

Influence of Estrogen Plus Testosterone Supplementation on Breast Cancer

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Background: Concern that the use of exogenous testosterone may increase breast cancer risk coexists with rising use of this medication in the United States. We sought to examine the relationship between the use of estrogen plus testosterone (E + T) therapy (esterified estradiol plus methyltestosterone) and the occurrence of breast cancer.

Methods: A total of 31 842 postmenopausal participants in the Women's Health Initiative Observational Study were followed for a mean of 4.6 years. At the 3-year visit, E + T users were compared with non-hormone therapy users for time to incident invasive breast cancer. Cox proportional hazards estimates were adjusted for known predictors of breast cancer including prior hormone use and screening mammography.

Results: Thirty five women using E + T at visit 3 developed invasive breast cancer. Use of E + T had a nonsignificant impact on invasive breast cancer risk (adjusted hazard ratio, 1.42; 95% confidence interval, 0.95-2.11). The most commonly used E + T preparation, Estratest, was associated with a significant elevation in invasive breast cancer (adjusted hazard ratio, 1.78; 95% confidence interval, 1.05-3.01). However, rates of breast cancer were lower in longer-term E + T users than in shorter-term E + T users.

Conclusion: Although our results have less strength than an initial report linking E + T to breast cancer, we found a modest, albeit nonsignificant, elevation in breast cancer risk associated with E + T use.

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SINCE IT CAME ON THE US MARKET in 1965, over 36 million prescriptions have been written for the methyltestosterone and estrogen combination pill, Estratest (Solvay Pharmaceuticals, Marietta, Georgia).¹⁻³ Declining testosterone levels in women as they age, taken together with evidence that pharmacologic doses of testosterone might improve sexual function in women with dysfunction, has been used to advocate for the common use of testosterone supplementation for reduced libido.⁴⁻⁷ Moreover, small clinical trials in which estrogen plus testosterone (E + T) therapy was superior to estrogen alone in increasing lean body mass, decreasing body fat, and producing increases in bone formation markers and bone mineral density, have triggered demand for testosterone in the hopes of preventing frailty.⁸⁻¹²

Little is known, however, about long-term adverse events associated with exogenous testosterone use. Breast cancer has been linked to elevations in endogenous androgen levels, raising the concern that the use of exogenous testosterone may increase breast cancer risk. Indeed, in the Study of Osteoporotic Fractures, serum testosterone levels were predictive of estrogen receptor-positive invasive breast cancer, and this relationship was independent

of serum estradiol concentrations.¹³ Combined data from 9 cohorts indicate that elevated blood testosterone levels were as predictive of increased breast cancer risk as were elevated estrone or estradiol levels.¹⁴ Data from the Nurses' Health Study, showing that women with natural menopause and using E + T therapy had a 2.5-fold elevated breast cancer risk compared with never users of hormone therapy (HT), reinforces this concern.¹⁵ To our knowledge, this observation has not been replicated.¹⁶

We sought to examine the relationship between reported use of methyltestosterone and esterified estradiol and incident breast cancer in a large cohort of women participating in the Women's Health Initiative (WHI) Observational Study.

METHODS

STUDY POPULATION

A total of 93 676 women were enrolled in the WHI Observational Study between October 1993 and December 1998. Details of the design and baseline characteristics of the study cohort have been previously published.^{17,18} Women were recruited from 40 US clinical centers either directly or by virtue of ineligibility or unwillingness to participate in the clinical trial components of WHI. Eligibility criteria in-

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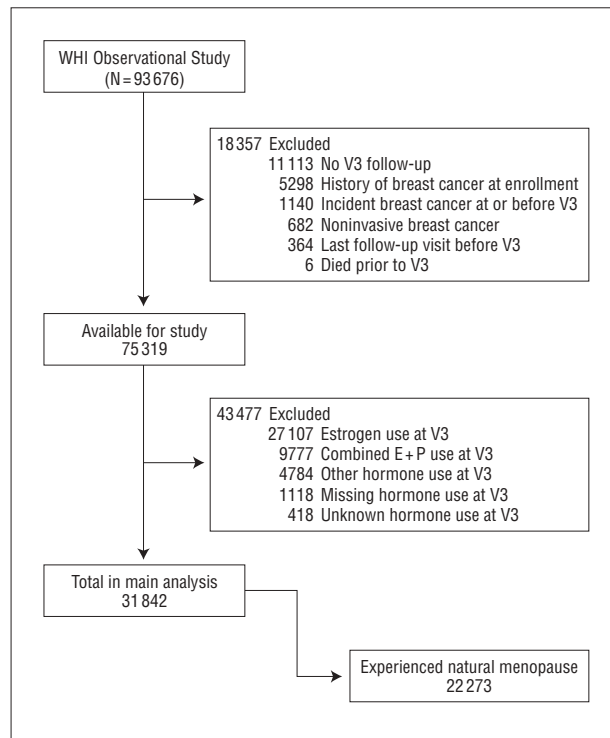


Figure 1. Exclusion cascade of observational study participants (Women's Health Initiative [WHI] Observational Study). E + P indicates estrogen plus progesterone; V3, year 3 visit.

cluded age 50 to 79 years; postmenopausal; planning to live in the clinical center area for at least 3 years; cognitively able to participate (without dementia); and free from serious conditions such as class IV congestive heart failure, or severe chronic liver, kidney, or lung disease. The present analyses are based on follow-up from the year 3 visit (V3) because this was the first time that women were asked about use of exogenous testosterone. We excluded women who reported a history of breast cancer at baseline or a diagnosis of invasive or noninvasive breast cancer diagnosed between V1 and V3; women who had no V3 because of loss to follow-up or death; and those missing information on hormone use. We report only on women who used E + T or no postmenopausal hormones (**Figure 1**). Thus, this analysis is based on 31 842 postmenopausal participants in the WHI Observational Study. Mean follow-up time since V3 was 4.6 years (maximum, 7.7 years).

EXOGENOUS TESTOSTERONE EXPOSURE

The most common exogenous testosterone exposure reported among women enrolled in the WHI Observational Study was use of combination methyltestosterone (1.25 mg) and esterified estradiol (0.625 mg) therapy. At the time of cohort enrollment, injectable testosterone use was exceedingly rare and transdermal testosterone was not approved by the US Food and Drug Administration. At each annual follow-up, women were asked to complete a self-administered questionnaire that included questions about HT. Data from V3 through V8 were available for this analysis. In the V3 questionnaire, women were asked about HT over the past 2 years, whereas in the V4 through V8 questionnaires, they were asked about use in the last year. Questions directed women to report HT pill use in all years (V1-V8) and to report the use of pill, patch, and cream forms of estrogen plus progesterone therapy at V6 through V8.

The disease end point in our analysis was invasive breast cancer. On each annual follow-up outcomes questionnaire, women were asked: "Has a doctor told you for the first time that you have a new cancer or a malignant tumor? What kind of cancer or malignant tumor was it?" In addition, all hospitalizations and breast surgical procedures were investigated, and any indication of a possible breast cancer diagnosis was validated. Validation of breast cancer diagnoses were based on pathology reports, discharge summaries, operative reports, and radiology reports for both breast biopsies and breast surgical procedures. Central adjudication by physicians and cancer coders classified cases according to the National Cancer Institute Surveillance Epidemiology and End Results guidelines.

STATISTICAL ANALYSES

After enumerating the reported prevalence of HT use by type at each visit, the first analytic step was to describe users of E + T therapy and no HT use at V3 on the basis of race/ethnicity, socioeconomic demographics, lifestyle factors, reproductive characteristics, and personal and family breast disease. We used analysis of variance (continuous variables) and χ^2 tests (categorical variables) for these analyses.

Kaplan-Meier survival analysis was the main strategy for comparing time to invasive breast cancer among E + T and non-HT users at V3. Follow-up time for each woman was calculated from the date of V3 to the date of breast cancer diagnosis, death from a non-breast cancer cause, loss to follow-up, or to the end of follow-up (September 12, 2005). Secondary analyses considered type of E + T pill and duration of E + T use in relation to breast cancer rates. Notably, because cell sizes were expected to be small, these analyses were exploratory.

Cox proportional hazards regression models were used to adjust for potentially confounding covariates. Age was used as the timescale.¹⁹ Age at entry in days was calculated as a woman's age at V3 in years multiplied by 365.25. Age at exit in days was calculated as V3 age in days plus the total number of days of follow-up from V3. We first fit basic models including hormone-only use (E + T vs none). Next, we selected the covariates for our initial model based on univariate statistical significance ($P = .10$) and scientific importance.²⁰ We then embarked on model construction involving a manual procedure of removing variables with statistical significance greater than $P = .10$. Owing to the small number of cases, categories were collapsed when appropriate to conserve power. When applicable, dummy variables were coded. The following covariates were considered for entry into the model: race/ethnicity (non-Hispanic white, black/African American, Hispanic/Latino, or other); education ($\leq$ high school graduate or $>$ high school); body mass index (BMI) (calculated as weight in kilograms divided by height in meters squared) (<30 or ≥ 30); current or not current cigarette smoking; current or not current alcohol use; physical activity (metabolic equivalent tasks per week); age at menarche (≤ 13 or ≥ 14 years); number of mammograms in the 5 years before study enrollment (none, 1-4, or ≥ 5); age at first birth (no term pregnancy, <20 , 20-29, or ≥ 30 years); breast-fed (for at least 1 month ever or never); age at menopause (<40 or ≥ 40 years); benign breast disease (yes or no); and first-degree relative with breast cancer (yes or no). On completion of the elimination process, each removed covariate was individually reintroduced into the model to verify its continued nonsignificance. All first-level interactions were assessed ($P < .05$ was considered significant). The resulting models are termed initially adjusted multivariate models.

Additional multivariate adjustments included prior hormone use (none, estrogen use, estrogen plus progesterone use,

and both estrogen and estrogen plus progesterone). Moreover, in these final models, women without a mammogram for 2 or more years were censored at that time point. These models were considered finally adjusted multivariate models. Interpretations of the initial and final adjusted models are at a significance level of $P = .05$. All analyses were completed in SAS version 9.1.3 (SAS Inc, Cary, North Carolina).

In addition, we conducted a Cox proportional hazards regression analysis restricted to women who reported having experienced natural menopause (ie, women who had a hysterectomy or bilateral oophorectomy were excluded). Adjustment for potential confounders was conducted as previously described.

Prior to data analysis, we performed a priori effect size calculations. These assumed 1780 newly diagnosed cases of breast cancer occurring over a mean follow-up of 4.7 years among women recruited into the study.²¹ We also assumed an exposure frequency of 767 current E + T users and 100:1 nonusers to users, an α level of .05 (2-tailed), and a β level of 0.80. These assumptions would allow us to detect a doubling of the baseline rate of breast cancer development, in concert with the results found in the Nurse's Health Study.¹⁵

RESULTS

Among postmenopausal women in the WHI Observational Study, 1705 (2.3% of all participants; 5.4% of women in the present analysis) used E + T therapy. This prevalence of use remained relatively stable as a proportion of all HT use, although rates of HT use declined substantially over the study period (**Figure 2**). Of those who reported E + T use at V3, about half used E + T for up to 1 year, while the other half used E + T for more than a year.

Participants at V3 were, on average, in their mid-60s, and E + T users were younger than nonusers (**Table 1**). Most women were white, and E + T users were more likely to be white than nonusers. Compared with nonusers, E + T users were more likely to be highly educated, be physically active, drink 1 or more alcoholic drinks per week, have a lower BMI, have early age at menarche and menopause, have been pregnant, have breastfed, and be a past but not current smoker. They were less likely to have had a late age at first birth, a family history of breast cancer, and no mammograms over follow-up.

Thirty-five invasive breast cancer cases occurred among E + T users (**Table 2**). The unadjusted (hazard ratio [HR], 1.26; 95% confidence interval [CI], 0.89-1.78) and adjusted HRs (fully adjusted HR, 1.42; 95% CI 0.95-2.11) for invasive breast cancer were modestly, albeit not significantly, elevated compared with nonusers.

Users of E + T for less than 1 to 12 months had a significant elevation in the rate of invasive breast cancer (fully adjusted HR, 1.90; 95% CI, 1.16-3.12), whereas longer-term users (13-24 months) had no elevation in breast cancer rate (adjusted HR, 1.09; 95% CI, 0.61-1.93) (**Table 3**). Users of Estratest, the most commonly used E + T preparation, had an elevation in invasive breast cancer compared with nonusers (fully adjusted HR, 1.78; 95% CI, 1.05-3.01).

Finally, we examined rates of invasive breast cancer among women who experienced natural menopause because this was the group most affected in the previous prospective analysis in which E + T therapy was implicated in breast cancer risk (data not shown).¹⁵ Among women with natural menopause, those using E + T had a mod-

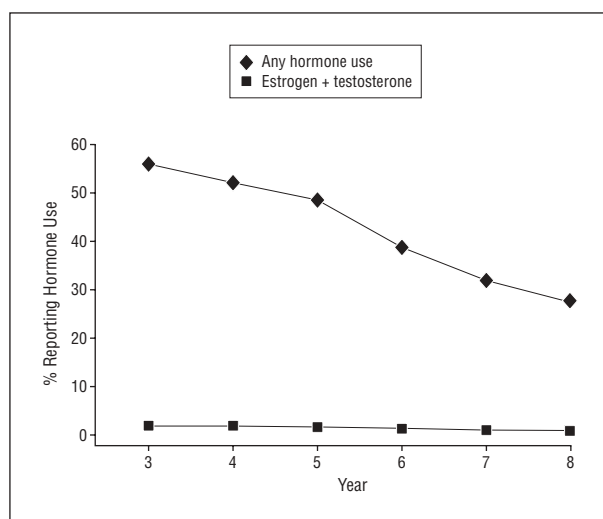


Figure 2. Percentage of women reporting postmenopausal hormone use by type and year.

est, albeit nonsignificant, elevation in the rate of breast cancer that was similar to the effect size seen in the study overall (fully adjusted HR, 1.57; 95% CI, 0.95-2.61).

COMMENT

Among postmenopausal women enrolled in the WHI Observational Study, we found a modest elevation in the rate of invasive breast cancer among E + T users compared with women not receiving HT after adjustment for relevant potential confounding variables. For the most common form of E + T therapy, Estratest, this association reached statistical significance. However, the duration response for E + T use went in the wrong direction, that is, shorter-term users were at higher risk than longer-term users. This suggests that use of E + T therapy was not strongly related to risk for invasive breast cancer among women in the WHI Observational Study; however, we cannot exclude a modest association.

The association between E + T use and breast cancer found in the present study was less pronounced than that reported in the only other cohort study (the Nurses' Health Study) to our knowledge to examine this relationship. Nonetheless, the relationship in both studies was in the direction of elevated risk. In the Nurses' Health Study, women currently using E + T compared with those never receiving HT had an adjusted risk of invasive breast cancer that was elevated by 77%.¹⁵ A dose-response relationship was suggested by the finding that those receiving E + T therapy for less than 5 years had an adjusted risk elevation of 80%, and those receiving E + T therapy for more than 5 years were at a 2-fold excess risk. Among women with natural menopause, the adjusted risk of breast cancer among E + T users was elevated 2.5-fold. There were many similarities between the Nurses' Health Study analysis and our own: the number of exposed women developing breast cancer was similar (29 in their analysis; 35 in ours); rates of E + T use were climbing rapidly during the years of the Nurses' Health Study to 2.2% in 2000. The V3 of the WHI occurred between 1997 and 2001, and the overall rate of use

Table 1. Characteristics by E + T Therapy or No Hormone Use Among Postmenopausal Women (Women's Health Initiative Observational Study)^a

Characteristic	E + T Therapy (n=1705)	No Hormone Use (n=30 137)	P Value
Age at V3, mean (SD), y	62.7 (7.0)	68.4 (7.1)	<.001
Race/ethnicity, %			
White	84.4	82.5	<.001
Black	6.1	9.7	
Hispanic	5.5	3.6	
American Indian	0.7	0.4	
Asian/Pacific Islander	2.6	2.5	
Unknown	0.8	1.3	
Education, %			
None–some high school	4.1	6.1	<.001
High school diploma/GED	10.2	19.0	
>High school diploma/GED	85.7	74.9	
Smoking, %			
Never	49.4	53.2	<.002
Past	44.5	40.1	
Current	6.1	6.7	
Alcohol use, %			
Nondrinker	9.7	13.0	<.001
Past drinker	14.2	20.2	
<1 drink/wk	31.3	31.7	
≥1 drink/wk	44.8	35.2	
Physical activity, mean (SD), MET/wk	16.2 (16.7)	13.2 (14.3)	<.001
BMI, %			
<25	47.3	36.2	<.001
25–29	34.2	34.4	
≥30	18.5	29.4	
Age at menarche, y, %			
≤11	23.0	21.7	<.001
12–13	57.3	54.4	
≥14	19.7	23.9	
Never pregnant, %	7.3	10.9	<.001
Age at first birth, y, %			
Never pregnant	8.0	12.2	<.001
No term pregnancy	3.8	2.8	
<20	15.9	11.6	
20–29	64.6	64.1	
≥30	7.7	9.2	
No. of live births, %			
Never pregnant	7.4	10.9	<.001
None	3.8	2.7	
1	10.6	8.9	
2–4	70.3	62.4	
≥5	8.0	15.1	
Breastfed, %	54.1	49.7	<.001
Age at menopause, y, %			
<40	10.3	6.9	<.001
40–49	45.2	34.9	
≥50	44.5	58.2	
Type of menopause, % ^b			
Natural	51.2	71.3	<.001
Surgical	26.2	12.2	
Other	22.6	16.5	
No. of mammograms, %			
None	2.6	9.2	<.001
1–2	11.8	25.0	
3–4	29.0	30.4	
≥5	56.6	35.5	
Benign breast disease, %	36.5	24.4	<.001
Family history of breast cancer, % ^c	11.9	17.4	<.001
Prior estrogen use, % ^d	50.6	17.9	<.001
Prior combined estrogen/progestosterone use, % ^d	48.9	11.1	<.001

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); E + T, estrogen plus testosterone (esterified estradiol plus methyltestosterone); GED, General Education Development; MET, metabolic equivalent tasks; V3, year 3 visit.

^aHormone use within the past 2 years as indicated at V3. Age is at V3; other variables are from V1.

^bSurgical indicates bilateral oophorectomy; other, hysterectomy with or without unilateral oophorectomy.

^cFamily history of breast cancer indicates self-reported breast cancer diagnosis in a first-degree female relative (ie, mother, sister, or daughter).

^dPrior hormone use indicates current or past use (pills or patches) as assessed at enrollment, approximately 3 years before this study's baseline (ie, V3).

Table 2. Incidence and Hazard Ratios of Invasive Breast Cancer by Postmenopausal E + T Therapy or No Hormone Use (Women's Health Initiative Observational Study)

Hormone Pill Use	Breast Cancer Cases, No.	Unadjusted HR (95% CI)	Initial Adjusted HR (95% CI) ^a	Final Adjusted HR (95% CI) ^b	P Value ^c
No HT use	558	1 [Reference]	1 [Reference]	1 [Reference]	.67
E + T	35	1.26 (0.89-1.78)	1.32 (0.91-1.93)	1.42 (0.95-2.11)	

Abbreviations: CI, confidence interval; E + T, estrogen plus testosterone (esterified estradiol plus methyltestosterone); HR, hazard ratio; HT, hormone therapy.

^aAdjusted for body mass index, age at menopause, history of breast cancer in a first-degree relative, and number of mammograms in the 5 years before study enrollment.

^bAdjusted for body mass index, age at menopause, history of breast cancer in a first-degree relative, number of mammograms in the 5 years before study enrollment, and prior HT use (estrogen, combined estrogen and progesterone use, or both); subjects were censored after 2 years without a mammogram.

^cP value for Kaplan-Meier log rank test.

Table 3. Incidence and Hazard Ratios (HRs) of Invasive Breast Cancer by Type and Duration of Prescribed Postmenopausal Combined E + T Therapy Use (Women's Health Initiative Observational Study)^a

E + T Therapy Variable	Breast Cancer Cases	Unadjusted HR (95% CI)	Initial Adjusted HR (95% CI) ^b	Final Adjusted HR (95% CI) ^c	P Value ^d
Type of E + T pill					.51
Estratest ^e	17	1.43 (0.88-2.33)	1.61 (0.97-2.68)	1.78 (1.05-3.01)	
Estratest HS	6	0.86 (0.38-1.94)	0.68 (0.25-1.84)	0.73 (0.27-1.99)	
Other	8	1.07 (0.53-2.15)	1.08 (0.51-2.29)	1.11 (0.52-2.38)	.50
Months of E + T use					
<1-12	18	1.47 (0.91-2.36)	1.77 (1.10-2.87)	1.90 (1.16-3.12)	
13-24	17	1.14 (0.70-1.85)	1.02 (0.58-1.78)	1.09 (0.61-1.93)	

Abbreviations: CI, confidence interval; E + T, estrogen plus testosterone (esterified estradiol plus methyltestosterone); HS, half strength.

^aNo hormone pill use is the reference category.

^bAdjusted for body mass index, age at menopause, history of breast cancer in a first-degree relative, and number of mammograms in the 5 years before study enrollment.

^cAdjusted for body mass index, age at menopause, history of breast cancer in a first-degree relative, number of mammograms in that 5 years before study enrollment, and prior hormone therapy use (estrogen, combined estrogen and progesterone use, or both); subjects were censored after 2 years without a mammogram.

^dP value for Kaplan-Meier log rank test.

^eManufactured by Solvay Pharmaceuticals, Marietta, Georgia.

was 2.3%. Age and current BMI of the women analyzed in our study were similar to those analyzed in the Nurses' Health Study. Both were dominantly white, but this was overwhelmingly true in the Nurses' Health Study. One possible explanation for the stronger results from the Nurses' Health Study may have been prior HT use and use of screening mammography. These may have differentially confounded results in the 2 cohorts. Recent articles from the WHI Observational Study demonstrate the power of these confounders.^{22,23}

There is good biological plausibility to the notion that E + T therapy would elevate breast cancer risk. Elevated serum or urinary androgen levels are associated with the development of postmenopausal breast cancer. At least 7 cohort studies have demonstrated a link between circulating androgen levels and breast cancer,²⁴⁻³⁰ whereas 2 cohort studies did not.^{31,32} The demonstrated associations were of similar magnitude to the established relationship between circulating estrogen levels and breast cancer. To detail the largest of these cohort studies, among 5000 women ascertained between 1961 and 1967 and followed for 37 years, resulting in 115 cases of breast cancer, the highest tertile of urinary androgen levels doubled the risk of subsequent breast cancer.²⁴ In the Nurses' Health Study,¹⁵ steroid hormones were measured in blood samples collected from 1989 to 1990 and compared be-

tween 147 women who developed breast cancer by 1994 and 299 controls. Testosterone levels in the highest vs the lowest quartile had an 1.4-fold elevated breast cancer risk after covariate adjustment.²⁹ An even higher risk for breast cancer (6.2-fold) was found among the 71 postmenopausal women developing the condition over 10 years of follow-up among those enrolled in the Columbia, Missouri, cohort with serum testosterone values in the highest vs lowest quartile.²⁵ The 2 cohort studies that did not report significant associations between circulating testosterone levels and breast cancer risk were both small (15 and 39 cases), and 1 study involved premenopausal women.^{31,32} A pooled analysis of cohort studies estimated that the relative risk of breast cancer in women with testosterone in the top compared with the bottom quintile was 2.2 (95% CI, 1.6-3.1) and that the dose-response relationship between testosterone and breast cancer risk was statistically significant.³³

Risks of androgen supplementation are generally unknown. In 2 review articles in leading gynecology and geriatrics journals, potential risks were said to include virilization, hirsutism, acne, voice changes, erythrocytosis, liver toxic effects, and lipid alterations but did not mention breast cancer risk.^{2,4} Comments concerning possible breast cancer risk were that androgen receptors are frequently found on breast cancer tissue and associated

with a good prognosis, and no data have linked androgen supplementation to breast cancer.

Our study had both strengths and limitations. The study population was large and racially and ethnically heterogeneous, representing postmenopausal American women relatively well. The prospective nature of data collection and adjudication of breast cancer diagnoses are also strengths. Nonetheless, the modest proportion of women using E + T therapy and the small number of exposed women developing breast cancer are clear limitations. Moreover, the WHI Observational Study involved no validation of self-reported E + T therapy use. Previous studies have shown that self-report of ever use of HT is accurate, although details of use (eg, duration, formulation) are less so.³⁴

Our finding of a modest, nonsignificant association between E + T use and breast cancer, in contrast to the stronger association previously reported, leaves the association between E + T use and breast cancer risk in doubt. Only with additional accrued experience with this medication will clear evidence emerge for hazard vs safety.

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