Hormone Replacement Therapy and Lung Cancer Risk: A Case-Control Analysis

Matthew B. Schabath,[1] Xifeng Wu,[1] Rena Vassilopoulou-Sellin,[2] Ara A. Vaporciyan,[3] and Margaret R. Spitz[1]

[1]Departments of Epidemiology, [2]Endocrine Neoplasia and Hormonal Disorders, and [3]Thoracic and Cardiovascular Surgery, The University of Texas M. D. Anderson Cancer Center, Houston, Texas

ABSTRACT

Purpose: **To date, there are few published data regarding the use of hormone replacement therapy (HRT) and lung cancer risk. Therefore, we analyzed data regarding HRT use from a large case-control study designed to study genetic susceptibility to lung cancer to determine whether HRT affected risk of lung cancer.**

Experimental Design: **In a secondary analysis, we compared self-reported HRT use among 499 women with lung cancer and 519 healthy age-matched controls.**

Results: **HRT use was associated with an overall reduced risk of 34% [odds ratio (OR), 0.66; 95% confidence interval (CI), 0.51–0.89] of lung cancer, after adjusting for age, ethnicity, smoking status, education, body mass index, and menopausal status. The use of estrogen replacement therapy alone was associated with a 35% reduction in lung cancer risk (OR, 0.65; 95% CI, 0.47–0.89) and the use of combination therapy (estrogen and progestin) was associated with a 39% reduction in lung cancer risk (OR, 0.61; 95% CI, 0.40–0.92). HRT use was also associated with a statistically significantly reduced risk of lung cancer in current smokers (OR, 0.59; 95% CI, 0.38–0.92), but the risk estimates were not statistically significant in never (OR, 0.72; 95% CI, 0.37–1.40) or former smokers (OR, 0.73; 95% CI, 0.46–1.15). In addition, as the cigarette pack-years increased among ever smokers, the protective effect diminished, so that light smokers appeared to benefit the most from HRT use. Decreased lung cancer risks were also evident when the data were stratified by age, ethnicity, and body mass index. The joint effects of HRT use and mutagen sensitivity suggest that HRT use modifies lung cancer risk for genetically susceptible women. HRT use was also associated with a lower risk of death and improved survival compared with the women not taking HRT. To provide a possible biological mechanism to explain our findings, we compared plasma levels of insulin-like growth factor I in users and nonusers, and demonstrated that HRT use was associated with statistically significantly lower insulin-like growth factor I levels for both cases and controls compared with non-HRT users.**

Conclusions: **These data suggest an association of HRT use with a decrease in lung cancer risk. However, there are several limitations to this secondary analysis, requiring that the data be viewed with caution, and confirmation is required in well-designed hypothesis driven studies. The biological role of HRT in lung cancer remains understudied, and only extensive research can yield new insights into the mechanisms underlying a protective effect of HRT for lung cancer.**

INTRODUCTION

Hormone replacement therapy (HRT) compensates for the loss of endogenous estrogen that follows menopause and is typically prescribed to treat the symptoms of menopause (1, 2). However, HRT has also been associated with several additional putative health benefits including reductions in the risk of coronary heart disease and osteoporotic fractures, improved cognitive function, and reduced risk of colorectal cancer (3). Nonetheless, controversy exists about the beneficial effects of HRT *versus* recently suggested harmful effects that include risk of coronary heart disease, stroke, and thromboembolic events (4). In fact, recent studies have failed to demonstrate the beneficial effects of HRT for probable dementia or prevention of mild cognitive impairment (5) and in the treatment or prevention of heart disease (6, 7). There are also concerns of whether HRT use may heighten cancer risk. Although sex hormones are not genotoxic, it is known that they can stimulate or inhibit cell proliferation and, thus, theoretically modulate tumor development and progression (8), and their metabolites do have mutagenic potential. In addition, in animal studies, estrogens have been shown to induce cancers of the breast, endometrium, kidney, and ovary (9).

Several observational studies and meta-analyses have shown a positive association between breast cancer risk and HRT use (4, 10–13). The largest meta-analysis of HRT use and breast cancer was performed by the Collaborative Group on Hormonal Factors in Breast Cancer that examined data from 51 epidemiological studies comprising 52,705 women with breast cancer and 108,411 controls (10). This analysis revealed a 2.3% [relative risk (RR), 1.023; 95% confidence interval (CI), 1.011–1.036] increase in the relative risk of breast cancer for each year of HRT use with a relative risk for breast cancer of 1.35 (95% CI, 1.21–1.49) for women who had used HRT for ≥5 years (10). In an intervention trial, conducted by the Women's Health

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Requests for reprints: Margaret R. Spitz, The University of Texas M. D. Anderson Cancer Center, Department of Epidemiology Unit 189, 1515 Holcombe Boulevard, Houston, TX 77030. Phone: (713) 792-3020; Fax: (713) 792-0807; E-mail: mspitz@mdanderson.org.

Initiative, that focused on defining the risks and benefits of specific health outcomes among postmenopausal women (14), the combination HRT (estrogen plus progesterone) arm was stopped at the interim analysis because invasive breast cancer was deemed an adverse effect of the treatment [hazard ratio (HR), 1.26; 95% CI, 1.26–1.59; Ref. 14]. Although recent data continue to support the association between HRT use and an increased risk for breast cancer, there have also been numerous other studies that did not demonstrate such an association (15–19). Observational studies remain inconsistent and unclear for other cancers such as non-Hodgkin's lymphoma, ovarian, liver, and other digestive system organs (20). At the same time, HRT use has been shown to be protective against colon cancer (4, 14, 21, 22).

To date, there have been relatively few data published regarding HRT use and lung cancer risk (23–29), and once again the data that have been published are inconsistent. For example, a Swedish population-based cohort study of 23,244 women with an average of 6.7 years of follow-up revealed a nonsignificantly slightly elevated risk of lung cancer (RR, 1.3; 95% CI, 0.9–1.7) in women taking estrogen replacement therapy (ERT; Ref. 23). However, a small case-control analysis of 180 women with lung adenocarcinoma reported a 70% excess risk of lung cancer [odds ratio (OR), 1.7; 95% CI, 1.0–2.8] associated with ERT use (24), with the OR increasing 2-fold for ≥25 months of usage (OR, 2.0; 95% CI, 0.9–4.4). There have also been studies showing no relationship between HRT use and lung cancer. For example, a population-based case-control study in Los Angeles County, CA, found no substantial relationship (OR, 1.3; 95% CI, 0.8–2.2) between HRT use in 336 women with adenocarcinoma (25). Similarly, the Women's Health Initiative reported recently that lung cancer incidence was not affected by HRT use (HR, 1.04; 95% CI, 0.71–1.53) in a cohort of 16,690 women ages 50–79 (14). Blackman *et al.* (26) analyzed ERT use among 662 women with lung cancer and 4671 controls participating in a hospital-based case-control surveillance study conducted at four United States centers from 1976 to 2001. These investigators found that the OR for lung cancer was 1.0 for both ERT users (95% CI, 0.8–1.4) and the users of conjugated estrogens (95% CI, 0.7–1.4). In contrast, Ettinger *et al.* (27) observed a decrease in the risk of lung cancer mortality (RR, 0.22; 95% CI, 0.04–1.15) in a retrospective analysis of 232 ERT users and 222 nonusers. Likewise, a case-control study of 811 female lung cancer patients and 912 population-based controls showed a reduction in lung cancer risk associated with HRT use (OR, 0.83; 95% CI, 0.64–1.09), which was additionally decreased (OR, 0.59; 95% CI, 0.37–0.93) among long-term users (≥7 years) than nonusers (28). Recently, a population-based cohort study of 29,508 Swedish women found that HRT use was associated with a decrease in the incidence of lung cancer (standardized incidence ratio, 0.73; 95% CI, 0.31–1.43) and long-term HRT users who smoked had a decrease in the incidence of smoking-related cancers (standardized incidence ratio, 0.24; 95% CI, 0.08–0.76), including head and neck, lung, cervix, and bladder (29).

To shed more light on this role, we analyzed the association between HRT use and lung cancer risk among 499 women with lung cancer and 519 healthy age-matched controls participating in a case-control study designed to study genetic susceptibility to lung cancer. We also incorporated data on plasma levels of insulin-like growth factor I (IGF-I), a peptide hormone involved in regulating cell proliferation and differentiation, because plasma levels have been noted to be reduced in women treated with ERT (30). Furthermore, we incorporated data from a phenotypic assay, the mutagen sensitivity assay, that measures genetic instability after *in vitro* challenge of lymphocyte cultures. This assay measures the extent of mutagen-induced chromosome damage and provides a summary measure to indirectly infer DNA repair capacity. We speculated that any adverse or beneficial effects of HRT might be additionally modified by latent mutagen sensitivity, a trait we have shown previously to be an independent risk factor for lung cancer (31–34).

MATERIALS AND METHODS

Subject Recruitment. The women in this analysis were accrued from an ongoing and previously described (35) molecular epidemiological study on lung cancer susceptibility markers. At the time of this analysis, data were available on a total of 1018 women. There were 499 patients with histologically confirmed incident lung cancer recruited before any radiotherapy or chemotherapy from The University of Texas M. D. Anderson Cancer Center. There were no age, ethnic, histological, or stage restrictions. Healthy women ($n = 519$) without a previous diagnosis of cancer were recruited from the Kelsey-Seybold Clinics, Houston's largest private multispecialty physician group, that includes a network of 23 clinics and >300 physicians. Matching criteria were age, gender, ethnicity, and smoking status (never, former, or current). The potential control subjects were first surveyed by a short questionnaire to determine their willingness to participate in an epidemiological study and to provide preliminary data on matching characteristics. The potential control subjects were then contacted by telephone to confirm their willingness to participate, and if they were willing, an appointment was scheduled at a Kelsey-Seybold Clinic convenient to the participant. If the person refused to participate or was deemed ineligible, another potential control was contacted. The overall response rate among both the lung cancer patients and the controls has been ~75%. This research was approved by the M. D. Anderson and Kelsey-Seybold Institutional Review Boards and in accordance with an assurance filed with, and approved by, the United States Department of Health and Human Services.

The date of death was obtained from the patient medical record. If vital status was not available in the medical record, the date of death was sought from the tumor registry at M. D. Anderson and/or from the National Death Index. Date of death for a patient was taken from the Death Index only if the patient name, date of birth, and social security number were all in agreement with their medical record. If the vital status was confirmed as living, the date of last follow-up was recorded for an end point. Of the 499 patients available for the case-control analysis in the study, follow-up data were available on 454 patients.

Collection of Epidemiological Data. After study participants were briefed on the premise of the study and signed an informed consent, a 45-min personal interview was conducted by M. D. Anderson research interviewers, during which they

obtained information on sociodemographic characteristics, cigarette smoking, highest level of education, and history of birth control usage and HRT. Women were asked whether they had taken birth control pills and/or HRT in the previous 6 months. If known, the type(s) of birth control pill and HRT was also recorded. The body mass index [BMI = (weight in kilograms/height in meters^2)] was calculated from the self-reported current height and weight of the participant. Women were also asked if they had reached menopause and if so, at what age. The number of miscarriages, if any, was also recorded.

Mutagen Sensitivity. *In vitro* mutagen sensitivity was measured in peripheral blood lymphocytes by counting the chromatid breaks induced by *in vitro* exposure to mutagen challenges [bleomycin and benzo(*a*)pyrene-*r*-7,*t*-8-dihydrodiol-*t*-9,10-epoxide(±); (BPDE)] as described previously (36, 37). Briefly, phytohemagglutinin-stimulated blood cultures were incubated for 3 days and then treated with bleomycin (0.03 units/ml) for 5 h before harvesting. BPDE was added to separate phytohemagglutinin-stimulated tissue cultures at a final concentration of 2 μM 24 h before harvesting (32). Chromatid breaks were counted in 50 metaphases per sample and recorded as the mean number of breaks/cell. The laboratory personnel read the slides without knowledge of the case-control status of the individual.

Measurement of IGF-I. Plasma levels of IGF-I were determined as described previously (38). Briefly, blood was centrifuged at 3000 × *g* for 10 min at room temperature to separate the fraction containing the plasma. Collected plasma was stored at −80°C. The levels of IGF-I were determined by an ELISA (Diagnostic Systems Laboratories, Inc., Webster, TX).

Statistical Analyses. All of the statistical analyses were performed using the Intercooled Stata 8.0 statistical software package (Stata Corporation, College Station, TX). Pearson's χ^2 test was used to test the differences between the case and control subjects in terms of ethnicity, smoking status, education, menopausal status, current use of HRT and/or birth control use, and number of miscarriage(s). Student's *t* test was used to test differences between the cases and control subjects in terms of the mean age, mean age at menopause, mean cigarette pack-years, number of miscarriages, BMI, mutagen sensitivity, and IGF-I level. ORs and 95% CIs were calculated as an estimate of the relative risk. For all of the postmenopausal women currently using HRT, a surrogate value for the duration of HRT use was calculated on the basis of the difference between the current age of the woman and her age at menopause. From the standpoint of mutagen sensitivity, a woman was considered sensitive to BPDE or bleomycin if the number of breaks per cell was $\geq 75^{th}$ percentile of breaks per cell value in the controls. An unconditional multivariate logistic regression analysis was performed to control for confounding by age (continuous), smoking status, ethnicity, education, BMI (continuous), and menopausal status, where appropriate. Generally, two multivariate logistic regression models were used to generate risk estimates. The first model adjusted for known lung cancer confounding variables such as age, smoking status, ethnicity, and education, where appropriate. The second model included covariates that could affect hormonal status (*i.e.* BMI and menopausal status) as well as the known lung cancer confounding variables. Trend tests were performed using Woolf's test for heterogeneity for two or more subgroup ORs (39). All of the statistical tests were two-sided.

To evaluate whether HRT use impacted survival of the lung cancer patients, Cox proportional hazards models were used to assess the association between HRT and survival. Multivariate models were used to adjust for age, ethnicity, smoking status, and clinical stage. The log-rank test was used to test for the equality of survivor functions, and the Kaplan-Meier survivor functions for days of follow-up or days until death were plotted by HRT use *versus* no HRT use

A women who had smoked at least 100 cigarettes in her lifetime was defined as an ever-smoker. Ever smokers included former smokers, current smokers, and recent quitters. A former smoker for cases had quit smoking at least 1 year before diagnosis. Among controls, a former smoker had quit smoking at least 1 year before the interview. Pack-years were calculated using the average number of cigarette packs smoked per day and the numbers of years smoked.

RESULTS

Because this study is still ongoing, perfect matching has not yet been achieved. Caucasian women constituted ~82% of the lung cancer patients and 71% of the control subjects (Table 1). There were statistically significant differences between the cases and controls in terms of ethnicity, smoking status, and education ($P < 0.01$). Seventeen percent of the cases were never-smokers compared with 25% for the controls. Borderline statistically significant differences were also observed for HRT use (46.5% *versus* 52.6% for cases and controls; $P = 0.051$) and the reported number of miscarriages ($P = 0.057$). There was no difference between the cases and the controls in terms of mean age at menopause. As predicted, the cases were heavier smokers (mean pack-years = 43.2 ± 27.7 SD) than the control subjects (mean pack-years = 34.9 ± 24.5 SD; $P < 0.001$); the lung cancer patients also had a statistically significant lower BMI (25.5 ± 5.4 SD) than the control subjects (28.1 ± 6.2 SD; $P < 0.001$). As predicted by the age of the study population, very few women were currently using oral contraceptives. Mutagen sensitivity data were only available on a subset of these women. Nonetheless, the case patients showed a higher number of breaks per cell than did the control subjects for both bleomycin and BPDE sensitivity; however, only the difference for bleomycin sensitivity was statistically significant ($P < 0.001$).

HRT use was associated with a 34% overall reduced risk (OR, 0.66; 95% CI, 0.51–0.89) of lung cancer (Table 2), after adjusting for age, ethnicity, smoking status, education, BMI, and menopausal status. When HRT use was dichotomized according to the type of HRT, ERT use was found to be associated with a 35% reduction in lung cancer risk (OR, 0.65; 95% CI, 0.47–0.89), and combination HRT consisting of estrogen and progestin was associated with 39% reduction in the lung cancer risk (OR, 0.61; 95% CI, 0.40–0.92). Sixteen lung cancer patients and 11 control subjects did not provide data on the type of HRT used. For all of the subsequent analyses, HRT use was defined as the women using either ERT or a combination of estrogen and progestin.

The adjusted lung cancer risks associated with HRT use for Caucasian, Hispanic, and African-American women were 0.74

Table 1 Characteristics of the women with lung cancer and the healthy controls

Characteristic	Case patients (n = 499)	Control subjects (n = 519)	P^a
Ethnicity, no. (%)			
Caucasian	407 (81.6)	370 (71.3)	
Hispanic	24 (4.8)	35 (6.7)	
African-American	68 (13.6)	114 (22.0)	0.001
Smoking status, no. (%)			
Never	86 (17.2)	128 (24.7)	
Former	189 (37.9)	207 (39.9)	
Current	224 (44.9)	184 (35.4)	0.002
Ever	413 (82.8)	391 (75.3)	0.004
Education, no. (%)			
<12 years	412 (82.6)	465 (89.6)	
≥12 years	87 (17.4)	54 (10.4)	0.001
Menopausal status, no. (%)			
Premenopausal	62 (12.5)	82 (15.9)	
Postmenopausal	436 (87.5)	434 (84.1)	0.117
Use of hormone replacement therapy (HRT), no. (%)			
HRT use	232 (46.5)	273 (52.6)	
No HRT use	267 (53.5)	246 (47.4)	0.051
Use of birth control (BC) pills, no. (%)			
BC use	9 (1.8)	12 (2.3)	
No BC use	489 (98.2)	507 (97.7)	0.506
Reported miscarriage(s), no. (%)			
Yes	149 (30.4)	130 (25.0)	
No	341 (69.6)	389 (75.0)	0.057
Age (years), mean (SD)	59.7 (10.3)	58.6 (10.7)	0.089
Age (years) at menopause, mean (SD)	44.5 (8.3)	44.9 (7.4)	0.392
Pack-years smoked, mean (SD)	43.2 (27.7)	34.9 (24.5)	<0.001
Number of miscarriages, mean (SD)	1.9 (1.4)	1.7 (1.4)	0.281
Body mass index, mean (SD)	25.5 (5.4)	28.1 (6.2)	<0.001
Bleomycin sensitivity, no., mean breaks/cell (SD)	345, 0.778 (0.44)	333, 0.634 (0.32)	<0.001
BPDF[b] sensitivity, no., mean breaks/cell (SD)	250, 0.707 (0.36)	256, 0.653 (0.41)	0.115

[a] *P*s were derived from the χ^2 test for categorical variables and Student's *t* test for continuous variables. All *P*s are two-sided.
[b] BPDE, benzo(*a*)pyrene-*r*-7,*t*-8-dihydrodiol-*t*-9,10-epoxide.

(95% CI, 0.54–1.02), 0.38 (95% CI, 0.07–2.12), and 0.38 (95% CI, 0.18–0.79), respectively. Histology information on the tumor types was available for 80% of the lung cancer patients. HRT use was associated with a reduction in the risk of non-small cell lung cancer (OR, 0.69; 95% CI, 0.50–0.96), but not small cell lung cancer (OR, 1.55; 95% CI, 0.69–3.45).

When the HRT data were analyzed by smoking status (Table 3), HRT use was associated with a statistically significantly reduced risk of lung cancer in current smokers (OR, 0.59; 95% CI, 0.38–0.92), but the risk estimates were not statistically significant in never- (OR, 0.72; 95% CI, 0.37–1.40) or former smokers (OR, 0.73; 95% CI, 0.46–1.15). In both current and former smokers, the apparent benefits of HRT use diminished with increased cigarette pack-years. Among current smokers, HRT use was associated with a 60% (OR, 0.40; 95% CI, 0.12–1.35) reduction in lung cancer risk among the lightest smokers (<22.0 pack-years), compared with 0.49 (95% CI, 0.23–1.06) for moderate current smokers (≥22.2 to <42.0 pack-years) and 0.89 (95% CI, 0.46–1.76) for the heaviest current smokers (≥42.0 pack-years; *P for trend* = 0.696). This trend was similar for the former smokers with no benefit evident in former smokers with the heaviest pack-year history.

There was also an association between the age at the onset of lung cancer and HRT use (Table 4). Specifically, there was no apparent beneficial affect of HRT use in the youngest patients (<50 years; OR, 1.02; 95% CI, 0.41–2.49), which is probably explained by the fact that only a small proportion of these women were taking HRT. There was, however, a protective effect seen in the oldest women (≥70 years; OR, 0.46; 95% CI, 0.23–0.94), with a lesser effect in the two intermediate age groups. In addition, the apparent benefit of HRT was somewhat attenuated as the BMI increased. Specifically, for normal and underweight women, HRT use was statistically significantly protective (OR, 53; 95% CI, 0.34–0.82), whereas the protective effects were attenuated in the overweight women (OR, 0.78; 95% CI, 0.48–1.26) and obese women (OR, 0.81; 95% CI, 0.45–1.44).

Bleomycin sensitivity data were available for 64% (n = 333) of the control subjects and 69% (n = 345) of the case patients; BPDE sensitivity data were available for 49% (n = 256) of the control subjects and 50% (n = 250) of the case patients (Table 5). When the data were dichotomized at the 75th percentile in control subjects, mutagen sensitivity (defined as the breaks per cell above the cut point) was associated with an OR of 1.86 (95% CI, 1.32–2.64) for bleomycin, and BPDE sensitivity was associated with an OR of 1.54 (95% CI, 1.01–2.35). In a stratified analysis in which women who were not mutagen sensitive and had a history of HRT use served as the referent group, bleomycin sensitivity together with a history of HRT use was associated with an OR of 1.31 (95% CI, 0.80–

Table 2 HRT[a] use and lung cancer risk

Characteristic	No. of case patients	No. of controls subjects	Univariate OR (95% CI)	Multivariate model adjusting for lung cancer confounding variables OR (95% CI)	Multivariate model adjusting for hormonal status and lung cancer confounding variables OR (95% CI)
Overall					
HRT use	232	273	0.78 (0.61–1.01)	0.74 (0.58–0.96)[b]	0.66 (0.51–0.89)[c]
No HRT use	267	246			
Therapy type					
No HRT use	267	246	Referent	Referent	Referent
Estrogen use only	156	184	0.78 (0.59–1.04)	0.75 (0.56–0.99)[b]	0.65 (0.47–0.89)[c]
Combined therapy	60	78	0.71 (0.48–1.05)	0.68 (0.46–1.01)[b]	0.61 (0.40–0.92)[c]
Ethnicity					
Caucasian					
HRT use	206	209	0.79 (0.59–1.06)	0.82 (0.62–1.09)[d]	0.74 (0.54–1.02)[e]
No HRT use	201	161			
Hispanic					
HRT use	6	12	0.64 (0.16–2.31)	0.62 (0.14–2.70)[d]	0.38 (0.07–2.12)[e]
No HRT use	18	23			
African-American					
HRT use	20	52	0.49 (0.25–0.98)	0.43 (0.22–0.85)[d]	0.38 (0.18–0.79)[e]
No HRT use	48	62			
Histology					
Non-small cell carcinoma[f]					
HRT use	137	273	0.82 (0.61–1.11)	0.78 (0.57–1.05)[b]	0.69 (0.50–0.96)[c]
No HRT use	150	246			
Small cell carcinoma					
HRT use	23	273	1.30 (0.64–2.69)	1.27 (0.62–2.63)[b]	1.55 (0.69–3.45)[c]
No HRT use	16	246			

[a] HRT, hormone replacement therapy; OR, odds ratio; CI, confidence interval; BMI, body mass index.
[b] Adjusted by age, ethnicity, smoking status, and education.
[c] Adjusted by age, ethnicity, smoking status, education, BMI, and menopausal status.
[d] Adjusted by age, smoking status, and education.
[e] Adjusted by age, smoking status, education, BMI, and menopausal status.
[f] Includes adenocarcinoma, squamous cell carcinoma, and large cell carcinoma.

2.14). In contrast, the risk was nearly 4-fold (OR, 3.98; 95% CI, 2.23–7.12) for women not using HRT who exhibited the sensitive phenotype. This pattern was less evident for BPDE sensitivity and HRT use.

Data for plasma levels of IGF-I (ng/ml) were available for 84 cases and 103 controls (Table 6). Overall, IGF-I levels were higher for the cases (mean IGF-I = 158.5 ± 75.5 SD) than the controls (mean IGF-I = 124.2 ± 55.3 SD; $P < 0.001$). Among the cases, the IGF-I levels were significantly lower for HRT users (mean IGF-I = 140.4 ± 69.0 SD) compared with women not using HRT (mean IGF-I = 175.0 ± 78.1 SD; $P = 0.035$). A similar pattern was evident among the controls for users (mean IGF-I = 104.5 ± 92.9 SD) compared with nonusers (mean IGF-I = 146.7 ± 59.8 SD; $P < 0.001$).

The Kaplan-Meier survivor functions stratified by HRT users and nonusers were plotted for months of follow-up or months to death for 454 lung cancer cases with complete follow-up information (Fig. 1). There was a borderline statistically significant difference in survival ($P = 0.063$) between HRT users and non-users. Overall, there was no difference in the mean follow-up time between HRT users (months = 18.9 ± 15.7 SD) and non-HRT users months (22.4 ± 23.3 SD; $P = 0.062$). The multivariate Cox proportional hazard model revealed a 0.86 relative risk of death for HRT use (HR = 0.87; 95% CI, 0.67–1.13) after adjusting for age, smoking status, ethnicity, and clinical stage (data not shown).

DISCUSSION

In this secondary analysis of data from an ongoing molecular epidemiology case-control study of lung cancer assessing the relationship between self-reported HRT use and lung cancer risk, the main finding was that HRT use was associated with a statistically significantly reduced risk of lung cancer. There was no discernible difference in the risk estimates between ERT use and combination (estrogen and progestin) HRT use. Although there were a limited number of Hispanic and African-American women, decreases in lung cancer risk were evident for all three of the ethnic groups (Table 2). To increase the statistical power, we combined the data from Caucasian, Hispanic, and African-American women for all of the subsequent analyses. Although we provide the results from the two different sets of multivariate logistic regression models, for clarity and brevity we will discuss primarily the findings from the models in which statistical adjustments were made for hormonal status and lung cancer confounding variables.

To date, no clinical trial has been conducted to assess the effects of HRT use on lung cancer risk, so the published data on the association of HRT use and lung cancer risk have all been derived from secondary data or exploratory analyses (23–29) including the results for the present study. In support of our findings, previous studies (27–29) have shown beneficial effects of HRT use for lung cancer risk. For example, Kreuzer *et al.*

Table 3 HRT[a] use, cigarette smoking and lung cancer risk

HRT use by smoking status and pack-years	No. of case patients	No. of controls subjects	Univariate OR (95% CI)	Multivariate model adjusting for lung cancer confounding variables OR (95% CI)[b]	Multivariate model adjusting for hormonal status and lung cancer confounding variables OR (95% CI)[c]
Never smokers					
HRT use	40	53	1.23 (0.68–2.21)	1.11 (0.62–2.01)	0.72 (0.37–1.40)
No HRT use	46	75			
Former smokers					
HRT use	95	116	0.83 (0.55–1.25)	0.85 (0.56–1.29)	0.73 (0.46–1.15)
No HRT use	92	91			
Pack-years smoking					
<22.0					
HRT use	33	36	0.79 (0.38–1.63)	0.68 (0.33–1.39)	0.49 (0.21–1.11)
No HRT use	36	31			
≥22.0 to <37.6					
HRT use	21	43	0.73 (0.31–1.75)	0.80 (0.34–1.88)	0.67 (0.25–1.79)
No HRT use	18	27			
≥37.6					
HRT use	43	37	1.00 (0.51–2.01)	1.28 (0.64–2.59)	1.13 (0.54–2.37)
No HRT use	38	33			
			P for trend = 0.194	*P for trend* = 0.473	*P for trend* = 0.795
Current smokers					
HRT use	95	104	0.57 (0.37–0.86)	0.58 (0.39–0.88)	0.59 (0.38–0.92)
No HRT use	129	80			
Pack-years smoking					
<22.0					
HRT use	10	32	0.51 (0.18–1.43)	0.49 (0.17–1.37)	0.40 (0.12–1.35)
No HRT use	17	28			
≥22.0 to <42.0					
HRT use	39	37	0.49 (0.23–1.03)	0.47 (0.23–0.97)	0.49 (0.23–1.06)
No HRT use	45	21			
≥42.0					
HRT use	46	35	0.61 (0.31–1.17)	0.77 (0.40–1.49)	0.89 (0.46–1.76)
No HRT use	67	31			
			P for trend = 0.113	*P for trend* = 0.641	*P for trend* = 0.696

[a] HRT, hormone replacement therapy; OR, odds ratio; CI, confidence interval.
[b] Adjusted by age, ethnicity, and education.
[c] Adjusted by age, ethnicity, education, body mass index, and menopausal status.

(28) observed HRT use to be associated with a reduction in lung cancer risk (OR, 0.83; 95% CI, 0.64–1.09), which was more evident (OR, 0.59; 95% CI, 0.37–0.93) in long-term users (≥7 years) compared with nonusers.

The potential of estrogens to initiate and promote tumor growth in female reproductive organs by interacting with estrogen receptors, which are also present in both normal (40) and malignant lung tissue (40–42), is well established (41, 43, 44). However, their role in lung cancer tumorigenesis is unclear. One possible explanation for the reduction in lung cancer risk may be the ability of estrogen receptors to bind various substrates other than estrogen (45, 46), including the carcinogenic polycyclic aromatic hydrocarbons from cigarette smoke. In particular, in women who received HRT, the exogenous hormones may more readily bind to the estrogen receptors than the polycyclic aromatic hydrocarbons, thereby limiting the carcinogenic potential of the polycyclic aromatic hydrocarbons in lung tissue, which are substrates for specific cytochrome P450s, such as CYP1B1 (47). By binding instead to the estrogen receptors, estrogen inhibits the activation of polycyclic aromatic hydrocarbons. This might explain why the protective effect was strongest in current smokers in our study.

In addition, as the cigarette pack-years increased among ever smokers, the protective effect diminished, so that light smokers appeared to benefit the most from HRT use. This may be explained by the fact that cigarette smoking alters the metabolism of estrogen and tends to suppress estrogen levels (48). Although HRT use is a risk factor for endometrial cancer, this antiestrogenic effect of smoking (49, 50) has been associated with an ~50% reduction in the risk of endometrial cancer (51–55). This antiestrogenic effect may be due to the decreased ovarian production of estrogens or altered estrogen metabolism (55, 56). In support of our findings, Olsson *et al.* (29) found that among women who had ever used HRT, smokers had lower smoking-associated cancer risks, and for lung cancer, HRT use lowered the risk for smokers to 1.11 (95% CI, 0.45–2.29) from 1.68 (95% CI, 1.21–2.28) for nonusers.

HRT use provided the greatest benefit for women who were of normal weight or underweight (BMI <25; Table 4), and was of less benefit in overweight (BMI ≥25 to <30) and obese women (BMI ≥30). Increased body weight has been associated with increased death rates for many cancer types (57), and it has been proposed that obesity increases the risk of certain cancers as the result of the overproduction of endogenous estrogens

Table 4 HRT[a] use and lung cancer risk according to age and BMI

Characteristic	No. of case patients	No. of controls subjects	Univariate OR (95% CI)	Multivariate model adjusting for lung cancer confounding variables OR (95% CI)[b]	Multivariate model adjusting for hormonal status and lung cancer confounding variables OR (95% CI)[c]
Current age					
<50					
HRT use	19	21	1.26 (0.59–2.70)	1.34 (0.63–2.85)[b]	1.02 (0.41–2.49)[c]
No HRT use	61	85			
≥50 to <60					
HRT use	100	107	0.79 (0.48–1.32)	0.71 (0.43–1.17)[b]	0.69 (0.40–1.18)[c]
No HRT use	53	45			
≥60 to <70					
HRT use	91	112	0.61 (0.39–0.95)	0.65 (0.42–1.00)[b]	0.59 (0.37–0.93)[c]
No HRT use	90	68			
≥70					
HRT use	22	33	0.51 (0.25–1.03)	0.50 (0.25–0.99)[b]	0.46 (0.23–0.94)[c]
No HRT use	63	48			
			P for trend = 0.752	*P for trend* = 0.779	*P for trend* = 0.585
BMI (kg/m^2)					
<25					
(Underweight and normal)					
HRT use	121	100	0.66 (0.45–0.99)	0.69 (0.46–1.02)[d]	0.53 (0.34–0.82)[e]
No HRT use	138	75			
≥25 to <30					
(overweight)					
HRT use	72	98	0.82 (0.52–1.30)	0.78 (0.49–1.23)[d]	0.78 (0.48–1.26)[e]
No HRT use	75	84			
≥30					
(obese)					
HRT use	39	75	0.84 (0.48–1.45)	0.75 (0.43–1.31)[d]	0.81 (0.45–1.44)[e]
No HRT use	54	87			
			P for trend = 0.319	*P for trend* = 0.086	*P for trend* = 0.699

[a] HRT, hormone replacement therapy; BMI, body mass index.
[b] Adjusted by ethnicity, smoking status, and education.
[c] Adjusted by ethnicity, smoking status, education, BMI, and menopausal status.
[d] Adjusted by age, ethnicity, smoking status, and education.
[e] Adjusted by age, ethnicity, smoking status, education, and menopausal status.

(58). However, the issue of whether obesity is associated with lung cancer remains unresolved because an inverse association with BMI and lung cancer risk has been seen (59, 60), but obesity has also been shown to be associated with lung cancer (61). If, indeed, HRT use and leanness are independent factors that are inversely associated lung cancer, it would be expected that normal weight or underweight women may have the greatest benefit from HRT use. The problem of confounding by smoking on both BMI and lung cancer risk may obscure the true relation between BMI and lung cancer risk (61). Furthermore, cigarette smoking and genetic predisposition also contribute synergistically to affect obesity on cancer risk (62), which suggests competing roles by several risk factors in the development of lung cancer.

Older women also appeared to benefit the most from HRT use, but this may have to do with the duration of use, because older women may conceivably have used HRT for a longer time (Table 4). However, this is speculative, because we did not directly record information on the duration of HRT use but only inferred the duration (in years) by calculating the difference between the current age of the subject and age at menopause. We did, however, note that women (68 cases and 90 controls) with the longest duration of use (≥21 years) showed the greatest decrease in risk (OR, 0.55; 95% CI, 0.37–0.82; data not shown).

HRT use is also associated with a reduction in colon cancer risk (4, 14, 21, 22), and studies have suggested that exogenous estrogens reduce the risk either through direct effects on colonic mucosa (63, 64) or through decreases in the production of IGF-I (30, 65, 66). IGF-I is a peptide hormone with mitogenic activity, and is involved in the regulation of proliferation and differentiation of many normal and cancer cell types (67). We have reported previously that IGF-I-levels were significantly higher in cases than controls (38). However, a recent nested case-control study suggested no consistent patterns between IGF-I level and risk of lung cancer (68). Conversely, laboratory (69–72) and epidemiological studies (38, 73–75) have suggested that IGF-I is a potent mitogen for a variety of cancers such as breast, prostate, colon, liver, and lung. In our study, plasma levels of IGF-I in both the cases and controls were significantly lower for HRT users compared with non-HRT users (Table 6). Therefore, if a high level of IGF-I is a putative risk factor for lung cancer, HRT use appears to lower IGF-I levels, thereby providing another possible biological mechanism to explain our findings. Furthermore, a low level of IGF-I was associated with a slightly

Table 5 Mutagen sensitivity, HRT[a] use, and lung cancer risk

	Mutagen sensitivity	No. of case patients	No. of controls subjects	Univariate OR (95% CI)	Multivariate model adjusting for lung cancer confounding variables OR (95% CI)[b]	Multivariate model adjusting for hormonal status and lung cancer confounding variables OR (95% CI)[c]
	Bleomycin sensitive Overall					
	Yes	137	86	1.89 (1.34–2.66)	1.97 (1.40–2.76)	1.86 (1.32–2.64)
	No	208	247			
HRT use	No	107	132	1.0	1.0	1.0
No HRT use	No	101	115	1.08 (0.74–1.59)	1.14 (0.77–1.67)	1.25 (0.83–1.89)
HRT use	Yes	56	55	1.26 (0.78–2.02)	1.39 (0.86–2.24)	1.31 (0.80–2.14)
No HRT use	Yes	81	31	3.22 (1.93–5.43)	3.99 (2.35–6.81)	3.98 (2.23–7.12)
	BPDE sensitive Overall					
	Yes	75	59	1.43 (0.94–2.17)	1.59 (1.06–2.42)	1.54 (1.01–2.35)
	No	175	197			
HRT use	No	90	115	1.0	1.0	1.0
No HRT use	No	85	82	1.32 (0.86–3.04)	1.28 (0.84–1.94)	1.34 (0.85–2.12)
HRT use	Yes	34	25	1.74 (0.93–3.27)	1.87 (1.03–3.42)	1.82 (0.98–3.38)
No HRT use	Yes	41	34	1.54 (0.87–2.72)	1.73 (0.99–3.02)	1.84 (1.01–3.36)

[a] HRT, hormone replacement therapy; OR, odds ratio; CI, confidence interval; BPDE, benzo(*a*)pyrene-*r*-7,*t*-8-dihydrodiol-*t*-9,10-epoxide.
[b] Adjusted by age, smoking status, ethnicity, and education.
[c] Adjusted by age, smoking status, ethnicity, education, body mass index, and menopausal status.

better survival time among the women who died [18.7 months (±22.9 SD) for those with low IGF-I *versus* 15.8 months (±13.1 SD) for high IGF-I; $P = 0.570$], whereas the women who were still alive at the end of follow-up exhibited a lower mean IGF-I level at baseline compared with those who died [mean IGF-I was 149.4 (±70.6 SD) *versus* 162.5 (±79.7 SD); $P = 0.528$; data not shown].

Estrogens have also been shown to increase the expression of vitamin D receptors in the colon and to reduce estrogen-receptor gene methylation *in vitro*, which is equivalent to gene silencing and may also inhibit cell proliferation in colorectal carcinogenesis (76). Smirnoff *et al.* (76) have also suggested that estrogens protect against neoplastic transformation in the colon by interfering with CpG DNA methylation in the colonic mucosa, which prevents silencing of the vitamin D receptor gene. Clues to the action of HRT in reducing cancer risk have also come from animal studies. In particular, studies in nude mice showed that estradiol increases *p53* gene expression and decreases *bcl-2* gene expression, which reduced the tumor-doubling time in human endometrial adenocarcinoma cells grown in the mice (77, 78). Such increased p53 expression would prevent cells from entering S phase, allowing for DNA repair to occur and preventing the replication of damaged cells. To date, it is not clear how any of these biological mechanisms influence lung cancer tumorigenesis.

Table 6 Levels of insulin-like growth factor-I (IGF-I) among cases and controls

	No., Mean IGF-I ng/ml (SD)		
	Case patients	Control subjects	*P*
Overall	*84*, 158.5 (75.5)	*103*, 124.2 (55.3)	<0.001
HRT use	*44*, 140.4 (69.0)[a]	*55*, 104.5 (92.9)[b]	0.003
No HRT use	*40*, 175.0 (78.1)[a]	*48*, 146.7 (59.8)[b]	0.053

[a] $P = 0.035$ derived from Student's *t* test comparing the two mean values for case patients only.
[b] $P < 0.001$ derived from Student's *t* test comparing the two mean values for control subjects only.

Blackman *et al.* (26) speculated that HRT would have a more pronounced effect on tumors that possess estrogen receptors or estrogen binding sites (79–82), such as the non-small cell types, which are shown to be sensitive to estrogen. An additional consideration is that adenocarcinoma in women appears to be less associated with smoking than other types of lung cancer (83) and, therefore, could be influenced by non-tobacco-related risk factors. Hence, it is plausible that HRT could influence the risk of adenocarcinoma. Indeed, when our data were stratified by tumor histology (Table 2), HRT use was associated with a statistically significant reduced risk for non-small cell carcinoma (OR, 0.69; 95% CI, 0.50–0.96), but not for small cell carcinoma (OR, 1.55; 95% CI, 0.69–3.45). The non-small cell carcinomas in our analysis included adenocarcinoma ($n = 190$), squamous cell carcinoma ($n = 81$), and large cell carcinoma ($n = 16$). Furthermore, HRT use exhibited protective effects for each tumor stage stratum (data not shown).

Because we have shown mutagen sensitivity as a significant risk factor for lung cancer (31–34), we also explored the joint effects of this phenotype and HRT use for lung cancer risk in a subset of these women for whom laboratory data were available. A diverse array of DNA repair mechanisms from which we can indirectly infer DNA repair capacity are tested in this assay. We examined mutagen sensitivity using two independent mutagen challenges (BPDE and bleomycin) that activate different DNA repair pathways. BPDE, a metabolite of the tobacco smoke procarcinogen benzo(*a*)pyrene, induces DNA

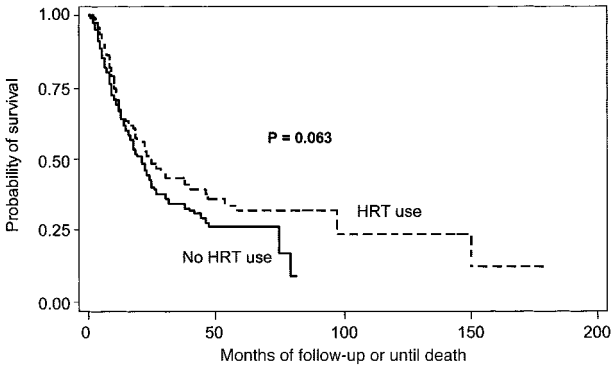

Fig. 1 Kaplan-Meier survival estimates for the lung cancer cases by hormone replacement therapy use.

adducts that require nucleotide excision repair to correct. Bleomycin is radiomimetic, and induces oxidative damage and initiates the base excision and recombination repair systems. The data suggest that HRT modifies lung cancer risk for genetically susceptible women, but it is not clear whether HRT has a biological influence on mutagen sensitivity. Estrogens are classified as epigenetic and nongenotoxic on the basis of their hormone receptor-mediated effects on cell proliferation, and physiological concentrations of estrogen are essential for the maintenance of cell growth (84). Therefore, it is likely that the effects of mutagen sensitivity and estrogen represent two distinct cellular activities.

The survival analysis was performed *ad hoc*, because the main focus of this analysis was to assess HRT use for lung cancer risk. Nonetheless, our data do suggest that the benefits of HRT use include better survival and reduced risk of death, as well as an overall reduced risk for lung cancer. In support of our findings, Ettinger *et al.* (27) compared all-cause and specific-cause mortality rates in postmenopausal women who were long-term ERT users with those in age-matched nonusers. Although the overall cancer mortality was similar in the two groups (RR, 0.85; 95% CI, 0.46–1.58), ERT use was associated with a lower risk of death from lung cancer (RR, 0.22; 95% CI, 0.04–1.15).

This analysis has certain limitations that need to be considered. First, the *a priori* hypothesis of the study was to investigate molecular markers for lung cancer risk, and, therefore, detailed assessment of HRT was not considered at the design phase of the study, and the questionnaire was not developed to comprehensively quantify HRT use. We did not account for the statistical power necessary to analyze the main effects of HRT use on lung cancer risk. We also do not know if postmenopausal women reached that status as the result of surgery or natural menopause. In addition, for the surrogate measure of duration of use, we estimated the duration of HRT use among postmenopausal women by subtracting the age of the woman at menopause from her current age. Although this is a reasonable assumption, this may not be the situation for all women. Therefore, we may have a misclassification bias in terms of the surrogate exposure, but we do not know how this may differ between the lung cancer patients and the healthy controls. Furthermore, we essentially defined these women as "current users" because data on HRT use were only collected for the previous 6 months. Thus, we cannot differentiate between current and former/never users of HRT. We also did not collect data on HRT dose, or if the cases continued HRT after their lung cancer diagnosis, so conclusions about risk of death and survival are ambivalent. Case-control studies such as ours are also subject to selection bias as well as recall bias. Finally, IGF-I data were only available on a small subsample of the study population, and previous studies remain inconsistent regarding the role of IGF-I and cancer risk.

In conclusion, we found that HRT had a statistically significant protective effect on lung cancer risk. However, because of the several limitations with this study, the data should be viewed with caution and require confirmation in well-designed hypothesis-driven studies. In addition, the biological role of HRT in lung cancer remains understudied, and only extensive research can yield new insights into the mechanisms underlying a protective effect of HRT for lung cancer.

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