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Improved breast cancer survival among hormone replacement therapy users is durable after 5 years of additional follow-up

Dara Christante, M.D., SuEllen Pommier, Ph.D., Jennifer Garreau, M.D., Patrick Muller, B.S., Brett LaFleur, B.S., Rodney Pommier, M.D.*

Division of Surgical Oncology, Department of Surgery, Portland, OR, USA

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Abstract

BACKGROUND: We previously reported that breast cancer patients who used hormone replacement therapy (HRT) had significantly lower stage tumors and higher survival than never-users. We present an update with longer follow-up, HRT use data, and in vitro research.

METHODS: Our database of 292 postmenopausal breast cancer patients was updated to include HRT type, duration, and disease status. In vitro effects of estrogen (E) and/or medroxyprogesterone (MPA) on breast cancer cell growth were measured.

RESULTS: Tumor prognostic factors were better and survival rates higher for both E and combination HRT users of any duration. Use greater than 10 years correlated with node-negative disease, mammographically detected tumors, and 100% survival. E supported minimal proliferation; MPA induced cell death; E+MPA results were similar to E alone.

CONCLUSIONS: HRT users, regardless of type or duration of HRT use, continued to have higher survival rates. In vitro results supported the clinical finding that outcomes for users of E and E+MPA were similar.

We previously reported that breast cancers in hormone replacement therapy (HRT) users were smaller, lower grade, more often node-negative, lower stage, and had significantly higher survival rates compared to those in never-users.[1] Recent events have raised concerns about the impact of HRT on breast cancer. Particular concern has been raised about the use of combinations of estrogen (E) and medroxyprogesterone acetate (MPA). Results from the Women's Health Initiative (WHI) trial indicated that breast cancers were more advanced among users of E and MPA (combination HRT) than among patients receiving placebo.[2] This would be expected to result in lower survival rates among users of combination HRT. Concerns also exist about duration of HRT use and breast cancer.[3,4]

Due to these concerns, we investigated if the higher survival rate we reported was durable after an additional 5 years of follow-up. We also investigated whether duration or type of HRT are associated with differences in tumor characteristics or survival rates. We have supplemented our clinical investigation with in vitro studies in which estrogen receptor (ER)-positive and -negative breast cancer cell lines were treated with various concentrations of E, MPA, or

Dr. Rodney Pommier and Dr. SuEllen Pommier are consultants for Wyeth Pharmaceuticals.

* Corresponding author. Tel.: +1-503-494-5550; fax: +1-503-494-7573.

E-mail address: pommierr@ohsu.edu

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combinations of E and MPA to determine their impacts on cellular proliferation.

Methods

Our database of 292 postmenopausal women diagnosed with breast cancer at Oregon Health & Science University between March 1994 and January 2002 from our previous publication[1] was updated by review of medical records. New data included the current disease status of each patient as well as the type and duration of HRT used. HRT was categorized as E alone, progestin alone, E and progestin combination, or other HRT. If E and progestin were ever taken by a patient, either serially or concomitantly, it was considered combination HRT. Approval for this retrospective review was obtained from the institutional review board.

Disease-specific survival curves were constructed using the Kaplan-Meier method[5] and statistical significance between survival distributions was determined by log-rank analysis. Significance of differences between groups of patients was determined by chi-square analysis, Student *t* test, analysis of variance (ANOVA), Mann-Whitney U test, or Kruskal-Wallis H test. Correlations between duration of HRT use and tumor size, number of positive lymph nodes, and stage were determined using Pearson or Spearman coefficients of correlation. The independent effects of prognostic factors and HRT use on survival was determined by Cox regression analysis.

For all laboratory experiments, 3 cell lines, T-47D, HCC1954, and HCC1937, were obtained from the ATCC (American Type Culture Collection, Manassas, VA) and maintained according to ATCC protocol. T-47D cells are ER-positive, progesterone receptor (PR)-positive; HCC1954 and HCC1937 cells are ER-negative, PR-negative; both are androgen receptor (AR)-positive.[6] Cell lines with these receptor profiles were selected for study because our group of HRT users with a higher survival rate was comprised of a mixture of patients with ER-positive and ER-negative tumors. Cells were plated onto 96-well plates with 10^4 cells per well, grown in hormone-depleted media for 3 days, and then treated with 17-β-estradiol (E) and medroxyprogesterone-17-acetate (MPA) (Sigma-Aldrich, St Louis, MO).

The dose-dependent effects of MPA were initially tested by treating ER-positive (T-47D) and ER-negative (HCC1937) breast cancer cells with a range of MPA (.01, .1, 1, 10, 100, and 250 nmol/L). In all subsequent experiments, T-47D and HCC1954 cells were treated with E alone at concentrations of 1 and 10 nmol/L or MPA alone at concentrations of 1, 10, 100, and 250 nmol/L. Cells were also treated with 1 or 10 nmol/L E in combination with 1, 10, 100, or 250 nmol/L MPA. For all experiments, cells grown in hormone-depleted media served as an untreated control. Cell proliferation was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and expressed as the relative percent change between treated and untreated cultures.[7] All experiments were performed in triplicate wells and repeated a minimum of 3 times. Statistical significance of differences in proliferation of cell cultures was determined by ANOVA and independent *t* tests.

Results

Of the 292 patients, 144 reported HRT use at the time of diagnosis or anytime in the past. Seventy-three patients (51%) used E alone, 54 (38%) used combination HRT, 3 (2%) used progestins alone, and 14 (9%) used another type HRT (Table 1). There were no significant differences in the types of HRT used between patients with ductal carcinoma in situ (DCIS) or invasive cancer. There were no significant differences in the incidence of ER-positive or PR-positive tumors between HRT users and never-users. Data on HER2 status was not available for many patients whose diagnosis preceded the advent of traztuzumab therapy.

Among patients with invasive cancers, mean tumor size, nodal status, ER and PR status, the presence of distant metastases, stage, and the method of detection did not vary significantly by types of HRT. Tumors of patients using either E alone or combination HRT were smaller than those of never-users ($P = .022$ and .003, respectively). There were significantly more T1 ($\leq$2.0 cm) tumors among patients using E ($P = .036$) and combination HRT ($P = .001$) compared with never-users. Negative lymph nodes were significantly more common in users of E ($P = .045$) and combination HRT ($P = .023$) than in never-users. The mean number of positive lymph nodes was also significantly lower in users of E ($P = .03$) and combination HRT ($P = .02$) compared with never-users.

There were no significant differences in the distribution of stage based on type of HRT use. Compared to never-users, users of E or combination HRT had significantly more cancers lower than stage II ($P = .01$ for both). The incidence of distant metastases did not differ between never-users and HRT users, whether compared collectively or by type of HRT.

Data were available on the duration of HRT use for 133 of 144 users (92%) and the median duration was 7 years. Forty-seven patients (33%) used HRT for less than 5 years, 36 patients (25%) used HRT from 5 to 10 years, and 50 patients (35%) used HRT for more than 10 years. DCIS was evenly distributed between groups of HRT duration. Tumor size, number of positive lymph nodes, and stage did not increase with increasing durations of HRT use, regardless of type. Lymph node status was more often negative in patients with greater than 10 years of HRT use compared to never-users ($P = .023$). E and combination HRT users were more often diagnosed by mammography than palpation compared to never-users ($P = .036$ and .01, respectively). Patients with any type of HRT use for more than 10 years were also significantly more likely to have their tumors detected by

Table 1 Comparison of prognostic factors and survival rates between never-users and HRT users by HRT type and duration

	HRT type*						HRT duration† (y)						
	Never-use	HRT	P‡	E	P‡	E+MPA	P‡	<5	P‡	5–10	P‡	>10	P‡
Totals													
All cases	148	144		73		54		47		36		50	
DCIS	14	23		12		9		8		5		8	
Invasive cancer	134	121		61		45		39		31		42	
Mode of detection													
All cases													
MMG	63	84	.01	42	.04	34	.01	26	NS	20	NS	33	.01
PALP	85	58		31		20		21		16		17	
Invasive cancer													
MMG	53	65	.02	31	NS	27	.02	19	NS	15	NS	27	.01
PALP	81	56		30		18		20		16		15	
Mean tumor size (cm)													
All cases	2.6	1.8	.001	1.2	.02	1.6	.003	2.0	.03	1.7	.02	1.7	.01
Invasive cancer	2.7	2.0	.003	2.0	.09	1.9	.004	2.2	.05	1.8	.03	1.8	.01
T0+T1 vs >T1													
All cases	71	97	.001	45	.04	39	.001	32	.02	25	NS	34	.01
Invasive cancer	57	74	.003	33	NS	30	.005	24	.04	20	.03	26	.01
Mean no. of positive nodes													
All cases	2.0	.9	.01	.86	.03	.9	.02	1.3	NS	.42	.048	.26	.01
Invasive cancer	2.2	1.1	.01	1.03	.047	1.0	.03	1.6	NS	.48	NS	.30	.01
Nodal disease													
yes vs no													
All cases	52	31	.03	16	.045	10	.02	11	NS	7	NS	9	.02
Invasive cancer	52	31	.03	16	.09	10	.04	11	NS	7	NS	9	.04
Metastases													
yes vs no													
Invasive	14	10	NS	7	NS	2	NS	3	NS	4	NS	2	NS
Stage 0+1 vs >1													
All cases	68	94	.001	47	.01	36	.01	30	.03	26	.01	33	.01
Invasive cancer	54	71	.003	35	.03	27	.02	22	NS	21	.01	25	.03
Median years of HRT													
All cases		7		10	.01§	5		3		7		20	
DCIS		8		10	.09§	3¶		3		8		25	
Invasive cancer		7		10	.045§	6		3		7		20	
5-Year survival rate													
All cases	84%	92%	.02	91%	NS	95%	.04	88%	NS	93%	NS	100%	.01
Invasive cancer	83%	91%	.04	89%	NS	95%	.04	86%	NS	92%	NS	100%	.01
Mode of detection													
All cases													
MMG	92%	100%	.02	100%	NS	100%	NS	100%	NS	100%	NS	100%	NS
PALP	78%	81%	NS	78%	NS	88%	NS	75%	NS	86%	NS	78%	NS
Invasive cancer													
MMG	90%	100%	.03	100%	NS	100%	NS	100%	NS	100%	NS	100%	NS
PALP	78%	81%	NS	77%	NS	87%	NS	75%	NS	86%	NS	78%	NS

NS = not significant; MMG = mammographic detection; PALP = detection by palpation.
*"Other" group and progesterone users are not included.
†Duration group does not include patients with missing data.
‡Compared to never-user group.
§These compare duration of HRT between E and combination HRT users.
¶Difference in duration of combination HRT between DCIS and invasive cancer is not significant ($P = .76$).

mammography than by palpation compared to never-users ($P = .01$).

After an additional 5-years of follow-up, the disease-specific 5-year survival rate of HRT users was 92% compared with 84% for never-users (Figure 1, $P = .02$). No patient with a diagnosis of DCIS had died of breast cancer. Among patients with invasive cancers, the 5-year survival rate of HRT users was 91% compared with 83% for never-users ($P = .04$). There were no significant differences in survival between patients grouped by HRT type. The 5-year survival rates for users of E and combination HRT were 89% and 95%, respectively. The difference in survival rates between combination HRT users and never-users was statistically significant ($P =$

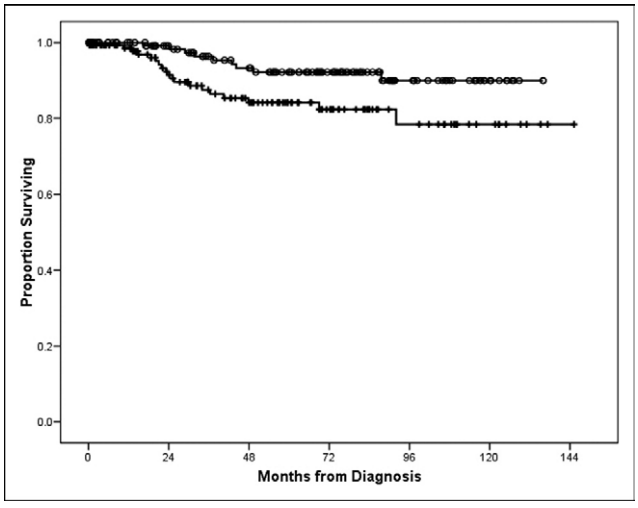

Figure 1 Survival curves for breast cancer patients who were HRT users (circles) compared with never-users (vertical dashes). The difference in survival was statistically significant ($P = .02$).

.04), but the difference in survival rates between users of E and never-users was not significant. Survival curves by type of HRT are shown in Figure 2 and compared with the curve for never-users.

Survival curves of patients grouped by durations of HRT use of <5, 5–10, and >10 years are compared to the survival curve of never-users in Figure 3. Patients with more than 10 years of use had a 100% survival rate compared to 84% among never-users ($P = .01$).

Among patients with tumors detected by palpation, there was no significant difference in 5-year survival rates between never-users and any group of HRT users (Table 1). Among patients with invasive tumors detected by mammography, the 5-year survival rate for HRT users was 100%

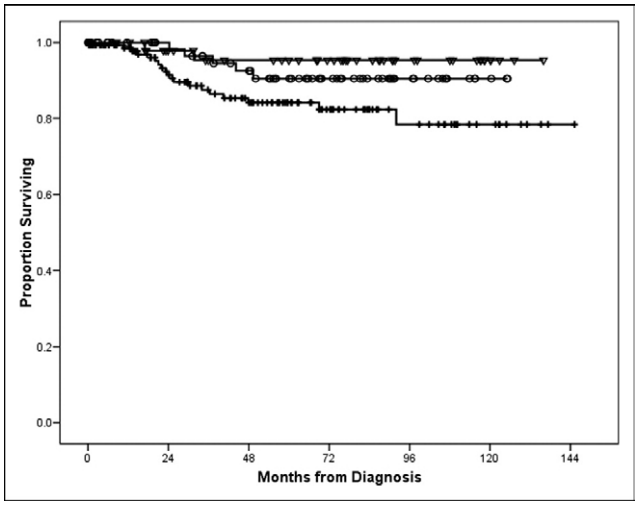

Figure 2 Survival curves for HRT users of E (circles) and combination therapy (triangles) compared with never-users (vertical dashes). The difference in survival between patients who used combination therapy and never-users was statistically significant ($P = .04$).

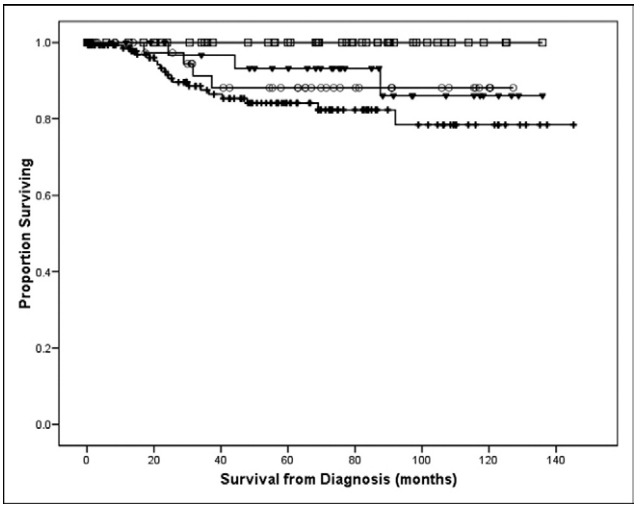

Figure 3 Survival curves for breast cancer patients based on duration of HRT use. Patients with varying durations of use <5 years (circles), 5–10 years (triangles), or >10 years (boxes) are compared to never-users (vertical dashes). The difference between patients with >10 years use and never-users was statistically significant ($P = .01$).

compared with 90% for never-users. (Figure 4, $P = .03$). To estimate the magnitude of difference in survival that could possibly be attributed to differences in adjuvant therapies between these 2 groups, data for each never-user with a mammographically detected tumor were entered into Adjuvant! Online, standard version 8.0.[8] The maximum potential benefit of adjuvant therapies was recorded for each patient. The calculated median and mean decreases in survival had maximum adjuvant therapies been completely withheld from all never-users with mammographically detected tumors were 3.0% and 5.6%, respectively, at 10 years.

Cox regression analysis was performed using variables of tumor size, tumor stage, nodal status, stage, mode of

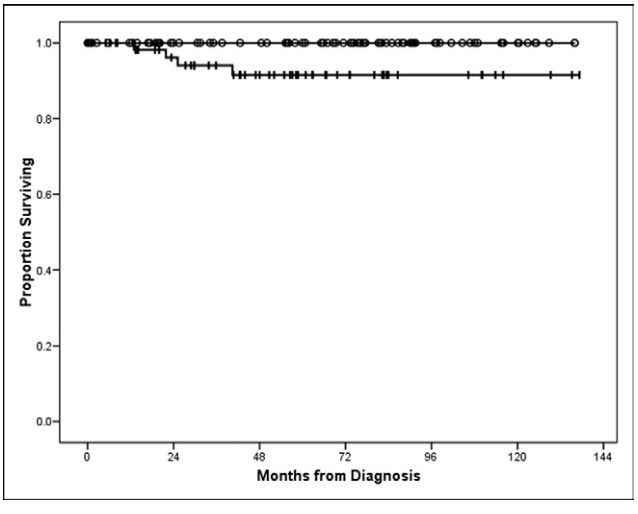

Figure 4 Survival curves for patients with breast cancers detected by mammography comparing HRT users (circles) to never-users (vertical dashes). The difference in survival between HRT users and never-users was statistically significant ($P = .02$).

detection, receptor status, and HRT use. The only variable that was a significant independent predictor of survival was stage.

In vitro responses to treatment with E and MPA

Exposure of the T-47D cells to 1 nmol/L E demonstrated 23% and 25% proliferation on days 5 and 8, respectively, compared to untreated cells. Treatment with 10 nmol/L E was inhibitory; cell growth decreased by 5% and 15% by days 5 and 8, respectively. MPA treatment alone did not have a proliferative effect at any concentration. Treatment with MPA and 1 nmol/L E resulted in 15% and 36% growth on days 5 and 8, respectively, which was not statistically different from the proliferation observed with 1 nmol/L E alone ($P = .13$ and .43, respectively). Treatment with MPA plus 10 nmol/L E resulted in significant cell death by day 8, an effect that increased with higher concentrations of MPA. Growth inhibition reached 50% and 42% by day 8 at concentrations of 100 nmol/L and 250 nmol/L MPA, respectively.

In HCC1954 cells, in contrast to what was observed in T-47D cells, exposure to both concentrations of E was cytotoxic. However, similar to what was observed with T-47D cells, MPA treatment alone did not induce proliferation at any concentration. When cells were treated with E and MPA in combination, some variation in response was observed. The addition of MPA, at all concentrations, blunted the cytotoxic effect observed with 1 nmol/L E treatment alone but did not induce significant proliferation. Treatment with MPA and 10 nmol/L E did not significantly alter the cytotoxicity observed with 10 nmol/L E alone. The mean responses of both T-47D and HCC1954 cells to the various hormonal treatments are summarized in Figure 5. There were no significant differences between the results seen with E alone or E+MPA.

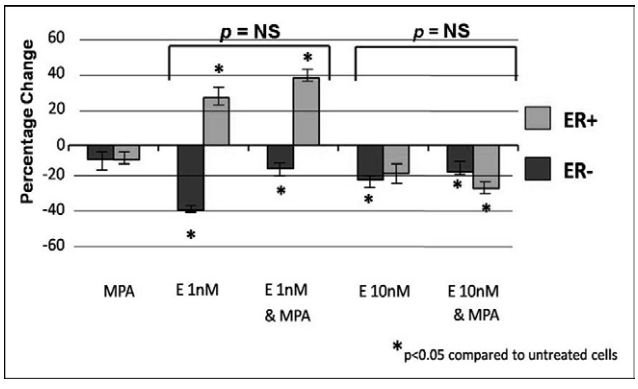

Figure 5 Mean percentage change in proliferation of HCC1954 (black) and T-47D (gray) cells compared to untreated cells after 8 days of treatment with 17-β-estradiol (E), or medroxyprogesterone-17-acetate (MPA), or E+MPA. *Statistically significant changes compared to the untreated group. Differences between cells treated with E alone and E+MPA were not statistically significant.

Dose-dependent cell death was observed in the ER-negative and ER-positive cell lines with increasing concentrations of MPA. By day 8, the percentages of cell death were −48%, −57%, −69%, −70%, and −73% for ER-negative cell line and −9%, −17%, −23%, −18%, and −16% for ER-positive cell lines at MPA concentrations of .01, .1, 1, 10, and 100 nmol/L, respectively.

Comments

After an additional 5 years of follow-up, the survival rates for HRT users were still significantly higher than for never-users. Shuetz et al[9] also recently reported significantly higher 5-year survival rates of 93% for HRT users compared to 82% for non-users. The 5-year survival rates for the 2 groups reported in that study were remarkably similar to the rates reported in this study. In their study of 1,072 women, Shuetz et al also found a decreased incidence of metastatic disease among HRT users compared to non-users. The presence of distant metastases did not differ between HRT users and never-users or among users of different types of HRT in this study, but there were relatively few stage IV patients in the database.

In this study, the higher survival rate was observed chiefly among patients with mammographically detected invasive tumors whose survival rate was still 100%, as it was in our previous report. In contrast, the 5-year survival rate for never-users with mammographically detected invasive tumors was significantly lower at 90%. Additional follow-up has not shown a change from our previous report in which there was no significant difference in survival rates based on HRT use among patients with invasive tumors detected by palpation.

Given that the higher survival rate was found among mammographically detected tumors and that the frequency of screening mammography between HRT users and never-users was equal,[1] the difference in survival is unlikely to be due to better screening of HRT patients. It is also unlikely that the higher survival rate is due to differences in adjuvant therapies. The calculated median and mean decreases in survival had maximum adjuvant therapies been completely withheld from all never-users with mammographically detected tumors were 3.0% and 5.6%, respectively, at 10 years. The observed difference in survival rates for mammographically detected tumors in our series was 10% and it occurred at less than 4 years. Thus, the difference in survival is greater in magnitude and occurs sooner than would be expected from differences in administration of adjuvant therapies.

Tumor size,[10] nodal status,[10] and stage[11] are strong independent predictors of breast cancer survival in large databases. In our series, HRT users had smaller tumors, more node-negative tumors, and lower stage disease than never-users. Regression analysis revealed that only stage was an independent predictor of survival in our database. This

indicates that the higher survival rate of HRT users is chiefly attributable to lower stage disease resulting from combinations of small tumors and node-negative tumors.

Several investigators have reported tumor size to be significantly smaller among HRT users[12–16] but did not differentiate by HRT type. Others have reported lower incidences of positive nodes among HRT users.[13,15,16] It is the report by Chlebowski et al[2] from the WHI trial that has been in contradiction to most published observational studies. They reported more advanced tumors among users of combination HRT compared to women taking placebo (patients in the placebo group were not required to be never-users). Combination HRT users had a mean tumor size of 1.7 cm and a 26% incidence of positive nodes of compared with a mean tumors size of 1.5 cm and a 16% incidence of positive nodes in the placebo group. Although the 2-mm difference in mean tumor sizes was statistically significant, it may be of little clinical significance because both would correspond to T1c tumors.[11] The statement that tumors are more advanced in combination HRT users is therefore derived primarily from the 10% higher incidence of positive nodes. There is a strong relationship between mean tumor size and the incidence of positive nodes, so this large shift in the incidence of positive nodes with only a 2-mm difference in tumor size is difficult to explain. The interpretation of the data has been that the placebo group is normal and, by comparing the combination HRT group to them, we must conclude that HRT made the tumors more advanced for their size than they otherwise would have been. This conclusion appears strengthened by the fact that it is derived from the only randomized prospective data available.

The validity of this conclusion can be tested against other databases. Based on data from 257,888 breast cancer patients in the Surveillance Epidemiology and End Results (SEER) database, the correlation between mean primary tumor size and the percentage of patients with positive lymph nodes has been accurately described by the equation: percentage of patients with positive lymph nodes = $65.6107594/1+8.336998433e^{-0.1005638804(\text{tumor size in mm})}$.[10] If one enters the mean tumor size of 17 mm for the combination HRT group into this equation, then the expected incidence of positive lymph nodes is 26%, which is precisely what was observed. This would indicate that the tumors in the combination HRT group were not more advanced for their size than other breast cancers. In contrast, if one enters the mean tumor size of 15 mm for the placebo group into the equation, then the expected incidence of positive lymph nodes is 23%. This does not agree with the observed rate of 16%. The data point for the placebo group falls well below the curve described by the equation. It is not plausible that placebo made tumors less aggressive than expected, so this indicates that the placebo group may not have been a typical sample of breast cancer patients. In light of the SEER equation, an alternative explanation of the WHI data is that the combination HRT group had typical breast cancers and the placebo group, for some reason, had an unusually low incidence of positive nodes. This had the effect of making the combination HRT group look worse than it actually was. The observed incidences of positive nodes for our patient groups were in close agreement with the predictions of the SEER equation, indicating that we had typical samples of breast cancer patients in our series.

Data on the impact of the duration of use of HRT on breast cancer prognostic factors and survival are limited, but no differences have been reported.[16] In our study, duration of use did not worsen any prognostic factor regardless of whether patients were considered together or grouped by type of HRT used. When patients were arranged in groups by durations of use, those with greater than 10 years of use were significantly more likely to have negative nodes and to have tumors that were detected by mammography.

Although there were no statistically significant differences in survival rates among HRT users based on the type of HRT used, patients who used combination HRT had significantly higher survival rates than never-users. Users of E HRT also had higher survival rates than never-users, but the difference did not achieve statistical significance.

Tissue culture results showed that 1 nmol/L E alone resulted in significant proliferation of ER-positive cells. In contrast, 10 nmol/L E alone consistently resulted in significant decreased proliferation of ER-positive cells. We have previously reported that concentrations between 1 nmol/L and 10 nmol/L support proliferation of ER-positive cell lines. Interestingly, in cultured breast cancer cells that have acquired anti-estrogen and aromatase inhibitor resistance, unexpected cytotoxicity has been observed in cells with normally proliferative concentrations of E, which may explain the observed results.[17,18] As expected, significant proliferation of ER-negative cells was not induced by treatment with E alone.

No concentration of MPA induced a proliferative effect in any of the cell lines. When cells were treated with combinations of MPA and E, the net effects were no different than when they were treated with E alone. For example, when ER-positive cells demonstrated proliferation with 1 nmol/L E, the addition of MPA did not alter this proliferative effect. Similarly, when E treatment induced growth inhibition in either ER-positive or ER-negative cells, the addition of MPA did not alter this effect to the point where significant cellular proliferation was observed. If E induced growth inhibition in either ER-positive or ER-negative cells, the addition of MPA never resulted in significant stimulation of proliferation. Our data indicate that observed net effects on growth were driven by the concentration of E used and not by MPA. Maximum serum concentrations of MPA in patients are dose-dependent and range from 1.2 pg/mL to 4.8 pg/mL (.003–.01 nmol/L).[19] In this study, the concentrations of MPA used for in vitro testing were within and above the range measured in patients.

A limitation to these experiments is that the only effect of hormonal treatment that was measured was cellular pro-

liferation. The effects of hormones on numerous other prognostic factors such as cellular differentiation, potential to metastasize to lymph nodes or distant organs, and interactions between tumor cells and supporting tissues were not tested in this in vitro model. The impact of hormones on these other factors was derived from the clinical outcomes measured in the study. Even the finding that E can support proliferation of ER-positive cells must be viewed in the context of an in vitro model. In this environment, cells are viable for only a short period of time when grown under minimal conditions that are supplemented with E only. Extending proliferation for longer periods requires the addition of growth factors and multiple other hormones to support the growth of breast cancer cells over time. However, the results of our in vitro studies support our clinical findings that the addition of MPA to E does not produce outcomes significantly different than those observed with E alone.

In this study, we found that the higher survival rates of breast cancer patients who used HRT were durable after an additional 5 years of follow-up. Higher survival rates of HRT patients were found chiefly among patients with mammographically detected tumors. Both E and combination HRT use were associated with smaller tumors, a lower incidence of positive lymph nodes, and lower stages of breast cancer. Greater than 10 years of HRT use was also associated with more mammographically detected tumors and a 100% survival rate. Users of combination HRT did not have more advanced tumors than users of E alone or never-users. These clinical findings are supported by in vitro experiments in which the addition of MPA to E did not alter the effects observed with E alone.

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