Effect of baseline oestradiol serum concentration on the efficacy of anastrozole for preventing breast cancer in postmenopausal women at high risk: a case-control study of the IBIS-II prevention trial

Jack Cuzick, Kim Chu, Brian Keevil, Adam R Brentnall, Anthony Howell, Nicholas Zdenkowski, Bernardo Bonanni, Sibylle Loibl, Kaija Holli, D Gareth Evans, Steve Cummings, Mitch Dowsett

Lancet Oncol 2024; 25: 108–16

Published Online December 6, 2023 https://doi.org/10.1016/S1470-2045(23)00578-8

See Comment page 8

Wolfson Institute of Population Health, Queen Mary University of London, London, UK (Prof J Cuzick PhD, K Chu MSc, A R Brentnall PhD)**; University South Manchester NHS Foundation Trust, Manchester, UK** (Prof B Keevil PhD)**; Paterson Institute for Cancer Research** (Prof A Howell MD) **and Centre for Genomic Medicine** (Prof D G Evans MD)**, University of Manchester, Manchester, UK; Faculty of Health and Medicine, University of Newcastle, Newcastle, Australia** (N Zdenkowski PhD)**; Division of Cancer Prevention and Genetics, European Institute of Oncology IRCCS, Milan, Italy** (Prof B Bonanni MD)**; German Breast Group, Goethe University of Frankfurt, Frankfurt, Germany** (Prof S Loibl PhD)**; Tampere University, Tampere, Finland** (K Holli MD)**; San Francisco Coordinating Center, California Pacific Medical Center Research Institute, San Francisco, CA, USA** (Prof S Cummings MD)**; Institute of Cancer Research, Royal Marsden Hospital, London, UK** (Prof M Dowsett PhD)

Correspondence to: Prof Jack Cuzick, Wolfson Institute of Population Health, Queen Mary University of London, London EC1M 6BQ, UK **j.cuzick@qmul.ac.uk**

Summary

Background An increased risk of breast cancer is associated with high serum concentrations of oestradiol and testosterone in postmenopausal women, but little is known about how these hormones affect response to endocrine therapy for breast cancer prevention or treatment. We aimed to assess the effects of serum oestradiol and testosterone concentrations on the efficacy of the aromatase inhibitor anastrozole for the prevention of breast cancer in postmenopausal women at high risk.

Methods In this case-control study we used data from the IBIS-II prevention trial, a randomised, controlled, double-blind trial in postmenopausal women aged 40–70 years at high risk of breast cancer, conducted in 153 breast cancer treatment centres across 18 countries. In the trial, women were randomly assigned (1:1) to receive anastrozole (1 mg/day, orally) or placebo daily for 5 years. In this pre-planned case-control study, the primary analysis was the effect of the baseline oestradiol to sex hormone binding globulin (SHBG) ratio (oestradiol–SHBG ratio) on the development of all breast cancers, including ductal carcinoma in situ (the primary endpoint in the trial). Cases were participants in whom breast cancer was reported after trial entry and until the cutoff on Oct 22, 2019, and who had valid blood samples and no use of hormone replacement therapy within 3 months of trial entry or during the trial. For each case, two controls without breast cancer were selected at random, matched on treatment group, age (within 2 years), and follow-up time (at least that of the matching case). For each treatment group, we applied a multinominal logistic regression likelihood-ratio trend test to assess what change in the proportion of cases was associated with a one-quartile change in hormone ratio. Controls were used only to determine quartile cutoffs. Profile likelihood 95% CIs were used to indicate the precision of estimates. A secondary analysis also investigated the effect of the baseline testosterone–SHBG ratio on breast cancer development. We also assessed relative benefit of anastrozole versus placebo (calculated as 1–the ratio of breast cancer cases in the anastrozole group to cases in the placebo group). The trial was registered with ISRCTN (number ISRCTN31488319) and completed recruitment on Jan 31, 2012, but long-term follow-up is ongoing.

Findings 3864 women were recruited into the trial between Feb 2, 2003, and Jan 31, 2012, and randomly assigned to receive anastrozole (n=1920) or placebo (n=1944). Median follow-up time was 131 months (IQR 106–156), during which 85 (4·4%) cases of breast cancer in the anastrozole group and 165 (8·5%) in the placebo group were identified. No data on gender, race, or ethnicity were collected. After exclusions, the case-control study included 212 participants from the anastrozole group (72 cases, 140 controls) and 416 from the placebo group (142 cases, 274 controls). A trend of increasing breast cancer risk with increasing oestradiol–SHBG ratio was found in the placebo group (trend per quartile 1·25 [95% CI 1·08 to 1·45], p=0·0033), but not in the anastrozole group (1·06 [0·86 to 1·30], p=0·60). A weaker effect was seen for the testosterone–SHBG ratio in the placebo group (trend 1·21 [1·05 to 1·41], p=0·011), but again not in the anastrozole group (trend 1·18 [0·96 to 1·46], p=0·11). A relative benefit of anastrozole was seen in quartile 2 (0·55 [95% CI 0·13 to 0·78]), quartile 3 (0·54 [0·22 to 0·74], and quartile 4 (0·56 [0·23 to 0·76]) of oestradiol–SHBG ratio, but not in quartile 1 (0·18 [–0·60 to 0·59]).

Interpretation These results suggest that serum hormones should be measured more routinely and integrated into risk management decisions. Measuring serum hormone concentrations is inexpensive and might help clinicians differentiate which women will benefit most from an aromatase inhibitor.

Funding Cancer Research UK, National Health and Medical Research Council (Australia), Breast Cancer Research Foundation, and DaCosta Fund.

Research in context

Evidence before this study

An increased risk of breast cancer associated with high serum concentrations of oestradiol and testosterone has been well established in postmenopausal women. However, little is known about the effects of these hormones on response to endocrine therapy to prevent or treat breast cancer. We searched PubMed for publications in English between Jan 1, 1996, and June 1, 2022, using the terms "oestradiol", "breast cancer", "hormone treatment", and "hormone prevention". Two breast cancer prevention trials were found that evaluated the role of oestradiol concentrations in the response to the selective oestrogen receptor modulators tamoxifen and raloxifene, with differing results. No trials were found that studied serum hormone impact on aromatase inhibitor treatment.

Added value of this study

This study, based on the IBIS-II prevention trial of the aromatase inhibitor anastrozole in postmenopausal women at high risk of breast cancer, found a clear relative benefit of anastrozole overall, but this benefit was limited to women with medium or high (quartiles 2–4) ratios of oestradiol to sex hormone binding globulin (SHBG), and there was no significant effect for those in the lowest quartile. To our knowledge, this is the first report of the effect of serum oestradiol concentrations on treatment with an aromatase inhibitor. These findings suggest a potential role for measuring oestradiol, testosterone, and SHBG more widely, both in determining which individuals are at high risk and the likely response to endocrine treatment.

Implications of all the available evidence

Overall, use of aromatase inhibitors offers the most effective option for treatment of early oestrogen receptor-positive breast cancer, and for preventing breast cancer in postmenopausal women at increased risk. The role of serum hormones in deciding whether preventive hormone agents are likely to be effective in individual women is a key question identified in the current study. An even more important question is the value of these measurements in choosing a treatment in the adjuvant setting. Measurement of serum hormones is inexpensive, and more routine use of hormone assays in high-risk clinics and for treatment of early breast cancer could substantially improve disease management.

Introduction

The increased risk of breast cancer associated with high serum concentrations of oestradiol and testosterone is well established in postmenopausal women.[1–6] A stronger effect has also been seen for oestrogen receptor-positive breast cancer than for all breast cancer.[7–9] Higher concentrations of sex hormone binding globulin (SHBG) have also been shown to reduce the risk of breast cancer.[4–6]

Although the association between oestradiol and breast cancer risk is well established, less is known about whether the concentrations of these hormones have an effect on the efficacy of preventive therapy with selective oestrogen receptor modulators or aromatase inhibitors in women at increased risk of developing breast cancer. Beattie and colleagues[10] found little effect of serum oestradiol concentrations on the response to tamoxifen in the P-1 breast cancer prevention trial. By contrast, Cummings and colleagues[11] found a strong protective effect of raloxifene for women at average risk of breast cancer (ie, who had high baseline concentrations [defined as >10 pmol/L] of oestradiol) in the MORE trial, but no protective effect was seen in those with undetectable concentrations, and suggested that this drug might not be effective for these women. To date, no results have been reported on the impact of endogenous hormone concentrations on the efficacy of aromatase inhibitors for either preventing or treating breast cancer in postmenopausal women.

In this study, we report on the effects of serum oestradiol, testosterone, and SHBG concentrations on the risk of breast cancer and the efficacy of anastrozole in the placebo-controlled IBIS-II breast cancer prevention trial, in postmenopausal women at high risk of breast cancer. Overall, the trial found a 49% reduction in the risk of new breast cancers with anastrozole.[12,13] In this pre-planned case-control analysis, we tested the hypothesis that, for women with a low oestradiol–SHBG ratio, anastrozole would provide little or no reduction in the risk of breast cancer.

Methods

Study design and participants

Briefly, the IBIS-II prevention trial is a randomised, double-blind, placebo-controlled trial investigating the effect of 5 years of anastrozole treatment on the risk of developing breast cancer. Postmenopausal women aged 40–70 years with increased risk of breast cancer were recruited from 153 breast cancer treatment centres across 18 countries. Major exclusion criteria were previous diagnosis of breast cancer, including ductal carcinoma in situ; current or planned use of hormone replacement therapy (HRT); and current or previous use of tamoxifen, raloxifene, or other selective oestrogen receptor modulators for a duration of 6 months or longer before trial entry, except for tamoxifen as part of IBIS-I and only used more than 5 years before trial entry. Full details of the IBIS-II trial method and study design have been described previously[13] and a protocol is available online.

For the **IBIS-II trial protocol** see https://www.qmul.ac.uk/wiph/people/profiles/cuzickjack.html#third

Women were randomly assigned to receive anastrozole (1 mg/day, oral) or placebo for 5 years. All participants provided written, informed consent to join the study, provide blood samples at baseline and at years 1 and 5 of follow-up, and have their past and future health records

examined. The primary outcome was the development of histologically confirmed breast cancer.

The trial was approved by the UK North West Multi-Centre Research Ethics Committee, and was done in accordance with the Declaration of Helsinki (1996 revision), under the principles of Good Clinical Practice. The study was monitored, reviewed, and approved annually by a trial steering committee while patients were still being treated (appendix p 11).

See Online for appendix

Described here is a pre-planned case-control study of the role of plasma oestradiol, testosterone, and SHBG concentrations at baseline on the development of breast cancer in untreated women and those treated with anastrozole.

Procedures

Women received 1 mg oral anastrozole or matching placebo every day for 5 years. Participants were seen once every 6 months for the first 5 years and blood samples were taken at baseline, 1 year, and 5 years. After the treatment period, women were followed up once a year to collect data on breast cancer incidence, other cancers, death, and adverse events. Only those of female sex at birth were eligible, and no data on gender, race, or ethnicity were collected.

For the case-control study, all cases identified at or before the most recent follow-up on Oct 22, 2019, were eligible.[12] For hormone analyses, two controls who had not developed breast cancer were selected at random (using a computer random number generator) from those who matched each case by allocated treatment, age (within 2 years), and follow-up time (which had to be at least as long as the case to which they were matching).

All hormone assays were done in the laboratory of BK at the Manchester University NHS Trust, with masking to treatment group or case-control status. Oestradiol and testosterone concentrations were measured by liquid chromatography–tandem mass spectroscopy (Waters model TQ-X5, Wilmslow, UK),[14] and the SHBG assay used an electrochemiluminescence immunoassay (Elecsys SHBG Cobas E immunoassay analyser; Roche Diagnostics, Mannheim, Germany) on a Cobas analyser (Roche Diagnostics).[15] The assays used have been standardised and have previously been shown to be reproducible.[14]

Statistical analysis

The primary outcome (both for this case control study and the IBIS-II prevention trial) was breast cancer (invasive or ductal carcinoma in situ). Secondary outcomes in this case-control study were oestrogen receptor-positive breast cancer and breast cancer developing before or after 5·5 years from randomisation. Data on these outcomes were collected from health records.

Here, the primary, prespecified analysis evaluated the effect of the baseline oestradiol–SHBG ratio on the development of breast cancer over the entire 131-month median follow-up period. A predefined secondary analysis evaluated the effect of the baseline testosterone–SHBG ratio on the development of breast cancer

A priori, we hypothesised an effect of oestradiol–SHBG ratio in the placebo group, based on the Generations study,[5] and no effect in anastrozole group. For a planned sample size of 85 cases in the anastrozole group and 165 cases in the placebo group, we anticipated 89% power to show an effect in the placebo group and 41% power to test the interaction between groups (appendix pp 1–2), which was judged sufficiently informative to assay samples. Minimal bias related to treatment was anticipated, because this was a randomised trial. We did a complete case analysis for all cases with available data. Data were unavailable for very few cases, and those missing data were judged not to materially affect the results. HRT was not allowed during the trial, and those with HRT use within 3 months of trial entry were also excluded from this analysis, as they were not excluded at the design stage. Outliers with oestradiol concentrations greater than 120 pmol/L, or SHBG concentrations either greater than 200 nmol/L or less than 2 nmol/L were excluded; a testosterone exclusion cutoff value of greater than 3·5 nmol/L was also specified, but there were no exclusions on this basis. Units used were pmol/L for oestradiol and nmol/L for testosterone and SHBG, so units for oestradiol–SHBG ratios are (pmol/L)/(nmol/L) and units for testosterone–SHBG ratios are (nmol/L)/(nmol/L). To approximate free oestradiol and free testosterone, the primary analyses were based on the oestradiol–SHBG ratio and testosterone–SHBG ratio. These ratios were used as practical approximations of the free concentrations of these hormones, which are considered to be better markers of bioactivity than the crude levels of oestradiol and testosterone alone.[5] After exclusions, we categorised hormone values into quartiles separately for oestradiol–SHBG ratio and testosterone–SHBG ratio, using matched controls from the combined anastrozole and placebo groups to determine the quartile cutoffs.

For each treatment group, we applied a multinominal logistic regression likelihood-ratio trend test for the change in the proportion of cases for a one-quartile change in hormone ratio—ie, exp(α), where the probability (p) that a case is in quartile j=1,2,3,4 is $p_j=\exp(j\alpha) / \Sigma_{k=1:4} \exp(k\alpha)$. Note that when α=0, $p_i=0\cdot25$ for all j (ie, no trend), and, for example, when exp(α)=1·2, p_j=(0·186, 0·224, 0·268, 0·321, j=1,...,4). Matched controls were only used to determine quartile cutoffs. Profile likelihood 95% CIs were used to indicate the precision of this estimate. A test for interaction between randomised allocation and serum hormone concentration was also done, using a case-only analysis based on a Wilcoxon test of the distribution of hormone concentrations between the two groups. The benefit of anastrozole was quantified as 1 minus the relative risk of cancer in the anastrozole group versus placebo group (ie, 1–[number of cases in

the anastrozole group divided by number of cases in the placebo group]) for the oestradiol–SHBG ratio and testosterone–SHBG ratio. A thin-plate spline smoother[16] was used to estimate the benefit of anastrozole as a function of the oestradiol–SHBG ratio. Relative benefit of anastrozole versus placebo was calculated in the overall population and by oestradiol–SHBG ratio and testosterone–SHBG ratio quartiles. A post-hoc analysis of relative benefit of anastrozole compared quartile 1 with the combined quartiles 2–4 using an exact binomial test. We assessed the effect of baseline oestradiol–SHBG ratio or testosterone–SHBG ratio on the incidence of breast cancer and the relative benefit of anastrozole in a pre-planned subgroup analysis for oestrogen receptor-positive invasive breast cancer, and in a post-hoc subgroup analysis for cancers occurring within the first 5·5 years (treatment period plus 6 months) of follow-up versus those occurring subsequently. Spearman's correlation coefficient was used to assess the association between BMI and serum hormone concentration. All p values and 95% CIs are two-sided. A two-sided significance threshold of $p<0·05$ was used. Statistical analyses were done with R (version 4.1.2) and Stata (version 17.0).

The IBIS-II prevention trial had a data monitoring committee which met annually when women were still being treated and annually approved the continuation of the trial. IBIS-II is registered as an International Standard Randomised Controlled Trial (number ISRCTN31488319).

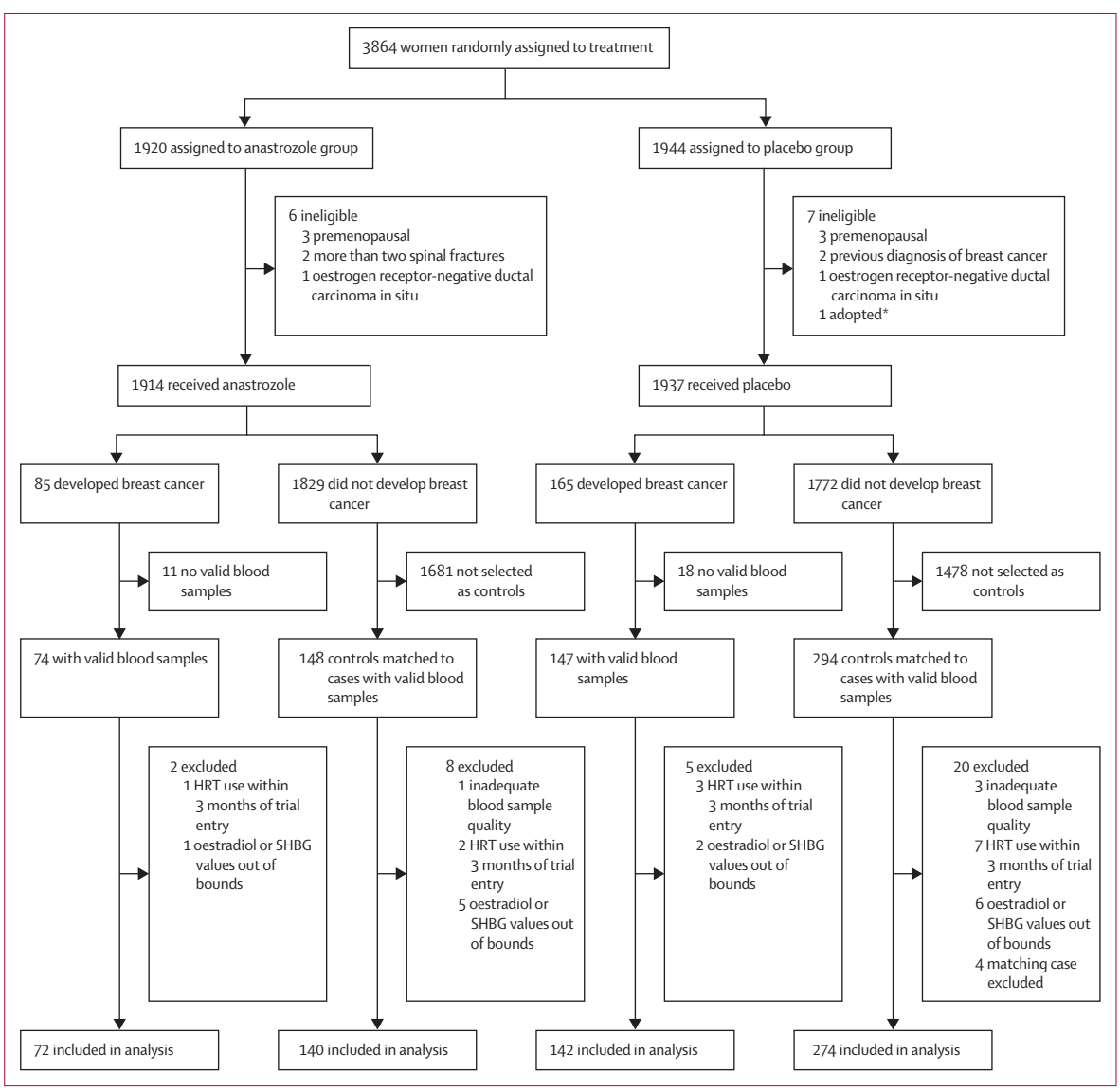

Figure: **Study profile**
HRT=hormone replacement therapy. SHBG=sex hormone binding globulin. *Unable to provide family history to establish high-risk status.

	Anastrozole group		Placebo group	
	Cases	Matched controls	Cases	Matched controls
Breast cancer cases identified among trial participants	85	..	165	..
Participants included in case-control analysis	72	140	142	274
Age at entry, years	61·0 (55·9–64·2)	61·3 (56·7–64·2)	58·9 (55·6–63·2)	58·8 (55·5–63·3)
BMI, kg/m²	27·8 (24·0–31·8)	27·0 (24·1–30·2)	28·7 (25·3–32·4)	27·5 (24·2–31·2)
Tyrer-Cuzick 10-year risk,[17] %	7·7% (5·9–10·8)	7·2% (5·5–9·9)	9·2% (6·5–12·7)	7·7% (6·0–9·7)
Breast cancer in a first-degree relative	20 (28%)	44 (31%)	36 (25%)	63 (23%)
Breast cancer oestrogen receptor status				
Positive	46 (64%)	..	102 (72%)	..
Negative	15 (21%)	..	24 (17%)	..
Unknown	11 (15%)	..	16 (11%)	..
HRT use >3 months before study entry	36 (50%)	71 (51%)	56 (39%)	121 (44%)
HRT use >3 months but ≤12 months before entry	3 (4%)	7 (5%)	12 (8%)	17 (6%)

Characteristics are shown only for participants included in the case-control analysis. Data are n, n (%), or median (IQR). HRT=hormone replacement therapy.

Table 1: **Characteristics of study participants by treatment group and case or control status**

	Breast cancer cases		Relative benefit of anastrozole (95% CI)*
	Anastrozole group	Placebo group	
Overall	72	142	0·49 (0·32 to 0·62)
By oestradiol–SHBG ratio			
Quartile 1 (<0·167)	18 (25%)	22 (16%)	0·18 (–0·60 to 0·59)
Quartile 2 (0·167 to <0·279)	14 (19%)	31 (22%)	0·55 (0·13 to 0·78)
Quartile 3 (0·279 to <0·617)	21 (29%)	46 (32%)	0·54 (0·22 to 0·74)
Quartile 4 (≥0·617)	19 (26%)	43 (30%)	0·56 (0·23 to 0·76)
Trend in risk (95% CI)†	1·06 (0·86–1·30), p=0·60	1·25 (1·08–1·45), p=0·0033	$p_{interaction}$=0·20‡
By testosterone–SHBG ratio			
Quartile 1 (<0·0077)	15 (21%)	26 (18%)	0·42 (–0·13 to 0·72)
Quartile 2 (0·0077 to <0·0111)	15 (21%)	31 (22%)	0·52 (0·08 to 0·76)
Quartile 3 (0·0111 to <0·0169)	18 (25%)	39 (28%)	0·54 (0·17 to 0·75)
Quartile 4 (≥0·0169)	24 (33%)	46 (32%)	0·48 (0·13 to 0·70)
Trend in risk (95% CI)†	1·18 (0·96 to 1·46), p=0·11	1·21 (1·05 to 1·41), p=0·011	$p_{interaction}$=0·85‡

Cases per group and quartile are presented as n (%). Quartile boundaries were based on data from 414 controls matched to breast cancer cases; ratios are based on oestradiol measured in pmol/L and SHBG and testosterone measured in nmol/L. SHBG=sex hormone binding globulin. *Relative benefit is calculated as 1 minus the ratio of cases in the anastrozole group to cases in the placebo group. †Increase per quartile on a multiplicative scale (1·00 corresponds to no linear trend in the proportion of cases by quartile). ‡p value for interaction between treatment and oestradiol–SHBG or testosterone–SHBG ratio.

Table 2: **Relative benefit of anastrozole versus placebo for breast cancer incidence overall and by quartiles of oestradiol–SHBG ratio and testosterone–SHBG ratio**

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

In the IBIS-II trial, 3864 postmenopausal women at increased risk of breast cancer were recruited between Feb 2, 2003, and Jan 31, 2012, and randomly assigned to receive anastrozole (n=1920) or placebo (n=1944; figure). At the last reported assessment, with cutoff date Oct 22, 2019,[12] the median follow-up time was 131 months (IQR 106–156), 85 (4·4%) participants in the anastrozole group and 165 (8·5%) in the placebo group were diagnosed with breast cancer, among whom 74 (87%) in the anastrozole group and 147 (89%) in the placebo group had valid blood samples. Two matched controls per case were randomly chosen, for a total of 442 controls (148 in the anastrozole group, 294 in the placebo group). After a total of 35 exclusions, 72 (97%) of 74 cases and 140 (95%) of 148 controls in the anastrozole group, and 142 (97%) of 147 cases and 274 (93%) of 294 controls in the placebo group were included in this analysis (figure). Details and demographics of the analysis sample are shown in table 1.

The distribution of hormone concentrations among controls was based on the combined control populations (n=414). Median values for oestradiol–SHBG ratio were 0·36 (IQR 0·20–0·74) for cases and 0·28 (0·17–0·62) for controls. For testosterone–SHBG ratio median values were 0·013 (0·090–0·019) for cases and 0·011 (0·008–0·017) for controls (appendix pp 3–5). As expected, oestradiol–SHBG ratio at baseline was moderately correlated with BMI (r_s=0·58 in controls).

The incidence of breast cancer by treatment group and quartiles of oestradiol–SHBG ratio is shown in table 2, and the relative benefit of anastrozole over placebo by oestradiol–SHBG ratio on a continuous scale is provided in the appendix (p 6). Overall, the relative benefit of anastrozole was 0·49 (95% CI 0·32–0·62; p<0·0001), matching the findings for the whole trial.[12] There was a clear trend of increasing risk of breast cancer with increasing quartile of oestradiol–SHBG ratio in the placebo group (trend per quartile 1·25 [95% CI 1·08–1·45], p=0·0033), but not in the anastrozole group (1·06 [0·86–1·30], p=0·60; table 2). In a post-hoc analysis,

the relative benefit of anastrozole for reduction of breast cancer incidence in quartiles 2–4 (overall 54 anastrozole cases *vs* 120 placebo cases) was 0·55 (95% CI 0·32–0·68; binomial p<0·0001), but no benefit was seen in the lowest quartile (0·18 (–0·60 to 0·59; binomial p=0·64; table 2). Little benefit from anastrozole was apparent for oestradiol–SHBG ratios below 0·10 (appendix p 6), but more data are needed to make specific recommendations about a cutoff, and the interaction between treatment and oestradiol–SHBG ratio concentration was not significant ($p_{interaction}$=0·20; table 2).

A weaker, but still significant, association with breast cancer risk was seen for the testosterone–SHBG ratio in the placebo group (trend 1·21 [95% CI 1·05–1·41], p=0·011), but not in the anastrozole group (1·18 [0·96–1·46], p=0·11), and there was no evidence of a significant interaction between treatment and testosterone–SHBG ratio ($p_{interaction}$=0·85; table 2) . SHBG concentrations were not significantly associated with breast cancer in the placebo group (p=0·060) or the anastrozole group (p=0·92), and there was no significant interaction between treatment and SHBG concentration ($p_{interaction}$=0·24; appendix p 7). Further details for oestradiol and testosterone concentrations alone are in the appendix (p 7).

Hormone receptor status was known for 61 (85%) of 72 breast cancer cases in the anastrozole group, of which 46 (75%) were oestrogen-receptor positive. In the placebo group, hormone receptor status was known in 126 (89%) of 142 cases, of which 102 (81%) were oestrogen-receptor positive. In pre-planned secondary analyses, trends in incidence by oestradiol–SHBG ratio for oestrogen receptor-positive cancers were very similar to those for all breast cancers, with no trend in the anastrozole group (trend 1·05 [95% CI 0·81–1·37], p=0·69) but a clear trend in the placebo group (1·32 [1·11–1·59], p=0·0018; table 3). Similar but slightly stronger trends than for all breast cancers were seen for the testosterone–SHBG ratio in oestrogen receptor-positive cancers (anastrozole group trend 1·26 [0·97–1·65], p=0·085; placebo group 1·34 [1·12–1·60], p=0·0013; table 3). Further details for SHBG are in the appendix (p 8). No effect was seen for oestrogen receptor-negative invasive cancers, but the numbers were too small (15 anastrozole, 21 placebo; table 1) to draw any firm conclusions (data not shown).

In post-hoc secondary analyses, the association of oestradiol–SHBG ratio with breast cancer incidence was only seen for cancers diagnosed in the first 5·5 years of the study (period of active treatment plus 6 months), and not subsequently, in the placebo group (table 4). However, the association of testosterone–SHBG ratio with breast cancer incidence was only seen in the subsequent 6-year median follow-up period in the placebo group (table 4). A test for interaction between cancer incidence in the two follow-up periods and the oestradiol–SHBG ratio or testosterone–SHBG ratio quartiles was not significant for either treatment group (p=0·64 for anastrozole and p=0·90 placebo for oestradiol–SHBG ratio, and p=0·64 and p=0·42 for testosterone–SHBG ratio; table 4), and these effects remain speculative.

Further details for SHBG, oestradiol, and testosterone alone are in the appendix (pp 9–10).

	Oestrogen receptor-positive breast cancer cases		Relative benefit of anastrozole (95% CI)*
	Anastrozole group	Placebo group	
Overall	46	102	0·55 (0·36 to 0·69)
By oestradiol–SHBG ratio			
Quartile 1 (<0·167)	10 (22%)	13 (13%)	0·23 (–0·90 to 0·70)
Quartile 2 (0·167 to <0·279)	10 (22%)	22 (22%)	0·56 (0·00 to 0·81)
Quartile 3 (0·279 to <0·617)	16 (35%)	35 (34%)	0·54 (0·15 to 0·76)
Quartile 4 (≥0·617)	10 (22%)	32 (31%)	0·69 (0·35 to 0·86)
Trend in risk (95% CI)†	1·05 (0·81 to 1·37); p=0·69	1·32 (1·11 to 1·59); p=0·0018	$p_{interaction}$=0·16‡
By testosterone–SHBG ratio			
Quartile 1 (<0·0077)	8 (17%)	13 (13%)	0·38 (–0·60 to 0·78)
Quartile 2 (0·0077 to <0·0111)	11 (24%)	25 (25%)	0·56 (0·07 to 0·80)
Quartile 3 (0·0111 to <0·0169)	10 (22%)	28 (28%)	0·64 (0·24 to 0·85)
Quartile 4 (≥0·0169)	17 (37%)	36 (35%)	0·53 (0·14 to 0·75)
Trend in risk (95% CI)†	1·26 (0·97 to 1·65); p=0·085	1·34 (1·12 to 1·60); p=0·0013	$p_{interaction}$=0·72‡

Cases per group and quartile are presented as n (%). Quartile boundaries were based on data from 288 controls matched to oestrogen receptor-positive breast cancer cases; ratios are based on oestradiol measured in pmol/L and SHBG and testosterone measured in nmol/L. SHBG=sex hormone binding globulin. *Relative benefit is calculated as 1 minus the ratio of cases in the anastrozole group to cases in the placebo group. †Increase per quartile on a multiplicative scale (1·00 corresponds to no linear trend in the proportion of cases by quartile). ‡p value for interaction between treatment and oestradiol–SHBG or testosterone–SHBG ratio.

Table 3: **Relative benefit of anastrozole versus placebo for incidence of oestrogen receptor positive breast cancer, overall and by quartiles of oestradiol–SHBG ratio and testosterone–SHBG ratio, preplanned secondary analysis**

Discussion

The results from the placebo group of this study confirm the increasing risk of breast cancer associated with higher oestradiol and testosterone concentrations, and a decreasing risk associated with increasing SHBG concentrations in women who were not randomly allocated to receive anastrozole.[3,4] However, to our knowledge, this is the first report of the effect of low concentrations of oestradiol or testosterone on a lack of response to aromatase inhibitor treatment, either as a preventive measure or in the adjuvant setting. These data provide support for the hypothesis that preventive therapy with an aromatase inhibitor is likely to be most effective for women with higher oestradiol–SHBG ratios and, conversely, of little or no benefit for those with low oestradiol–SHBG ratios. Measurement of oestradiol and SHBG concentrations might be helpful in making decisions about using inhibitors both for treatment and prevention.

Reliable SHBG assays are widely available, but many oestradiol assays in routine use are ill suited for measuring the low concentrations present in the

	Breast cancer cases in ≤5·5 years			Breast cancer cases in >5·5 years			Time period $p_{interaction}$*	
	Anastrozole group	Placebo group	Relative benefit of anastrozole (95% CI)†	Anastrozole group	Placebo group	Relative benefit of anastrozole (95% CI)*	Anastrozole group	Placebo group
Overall	35	88	0·60 (0·41 to 0·74)	37	54	0·31 (–0·06 to 0·56)	..	..
By oestradiol–SHBG ratio								
Quartile 1 (<0·167)	10 (29%)	12 (14%)	0·17 (–1·10 to 0·68)	8 (22%)	10 (19%)	0·20 (–1·25 to 0·73)	..	..
Quartile 2 (0·167 to <0·279)	4 (11%)	22 (25%)	0·82 (0·46 to 0·95)	10 (27%)	9 (17%)	–0·11 (–2·09 to 0·59)	..	..
Quartile 3 (0·279 to <0·617)	10 (29%)	27 (31%)	0·63 (0·21 to 0·84)	11 (30%)	19 (35%)	0·42 (–0·28 to 0·75)	..	..
Quartile 4 (≥0·617)	11 (31%)	27 (31%)	0·60 (0·15 to 0·82)	8 (22%)	16 (30%)	0·50 (–0·24 to 0·81)	..	..
Trend in risk (95% CI)‡	1·11 (0·82 to 1·50), p=0·50	1·26 (1·04 to 1·53), p=0·017	Treatment $p_{interaction}$=0·48§	1·01 (0·76 to 1·35), p=0·94	1·23 (0·97 to 1·58), p=0·087	Treatment $p_{interaction}$=0·30§	0·64	0·90
By testosterone–SHBG ratio								
Quartile 1 (<0·0077)	7 (20%)	16 (18%)	0·56 (–0·12 to 0·85)	8 (22%)	10 (19%)	0·20 (–1·25 to 0·73)	..	..
Quartile 2 (0·0077 to <0·0111)	8 (23%)	21 (24%)	0·62 (0·10 to 0·85)	7 (19%)	10 (19%)	0·30 (–1·04 to 0·77)	..	..
Quartile 3 (0·0111 to <0·0169)	6 (17%)	26 (30%)	0·77 (0·43 to 0·92)	12 (32%)	13 (24%)	0·08 (–1·19 to 0·62)	..	..
Quartile 4 (≥0·0169)	14 (40%)	25 (28%)	0·44 (–0·12 to 0·73)	10 (27%)	21 (39%)	0·52 (–0·06 to 0·80)	..	..
Trend in risk (95% CI)‡	1·25 (0·92 to 1·70), p=0·15	1·16 (0·96 to 1·40), p=0·42	Treatment $p_{interaction}$=0·68§	1·13 (0·84 to 1·51), p=0·13	1·31 (1·03 to 1·69), p=0·028	Treatment $p_{interaction}$=0·43§	0·64	0·42

Quartile boundaries were based on data from 414 controls matched to breast cancer cases; ratios are based on oestradiol measured in pmol/L and SHBG and testosterone measured in nmol/L. SHBG=sex hormone binding globulin. *p value for interaction between follow-up period (≤5·5 years *vs* >5·5 years) and oestradiol–SHBG ratio or testosterone–SHBG ratio quartiles for cancer risk. †Relative benefit is calculated as 1 minus the ratio of cases in the anastrozole group to cases in the placebo group. ‡Increase per quartile on a multiplicative scale (1·00 corresponds to no linear trend in the proportion of cases by quartile). §p value for interaction between treatment and oestradiol–SHBG or testosterone–SHBG ratio.

Table 4: **Relative benefit of anastrozole versus placebo for incidence of breast cancer, in the first 5·5 years of follow up and after 5·5 years, overall and by quartiles of oestradiol–SHBG ratio and testosterone–SHBG ratio, preplanned secondary analysis**

plasma in postmenopausal women.[18] We used a very sensitive liquid chromatography–tandem mass spectroscopy assay (lower limit of sensitivity of 3 pmol/L), which allowed us to accurately measure the low concentrations of oestradiol and SHBG in the serum samples from our population of postmenopausal women. Wider use of this type of assay or a similar assay will be necessary to implement any of the actions suggested by this study.

This study of the aromatase inhibitor anastrozole adds substantial information to the previous literature on the effect of serum oestradiol concentrations on response to the selective oestrogen receptor modulators tamoxifen (P1 trial)[10] and raloxifene (MORE trial)[11] in the preventive setting. Oestradiol had very little effect in on the efficacy of tamoxifen treatment in the P1 trial, but showed a strong effect in predicting response to raloxifene in the MORE trial. Two studies have reported that lower doses of tamoxifen lead to similar preventive effects, but with fewer side-effects. A Swedish study[19] found that doses of 2·5, 5, and 10 mg/day of tamoxifen all had the same effect on reducing mammographic density, a known risk factor for breast cancer, as did 20 mg, but with only half the number of severe vasomotor symptoms (hot flashes, cold sweats, and night sweats). Additionally, an Italian trial[20] found that low-dose tamoxifen at 5 mg/day for 3 years in women with intraepithelial neoplasia reduced the occurrence of invasive breast cancer or ductal carcinoma in situ by 52% compared with placebo after a median 9·7 years of follow-up, without additional adverse events. Further study of tamoxifen is ongoing in the IBIS-I prevention trial (ISRCTN91879928).[21]

In the ATAC trial, adjuvant anastrozole resulted in a greater reduction in distant recurrence than did tamoxifen in a direct randomised comparison,[22] and also in breast cancer deaths in an overview of nine randomised trials;[23] however, to our knowledge, no studies have investigated the effect of oestradiol or other serum hormones on the efficacy of anastrozole in the adjuvant setting. The POETIC study[24] showed that 2 weeks of use of an aromatase inhibitor as a preoperative treatment in women with oestrogen receptor-positive cancer can lead to an important reduction in Ki-67 concentration in the tumour tissue, which in turn predicts better treatment response in this setting. Concordant with the current report, higher plasma oestradiol concentrations were recently reported to be associated with a significantly greater response to aromatase inhibition in the POETIC study.[25]

Although IBIS-II has established a 50% reduction in cancer incidence with anastrozole overall in the preventive setting, the current analysis indicates a much weaker effect when oestradiol concentrations are low. Further work is needed to accurately assess whether there is any benefit of anastrozole in these circumstances and, if so, if such a benefit is sufficient to justify the side-effects of treatment.[22] These results also raise the question of whether serum hormones should be

measured more routinely and integrated into risk models to help decide on possible preventive measures, including the need for more (or less) screening.

A major strength of this study is that it was conducted within the context of a large clinical trial. As this was a randomised trial, bias should be minimal. The population studied was identified prospectively, and a standard format was used for case identification and assay collection, which was independent of breast cancer risk factors or hospital contact.

This study has a number of limitations. Although it is the largest study to date on the effect of serum hormone concentrations on endocrine-based preventive treatment, studies with larger numbers of cases and studies in the adjuvant setting will be needed to fully validate these findings. One other trial (MAP3) has been reported in the prevention setting, using an aromatase inhibitor (exemestane), but no results regarding hormone concentrations have been reported.[26] Another issue that needs further study is the role of serum hormones in deciding whether preventive hormone agents are likely to be effective for breast cancer prevention in individual women. An even more important question is the value of such measurements in choosing a treatment in the adjuvant setting. Another limitation of our study is that it only evaluated an aromatase inhibitor in the prevention setting, whereas the effects in an adjuvant setting remain an important area for further investigation. Although ethnicity was not recorded in the trial, we know that the study population comprised mainly White European women at high risk of breast cancer due to family history; therefore, our findings might not be generalisable to women with other ethnic backgrounds or at a lower risk.

A surprising finding from the ATAC adjuvant trial was that a low BMI was associated with a better response to anastrozole than to tamoxifen,[27] despite BMI being positively correlated with oestradiol concentrations. Unfortunately, no blood samples were taken in ATAC. Further work is also needed to assess whether there is a similar effect of serum hormones on the efficacy and side-effects of tamoxifen. Tamoxifen and the aromatase inhibitors have very different modes of action from one another, so the outcomes will be of considerable interest. Another question of interest is whether hormone concentrations can identify which postmenopausal women are more likely to benefit more from tamoxifen or an aromatase inhibitor. Future work will look at the issues studied here in the IBIS-I trial of tamoxifen for prevention of breast cancer in both premenopausal and postmenopausal women.[21] The differential side-effect profiles between these two classes of drugs according to hormone concentrations also require more study. Finally, the associations of these hormones with other risk factors, including BMI, requires further work.[28]

In conclusion, the results of this analysis suggest that measuring serum hormone concentrations could be an inexpensive method to help clinicians differentiate which women will benefit most from use of aromatase inhibitor for the prevention of breast cancer.

Contributors

JC, SC, and MD conceptualised the project and acquired funding. JC, KC, ARB, SC, and MD developed the methodology. KC and ARB wrote the software code and accessed, verified, and curated the data. BK assayed the samples. JC, KC, and ARB did the statistical analyses. AH, NZ, BB, SL, KH, and DGE recruited and managed participants in the trial and contributed resources. JC, KC, BK, and ARB curated the data. JC wrote the original draft of the manuscript and supervised the study. All authors had access to the study data. JC, KC, BK, ARB, AH, NZ, BB, SL, KH, DGE, SC, and MD reviewed and edited the manuscript. KC administrated the study.

Declaration of interests

JC reports royalties from Cancer Research UK for commercial use of the IBIS (Tyrer-Cuzick) breast cancer risk evaluation software. ARB reports grants from Breast Cancer Now, Cancer Research UK, and the National Institute for Health and Care Research during the conduct of study; royalties from commercial use of the IBIS (Tyrer-Cuzick) breast cancer risk evaluation software from Cancer Research UK; and consulting fees from King's College London. NZ reports payments from AstraZeneca, Novartis, Pfizer; receipt of support for conference travel from Novartis; participation on advisory boards for AstraZeneca and Novartis; and receipt of payment through the University of Newcastle from AstraZeneca. SL reports support for the manuscript through the University of Frankfurt from AZ Germany for the IBIS-II trial; receipt of grants through the University of Frankfurt from Amgen, AstraZeneca, AbbVie, DSI, Gilead, Molecular Health, Celegene/BMS, Novartis, and Pfizer; royalties through the University of Frankfurt from VM Scope; consulting fees for steering committees from AstraZeneca, BMS, Daiichi-Sankyo, Roche, and Stemline Menarini and for participation on an independent data monitoring committee from Parexel; payments through the University of Frankfurt from Amgen, AbbVie, AstraZeneca, DSI, Gilead, Celegene/BMS, Novartis, Pfizer, Seagen, Sanofi, Stemline Menarini, Relay, Olema, Eirgenix, Merck KG, Lilly, GSK, Pierre Fabre, Esai, MSD, Incyte, and Hexal; patents pending for EP14153692.0, EP21152186.9, EP15702464.7 for work not related to this trial; participation in the European Society for Medical Oncology (ESMO) Guideline Committee; and involvement in medical writing support for steering committees from Gilead, Novartis, Pfizer, Roche, Seagen, AstraZeneca, DSI, and Cellcuity. DGE reports consulting fees from AstraZeneca relating to poly (ADP-ribose) polymerase inhibitors and from EverythingGenetic for genetic testing. MD reports consulting fees from AstraZeneca, ROVI, Agilent, Lilly, Roche, and Besins; patents pending for AIR-CIS (Aromatase Inhibitor-Resistant CDK4/6 Inhibitor-Sensitive molecular signature, an RNA-based signature that we developed to identify women with primary oestrogen receptor-positive breast cancer that is resistant to an aromatase inhibitor but who have a greater chance than average patients to benefit from a CDK4/6 inhibitor; international patent application number PCT/EP2021/076368); being a Trustee and Chair of the Scientific Strategy Committee for Breast Cancer Now; and payments related to the invention of abiraterone from the Institute of Cancer Rewards for Innovations Scheme. All other authors declare no competing interests.

Data sharing

Data will be made available according to IBIS-II's data sharing plan. Requests for specific analyses or data can be submitted by email to j.cuzick@qmul.ac.uk. Details for data sharing policy and application process can be found on the website of Queen Mary University London (https://www.qmul.ac.uk/wiph/our-research/data-sharing-policy-/).

Acknowledgments

We thank Cancer Research UK (grant C569/A16891) and the National Health and Medical Research Council, Australia (project grant numbers 209811, 980381, 950319, 920876) for supporting the IBIS-I trial; the Breast Cancer Research Foundation for supporting the tissue biobank and the hormone assays; and DaCosta Fund for providing further support for the hormone assays.

References

1 Thomas HV, Key TJ, Allen DS, et al. A prospective study of endogenous serum hormone concentrations and breast cancer risk in post-menopausal women on the island of Guernsey. *Br J Cancer* 1997; **76:** 401–05.

2 Hankinson SE, Willett WC, Manson JE, et al. Plasma sex steroid hormone levels and risk of breast cancer in postmenopausal women. *J Natl Cancer Inst* 1998; **90:** 1292–99.

3 Key T, Appleby P, Barnes I, Reeves G. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. *J Natl Cancer Inst* 2002; **94:** 606–16.

4 Kaaks R, Rinaldi S, Key TJ, et al. Postmenopausal serum androgens, oestrogens and breast cancer risk: the European prospective investigation into cancer and nutrition. *Endocr Relat Cancer* 2005; **12:** 1071–82.

5 Schoemaker MJ, Folkerd EJ, Jones ME, et al. Combined effects of endogenous sex hormone levels and mammographic density on postmenopausal breast cancer risk: results from the Breakthrough Generations Study. *Br J Cancer* 2014; **110:** 1898–907.

6 Tin Tin S, Reeves GK, Key TJ. Endogenous hormones and risk of invasive breast cancer in pre- and post-menopausal women: findings from the UK Biobank. *Br J Cancer* 2021; **125:** 126–34.

7 Fourkala E-O, Blyuss O, Field H, et al. Sex hormone measurements using mass spectrometry and sensitive extraction radioimmunoassay and risk of estrogen receptor negative and positive breast cancer: Case control study in UK Collaborative Cancer Trial of Ovarian Cancer Screening (UKCTOCS). *Steroids* 2016; **110:** 62–69.

8 Zhang X, Tworoger SS, Eliassen AH, Hankinson SE. Postmenopausal plasma sex hormone levels and breast cancer risk over 20 years of follow-up. *Breast Cancer Res Treat* 2013; **137:** 883–92.

9 Tamimi RM, Byrne C, Colditz GA, Hankinson SE. Endogenous hormone levels, mammographic density, and subsequent risk of breast cancer in postmenopausal women. *J Natl Cancer Inst* 2007; **99:** 1178–87.

10 Beattie MS, Costantino JP, Cummings SR, et al. Endogenous sex hormones, breast cancer risk, and tamoxifen response: an ancillary study in the NSABP Breast Cancer Prevention Trial (P-1). *J Natl Cancer Inst* 2006; **98:** 110–15.

11 Cummings SR, Duong T, Kenyon E, Cauley JA, Whitehead M, Krueger KA. Serum estradiol level and risk of breast cancer during treatment with raloxifene. *JAMA* 2002; **287:** 216–20.

12 Cuzick J, Sestak I, Forbes JF, et al. Use of anastrozole for breast cancer prevention (IBIS-II): long-term results of a randomised controlled trial. *Lancet* 2020; **395:** 117–22.

13 Cuzick J, Sestak I, Forbes JF, et al. Anastrozole for prevention of breast cancer in high-risk postmenopausal women (IBIS-II): an international, double-blind, randomised placebo-controlled trial. *Lancet* 2014; **383:** 1041–48.

14 Owen LJ, Wu FC, Keevil BG. A rapid direct assay for the routine measurement of oestradiol and oestrone by liquid chromatography tandem mass spectrometry. *Ann Clin Biochem* 2014; **51:** 360–67.

15 Adaway J, Keevil B, Miller A, Monaghan PJ, Merrett N, Owen L. Ramifications of variability in sex hormone-binding globulin measurement by different immunoassays on the calculation of free testosterone. *Ann Clin Biochem* 2020; **57:** 88–94.

16 Wood SN. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *J R Stat Soc Series B Stat Methodol* 2011; **73:** 3–36.

17 Tyrer J, Duffy SW, Cuzick J. A breast cancer prediction model incorporating familial and personal risk factors. *Stat Med* 2004; **23**: 1111–30.

18 Lee JS, Ettinger B, Stanczyk FZ, et al. Comparison of methods to measure low serum estradiol levels in postmenopausal women. *J Clin Endocrinol Metab* 2006; **91:** 3791–97.

19 Eriksson M, Eklund M, Borgquist S, et al. Low-dose tamoxifen for mammographic density reduction: A randomized controlled trial. *J Clin Oncol* 2021; **39:** 1899–908.

20 Lazzeroni M, Puntoni M, Guerrieri-Gonzaga A, et al. Randomized placebo controlled trial of low-dose tamoxifen to prevent recurrence in breast noninvasive neoplasia: a 10-year follow-up of TAM-01 study. *J Clin Oncol* 2023; **41:** 3116–21.

21 Cuzick J, Sestak I, Cawthorn S, et al. Tamoxifen for prevention of breast cancer: extended long-term follow-up of the IBIS-I breast cancer prevention trial. *Lancet Oncol* 2015; **16:** 67–75.

22 Cuzick J, Sestak I, Baum M, et al. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 10-year analysis of the ATAC trial. *Lancet Oncol* 2010; **11:** 1135–41.

23 Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. *Lancet* 2015; **386:** 1341–52.

24 Smith I, Robertson J, Kilburn L, et al. Long-term outcome and prognostic value of Ki67 after perioperative endocrine therapy in postmenopausal women with hormone-sensitive early breast cancer (POETIC): an open-label, multicentre, parallel-group, randomised, phase 3 trial. *Lancet Oncol* 2020; **21:** 1443–54.

25 Schuster EF, Lopez-Knowles E, Alataki A, et al. Molecular profiling of aromatase inhibitor sensitive and resistant ER+HER2- postmenopausal breast cancers. *Nat Commun* 2023; **14:** 4017.

26 Goss PE, Ingle JN, Alés-Martínez JE, et al. Exemestane for breast-cancer prevention in postmenopausal women. *N Engl J Med* 2011; **364:** 2381–91.

27 Sestak I, Distler W, Forbes JF, Dowsett M, Howell A, Cuzick J. Effect of body mass index on recurrences in tamoxifen and anastrozole treated women: an exploratory analysis from the ATAC trial. *J Clin Oncol* 2010; **28:** 3411–15.

28 Smith SG, Sestak I, Morris MA, et al. The impact of body mass index on breast cancer incidence among women at increased risk: an observational study from the International Breast Intervention Studies. *Breast Cancer Res Treat* 2021; **188:** 215–23.