodronate in women > 50 at time of randomization [21] and the GAIN investigators suggested improved outcomes in older women (aged > 60) with daily oral ibandronate [22].

The persistent benefits seen in AZURE at 10 years, with fewer bone relapses and improved DFS and breast cancer mortality in postmenopausal women, argue for inclusion of a bisphosphonate into adjuvant treatment programs from the outset. The lack of benefits in women who will have become menopausal, either through ageing or the effects of chemotherapy during the course of breast cancer treatment, should discourage the use of delayed intervention of a bisphosphonate in the hope that this will also bring about benefits in terms of breast cancer relapse. Such a strategy is worthy of appropriate clinical trials but, currently, there is no evidence to support such an approach.

To address relationships between treatment benefit and menopausal status more comprehensively, the EBCTCG conducted a meta-analysis of > 18,000 women included in randomized trials of adjuvant bisphosphonates with a specific aim to test the hypothesis that treatment benefits were related to menopausal status of the patient at the time bisphosphonates are initiated [6]. This individual patient meta-analysis identified relative risk reductions in both the development of bone metastases and death from breast cancer at 10 years of 28% ($P = 0.0002$) and 18% ($P = 0.002$) respectively in postmenopausal women but no suggestion of benefits in those who were premenopausal at initiation of treatment, even though many of these would have become postmenopausal during follow-up. Exclusion of the hypothesis generating studies (ABCSG-12 and AZURE) did not materially change

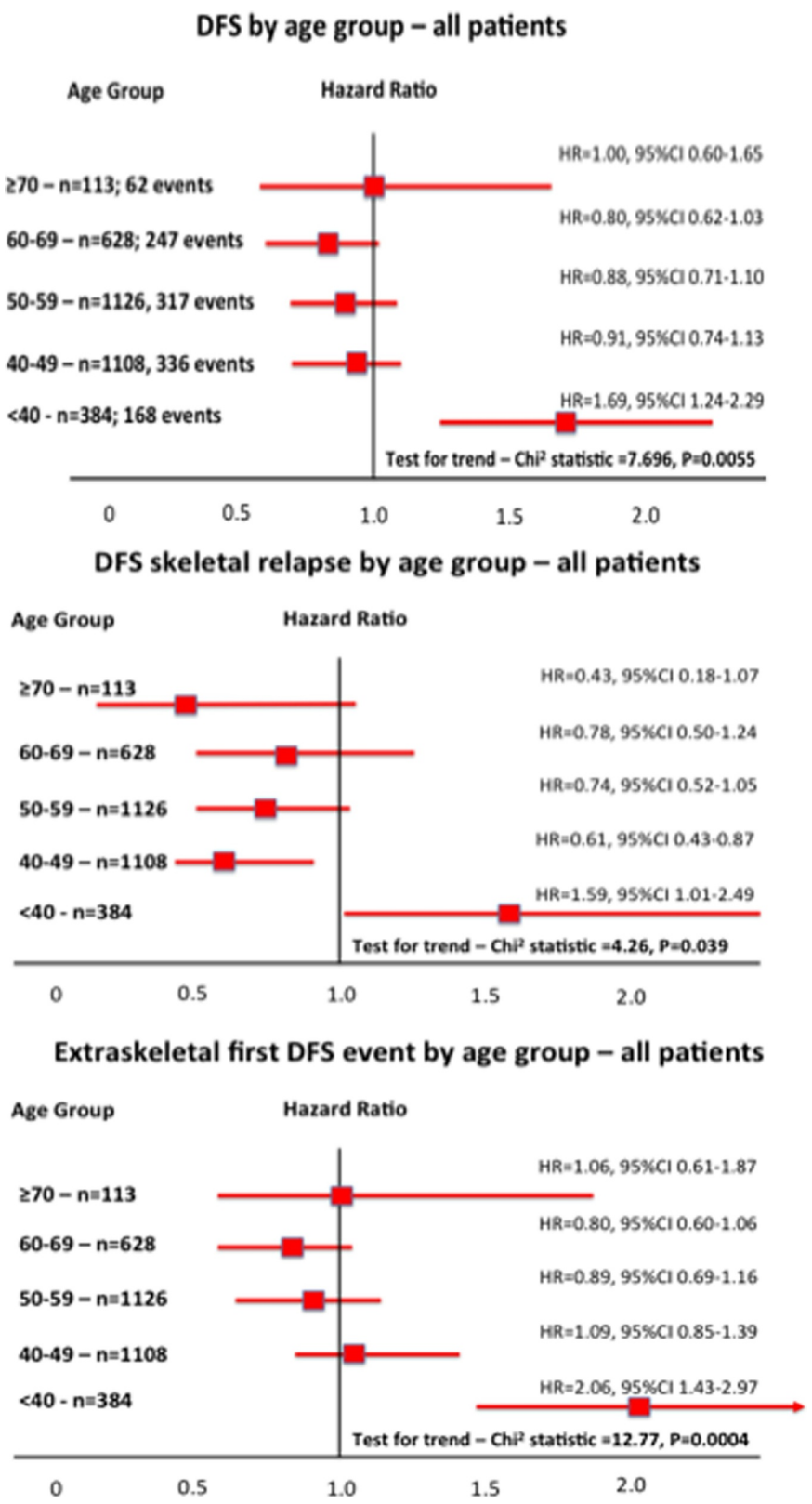


Fig. 6. Exploratory analyses investigating the impact of age at randomization on disease outcomes with or without adjuvant zoledronic acid: A:top – All DFS events; B:middle – bone DFS events; C:bottom – all DFS other than bone events.

the study findings.

These results have led to supportive clinical guidelines in Europe and North America [7,8], a consensus recommendation by a European expert group to incorporate adjuvant bisphosphonates into routine clinical care [23] and adoption of these recommendations in some health care systems. Despite the clinically important effects on breast cancer mortality, global acceptance has been slow, in part due a lack of regulatory approval for these generic medicines but also due to the

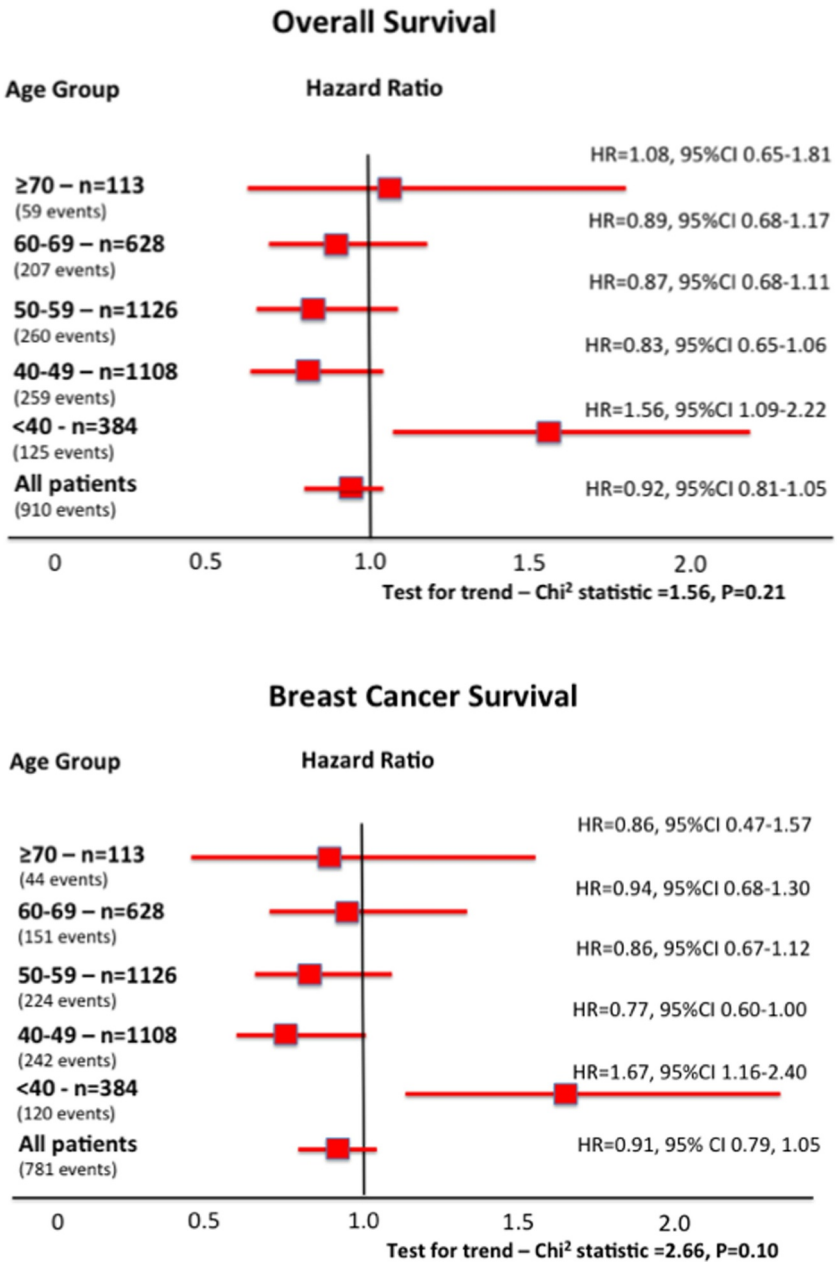


Fig. 7. Exploratory analyses investigating the impact of age at randomization on overall survival (top) and breast cancer survival (bottom) with or without adjuvant zoledronic acid.

finding that these benefits relate only to a subset of patients that is somewhat imprecise in its definition and also because mechanistic explanations for the findings remain unclear. The relationship between MAF status in the primary tumor and treatment outcome, if confirmed, may improve adherence to clinical guidelines.

There are some limitations to our study findings. Adjuvant systemic treatments have evolved since AZURE started in 2003. Most notably, the treatment of HER2 + breast cancer with trastuzumab has been standard treatment since 2006 and also the clear preference for the routine use of aromatase inhibitors over tamoxifen for postmenopausal ER + breast cancer is well established with extended (> 5years) use in some women. Less than half of the HER2 positive patients included in AZURE received trastuzumab and only around two thirds of women received an aromatase inhibitor, with most use being after a few years of tamoxifen rather than as initial endocrine treatment. However, the consistent benefits seen in the meta-analysis [6] within the postmenopausal population, irrespective of clinic-pathologic (no differences by histologic grade or nodal involvement) and biological subtypes (no differences by ER status) suggest that these results remain valid.

Finally, if an adjuvant bisphosphonate is to be used, which agent and schedule should be recommended? Both the meta-analysis and the initial report of the SWOG-0307 study [24] suggest the benefits are similar for zoledronic acid, daily oral clodronate and daily oral ibandronate. The AZURE and SWOG-0307 schedule of zoledronic acid is intensive and associated with a small but not insignificant rate of ONJ. In the absence of clear evidence of a dose response relationship, a less

Table 5
Frequency of skeletal morbid events at 10 years according to treatment allocation.

	Standard treatment alone $N = 1678$	Standard treatment + ZOL $N = 1681$	Total $N = 3359$
Patients experiencing a SRE (% of patients)			
• Yes	378 (22.5%)	284 (16.9%)	662 (19.7%)
• No	1300 (77.5%)	1397 (83.1%)	2697 (80.3%)
Type of SRE (% of skeletal event by treatment arm)			
• Fracture	272 (58%)	195 (42%)	467
• Before DFS event	193 (58%)	143 (42%)	336
• After DFS event	79 (60%)	52 (40%)	131
• Radiation therapy to bone	251 (67%)	125 (33%)	376
• Surgery to bone	106 (51%)	103 (49%)	209
• Spinal cord compression	34 (65%)	18 (35%)	52
• Hypercalcemia	36 (57%)	27 (43%)	63
• Unclassified	1	2	3
Total number of SRE	700 (60%)	470 (40%)	1170

SRE, skeletal related event; DFS, disease free survival

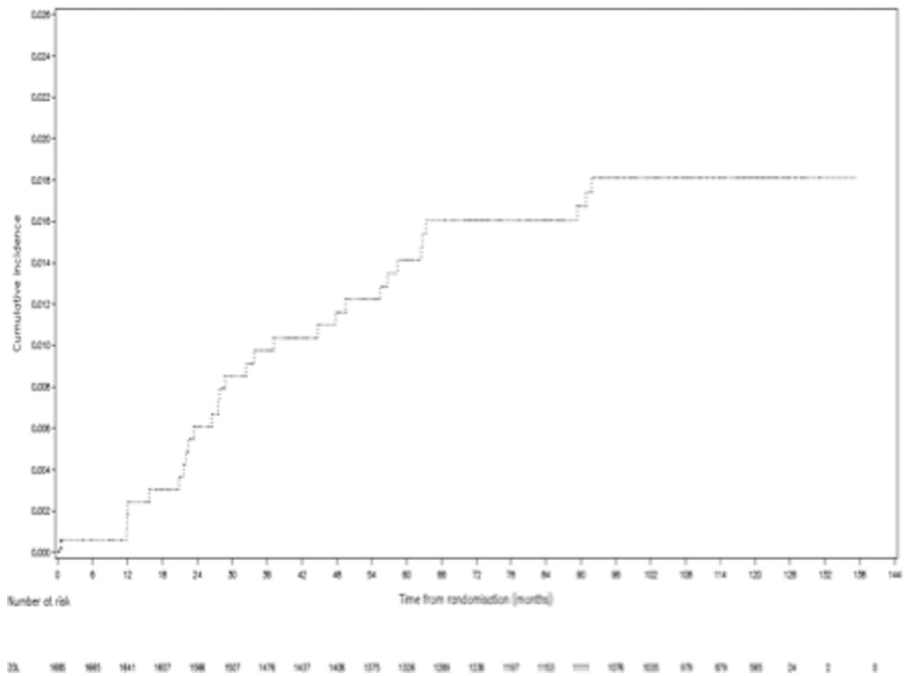


Fig. 8. Cumulative incidence function for time to confirmed osteonecrosis of the jaw for patients receiving zoledronic acid. No cases were reported in the control population.

intensive regimen of zoledronic acid, as pioneered by the ABCSG [21], or daily oral therapy with clodronate or ibandronate seem reasonable. Interestingly the use of adjuvant denosumab in early breast cancer, despite its ability to reduce the frequency of aromatase inhibitor associated fractures [25], failed in the large randomized D-CARE (NCT01077154) study to meet the primary objective of improving bone metastases free survival. IDFS and OS were also similar and there was no suggestion of a treatment interaction with menopausal status [26]. The lack of disease benefits from denosumab also suggest that inhibition of bone resorption is necessary but not sufficient to prevent metastases. Bisphosphonates have multiple other cellular effects beyond osteoclast inhibition including effects on tumor cell adhesion, angiogenesis and immune function [27]. Further research is required to understand whether some or all of these “off target” effects are clinically relevant.

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University of Birmingham), Australia (Victoria Co-operative Oncology Group), Ireland (Irish Clinical Oncology Research Group [now known as Cancer Trials Ireland]) and Spain (SOLTI Breast Cancer Research Group), the independent members and patient representatives on the Trial Steering and Data Monitoring Committees and Novartis Pharmaceuticals for their academic grant support.

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Conflicts of interest

Coleman reports grant funding to institution from Novartis, Bayer and Amgen, lecture fees from Amgen and a patent pending with Inbiomotion for the biomarker MAF. Brown reports grant funding from Novartis and Bayer and lecture/advisory fees from Amgen and Novartis. De Boer reports advisory board fees from Novartis. Gregory reports consultancy fees from Cologne and Janssen and a patent pending with Inbiomotion for the biomarker MAF. Collinson, Marshall and Liversedge all report grant support to their institution from Novartis. Tercero reports salary from Inbiomotion and patents pending related to the biomarker MAF. Jean-Mairet owns < 0.25% of Inbiomotion S.L and Gomis declares shares of Inbiomotion SL for a value of less than 10,000$ and patents pending related to the biomarker MAF. All other authors report no conflicts of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jbo.2018.09.008.

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