ORIGINAL ARTICLE

Investigation of Combination Treatment With an Aromatase Inhibitor Exemestane and Carboplatin-Based Therapy for Postmenopausal Women With Advanced NSCLC

Patricia A. Young, MD,[a,b,*] Diana C. Márquez-Garbán, MD,[a,b] Zorawar Singh Noor, MD,[a,b] Neda Moatamed, MD,[b,c] David Elashoff, PhD,[b,d] Tristan Grogan, PhD,[b,d] Tahmineh Romero, PhD,[b,d] Hironobu Sasano, MD, PhD,[e] Ryoko Saito, MD, PhD,[e] Rebecca Rausch, PhD,[b,f] Nalo Hamilton, PhD,[b,g] Steven M. Dubinett, MD,[b,h] Edward B. Garon, MD,[a,b] Richard J. Pietras, MD, PhD[a,b]

[a]*Division of Hematology-Oncology, Department of Medicine, UCLA David Geffen School of Medicine, Los Angeles, California*
[b]*Jonsson Comprehensive Cancer Center, University of California, Los Angeles, California*
[c]*Department of Pathology and Laboratory Medicine, UCLA David Geffen School of Medicine, Los Angeles, California*
[d]*Division of General Internal Medicine and Health Services Research, Department of Medicine, UCLA David Geffen School of Medicine, Los Angeles, California*
[e]*Department of Pathology, Tohoku University School of Medicine, Sendai, Japan*
[f]*Department of Neurology, UCLA David Geffen School of Medicine, Los Angeles, California*
[g]*UCLA School of Nursing, Los Angeles, California*
[h]*Division of Pulmonary and Critical Care Medicine, Department of Medicine, UCLA David Geffen School of Medicine, Los Angeles, California*

Received 21 August 2020; revised 7 January 2021; accepted 22 January 2021
Available online - 3 February 2021

ABSTRACT

Introduction: Estrogen receptors (ER) (ERα, ERβ) and aromatase (key enzyme for estrogen synthesis) are expressed in most human NSCLCs. High intratumoral estrogen levels and elevated aromatase expression in NSCLC predict poor outcome. This open-label, phase 1b, single-center study evaluated the safety and tolerability of escalating doses of the aromatase inhibitor, exemestane, in combination with carboplatin and pemetrexed in postmenopausal women with stage IV nonsquamous NSCLC.

Methods: Patients received exemestane (starting 1-wk before chemotherapy) at 25 mg orally (PO) daily (cohort 1) or 50 mg PO daily (cohort 2) combined with carboplatin (area under the curve 6 mg $\times$ min/mL) and pemetrexed (500 mg/m^2) intravenously every 3 weeks for four cycles. Thereafter, patients were eligible for continued therapy with exemestane and pemetrexed or pemetrexed alone.

Results: A total of 10 patients consented for therapy, and two patients failed in the screening. Four patients completed the therapy in cohort 1 and four patients in cohort 2. The median number of cycles administered was 15 (range: 1–54). Maximum tolerated dose was exemestane 50 mg PO daily with combination chemotherapy. Intention-to-treat analysis revealed an objective response rate (ORR) of 62.5% (five of eight patients with partial response) and a clinical benefit rate of 87.5% (seven of eight patients with

***Corresponding author.**

Drs. Young and Márquez-Garbán contributed equally to this work.

Disclosure: Dr. Dubinett reports serving as a consultant for the scientific advisory boards for EarlyDiagnostics, Johnson & Johnson Lung Cancer Initiative, LungLife AI, Inc., and T-Cure Bioscience, Inc., and receiving research funding from Johnson & Johnson. Dr. Garon reports serving as consultant for the advisory board and steering committee for Dracen, EMD Serono, GlaxoSmithKline, Merck, and Novartis and receiving research trial funding from AstraZeneca, Bristol Myers Squibb, Dynavax, Eli, EMD Serono, Genentech, Iovance, Mirati, Merck, Neon, and Novartis. Dr. Pietras as past consultant for AstraZeneca Pharmaceuticals, Genentech, and Enlibrium. The remaining authors declare no conflict of interest.

Address for Correspondence: Patricia A. Young, MD, Department of Medicine-Hematology & Oncology, University of California Los Angeles, 2020 Santa Monica Blvd, Suite 600, Santa Monica, CA 90404. E-mail: pyoung@mednet.ucla.edu

Cite this article as: Young, PA, Márquez-Garbán, DC, Noor ZS, et al. Investigation of combination treatment with an aromatase inhibitor exemestane and carboplatin-based therapy for postmenopausal women with advanced NSCLC. JTO Clin Res Rep. 2021;2:100150.

ISSN: 2666-3643

https://doi.org/10.1016/j.jtocrr.2021.100150

either stable disease or partial response). ORR was associated with aromatase expression ($p = 0.02$). Circulating estrogen levels decreased with exemestane use, and quality of life measurements did not significantly change during the treatment. There were no adverse events.

Conclusions: The combination of carboplatin, pemetrexed, and exemestane in postmenopausal women with metastatic NSCLC is safe and well tolerated. Biomarker studies revealed that ORR correlates with tumor aromatase expression. These findings support future clinical trials to confirm the antitumor efficacy with this combination therapy.

Keywords: Aromatase; Exemestane; Lung cancer; Estrogen; Estrogen receptor

Introduction

Lung cancer is the most common cause of cancer mortality in both female and male patients in the United States. NSCLC accounts for more than 80% of lung cancers at diagnosis. It is estimated that 180,000 new cases of NSCLC will be diagnosed this year in the United States and approximately 165,000 patients will succumb to NSCLC. Survival rates from advanced NSCLC are unacceptably low, and new therapeutic options are urgently needed.[1] Notably, marked increases in the incidence of lung cancer among women have now attained epidemic proportions that cannot be fully explained by sex differences in smoking behaviors.[2] Although many women affected by lung cancer are smokers, a considerable proportion are nonsmokers. There is clear evidence that tobacco smoking is a major cause of lung cancer. However, it is estimated that 15% of men and 53% of women with lung cancer *worldwide* are never smokers.[3] Etiologic factors other than tobacco may have an important role in the development of lung cancer.

Although sex steroid hormones were not previously considered to play a role in lung function,[4] many studies now provide strong evidence for the action of sex steroids not only in normal lung development and function[5] but also in the pathogenesis of lung cancer.[6-9] One of the first reports in the clinic for an association between estrogens and lung cancer risk was published as part of the Coronary Drug Project in 1973. This study assigned men who had suffered a myocardial infarction to a randomized, prospective trial in which they received either equine estrogen or placebo. It was expected that a decrease in cardiac events would be found in the estrogen arm, but instead the trial was stopped early when an increase in lung cancer mortality occurred in patients receiving estrogen.[10] More recent clinical studies confirm an increase in lung cancer mortality among postmenopausal women from use of combined estrogen–progestin as hormone replacement therapy,[11-13] including evidence for an increased incidence of lung cancer.[13] Furthermore, the increased risk of death from lung cancer observed during estrogen plus progestin use was attenuated after the discontinuation of the combined hormone therapy.[14]

Activated hormone receptors are expressed in lung cancer cells,[6-8,15] and a number of preclinical studies suggest that the development and progression of lung cancer may be promoted by estrogens in vitro and in animal models.[6-8,16] Thus, treatment with 17β-estradiol stimulates a marked increase in proliferation of diverse lung carcinoma cell lines in vitro, and exposure to 17β-estradiol also promotes progression of human lung tumor xenografts in vivo.[6,8,17-19] In addition, the actions of estrogens to stimulate NSCLC cell proliferation in vitro and lung tumor growth in vivo are inhibited by fulvestrant, a known ER antagonist and down-regulator of ERα in the breast.[8,16] Treatment of both male and female patients with advanced NSCLC with high-dose fulvestrant in combination with erlotinib in a phase 2 clinical trial was found to enhance progression-free survival (PFS) and overall survival (OS) in patients with wild-type *EGFR* tumors but not mutant *EGFR* tumors.[20] In this trial, tumor estrogen receptor (ER)α and progesterone receptor (PgR) expression was more likely to be positive in *EGFR* wild-type as compared with *EGFR* mutant groups. In contrast, a recent trial reports that addition of fulvestrant to erlotinib did not result in improved PFS or OS in postmenopausal female patients with *EGFR* mutated or wild-type *EGFR* tumors.[21] One major difference in the outcomes of these two trials may be due to the previous treatment status of the patients enrolled. In the trial by Garon et al.,[20] most patients were treatment-naive or had only one previous therapy. In contrast, all patients enrolled in the trial by Mazieres et al.[21] had previously received second, third, or more advanced lines of therapy. Hence, patients exposed to more previous antitumor treatments would have a greater likelihood of developing therapeutic resistance.

In addition to the role of estrogens in lung tumor development, high tumor aromatase level seems to correlate with poor survival. Mah et al.[22] published findings of aromatase protein expression in 422 patients with NSCLC, with results confirmed and validated on an independent patient cohort (n = 337). Lower levels of aromatase predicted improved survival in women 65 years and older, which implicates aromatase as an early stage predictor of survival in some women with

NSCLC.[22] Such findings predict that targeted treatment of women whose lung cancers have higher levels of aromatase may be good candidates for treatment with aromatase inhibitors.[7,9,22] In both male and female patients, approximately 73% of NSCLCs have higher levels of intratumoral estradiol in cancer tissues than in paired nonneoplastic lung tissues. Such results confirm that estradiol is locally produced in NSCLC by aromatase.

Furthermore, preclinical studies have revealed significant ($p < 0.001$) antitumor effects of aromatase inhibitors. NSCLC cells grown as xenografts in ovariectomized nude mice with and without the aromatase inhibitor, anastrozole, exhibited pronounced growth inhibition with aromatase inhibitor treatment as compared with controls.[9]

On the basis of relevant preclinical work and a number of clinical studies implicating estrogens in lung cancer pathogenesis,[6,7,9,10] a phase 1 investigator-initiated trial was undertaken to find the maximum tolerated dose (MTD) of exemestane when used in combination with carboplatin and pemetrexed therapy in postmenopausal women with advanced NSCLC. Standard chemotherapy for patients with metastatic or locally advanced NSCLC is a platinum-based regimen unless biomarkers for other targeted therapies are identified,[1,12,13,23] which is the basis for this combination therapy.

Materials and Methods

Patients and Samples

The study was conducted in accord with the Good Clinical Practice guidelines, applicable local regulatory requirements, and principles enunciated in the Declaration of Helsinki. The protocol and informed consent form were reviewed and approved by an Institutional Review Board at each study center before implementation. Patients provided written informed consent before enrollment. The study is registered on ClinicalTrials.gov, number NCT01664754. Key inclusion criteria included age more than 18 years, with pathologically proven advanced stage (stage IV), treatment-naive (with exception of tyrosine kinase inhibitor and immunotherapy), nonsquamous NSCLC, Eastern Cooperative Oncology Group performance status less than 1, measurable disease as defined by the Response Evaluation Criteria in Solid Tumors version 1.0, and postmenopausal status. Complete inclusion and exclusion criteria are provided in Supplementary Table 1. Postmenopausal status is defined as older than 50 years of age with no spontaneous menses for at least 12 months or 50 years of age or younger either with no spontaneous menses within 12 months of randomization (e.g., spontaneous or secondary to hysterectomy) and a follicle-stimulating hormone level within the postmenopausal range or with previous bilateral oophorectomy. Key exclusion criteria included untreated central nervous system involvement, major surgery 4 weeks before the therapy, and previous or concurrent investigational or standard therapy (with the exception of tyrosine kinase inhibitor and immunotherapy in the previous 4 wk).

Trial Design and Treatment

This was a phase 1b, open-label, single-center study (NCT01664754) that evaluated the safety and tolerability of escalating doses of exemestane in combination with carboplatin and pemetrexed in treatment-naive postmenopausal women with stage IV nonsquamous NSCLC. Patients received escalating doses of oral exemestane 1 week before starting chemotherapy with carboplatin and pemetrexed (lead-in cycle of 7 d ± 2 d before d 1 of chemotherapy with carboplatin and pemetrexed). Exemestane was administered at 25 mg orally (PO) daily (cohort 1) or 50 mg PO daily (cohort 2) combined with carboplatin (area under the curve [AUC] 6 mg × min/mL) and pemetrexed (500 mg/m^2) IV every 3 (q3) weeks for four cycles. After the four cycles, the patients were eligible for continued therapy with exemestane or pemetrexed alone (Fig. 1).

The secondary objectives of the trial were the following: (1) to find the objective tumor response rate (ORR) in the treated patients; (2) to evaluate the pharmacokinetic (PK) profile of pemetrexed, carboplatin, and exemestane; (3) to analyze tumor tissue and blood biomarkers for potential correlation with response; and (4) to assess the health-related quality of life (QOL) of individual patients during the course of the trial using the Functional Assessment of Cancer Therapy (FACT) specific to lung cancer (FACT-L) (version 4) and general health (FACT-G) instruments.[23]

Enrolled patients were treated in the dose-escalation cohorts in a standard 3 + 3 design. Dose escalation was guided by safety data from each subject during the first treatment cycle (d 1–21). Subjects who discontinued the study treatment before completing treatment cycle 1 and who did not experience a dose-limiting toxicity (DLT) were replaced in the same dose cohort.

Assessments

Tumor Response. Patients were evaluated for clinical response according to Response Evaluation Criteria in Solid Tumors version 1.1 guidelines. Computed tomography scans were obtained for tumor response (tumor measurement) at baseline and every 6 weeks (±3 d) as calculated from the first dose of the study treatment.

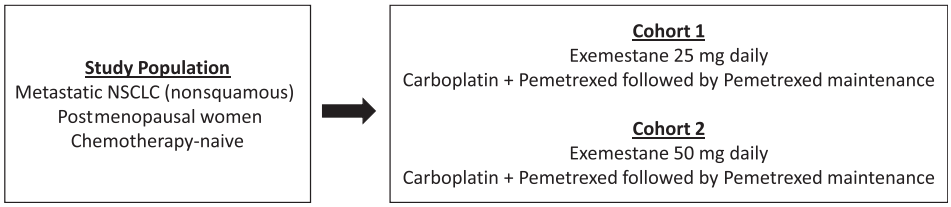


Figure 1. Study schematic.

Biomarker Assessment. Plasma and serum samples were collected for all trial participants and obtained at the start of therapy (C1D1) and on subsequent cycles C2D1 and C3D1 to evaluate the biomarkers (e.g., estrogens, androgens, sex hormone binding globulin, and albumin) related to aromatase blockade with exemestane. Assays were performed by a Clinical Laboratory Improvement Amendments-certified laboratory. Assays of the biomarkers in the tumor biopsy specimens were performed using formalin-fixed, paraffin-embedded tissues. Freshly cut sections were analyzed for expression of aromatase, ERα, and PgR using standard immunohistochemistry methods with validated antibodies and appropriate controls.[24,25]

QOL Assessments. QOL assessment questionnaires using the FACT-L (version 4) and FACT-G were recorded in several cycles of the treatment.[26,27] The FACT questionnaires asked patients to indicate, using a five-point scale, how true the statement has been for them during the past 7 days. The questionnaires were performed in the screening, on the first day of exemestane (d -7 ± 2), and on day 1 of each cycle during the combination treatments.

Evaluation of Safety and Tolerability

Toxicities were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0), with DLT assessed for each subject during the first treatment cycle (d 1–21) by the defined criteria. Toxicities expected after treatment with pemetrexed and carboplatin were not considered DLTs for purposes of exemestane dose escalation. The UCLA Jonsson Comprehensive Cancer Center Data Safety and Monitoring Board served as the Data Safety and Monitoring Board for this study.

Statistical Design and Analyses

AUC was extrapolated using linear trapezoidal methods. Safety was assessed through tabulation, grading, and attribution of severe adverse events and adverse events. The proportion of patients achieving a clinical response was estimated using the response assessment criteria in Section 2.3. Fisher's exact test was used for categorical markers to evaluate the relationship with response at specific time points. Log-rank test was used to evaluate an association between categorical markers and time to disease progression.

QOL measures were compared between response categories (analysis of variance), and the effect of time on therapy was assessed with mixed-effects models. A separate generalized linear model for repeated measurement using FACT-G and FACT-L for outcome was used for testing a trend in QOL over the cycle.

Results

Patient Characteristics

A total of 10 patients consented for therapy, and two patients failed in the screening. Four patients completed the therapy in cohort 1, and four patients were treated in cohort 2. One patient assigned to cohort 2 dosing was provided cohort 1 dosing and so was included in cohort 1 for PK and pharmacodynamic analysis. One patient in cohort 2 exited the trial for alternative therapy after only one partial treatment cycle. All participants received exemestane in combination with standard chemotherapy with pemetrexed (500 mg/m^2) and carboplatin (AUC 6), both given intravenously (IV) every 3 weeks. The median number of cycles given was 15 (range: 1–54). Patient characteristics are outlined in Table 1.

Toxicities

The reported adverse events are from all eight treated patients. There were no DLTs in any of the cohorts of combination carboplatin, pemetrexed, and exemestane. Adverse events related to exemestane were recorded as hot flashes and gastroesophageal reflux disease reported in 12.5% for both. The most common adverse events related to carboplatin pemetrexed combination were recorded as fatigue, nausea, and anemia reported in 25%, 37.5%, and 25%, respectively. Of the six patients reported with anemia, two were recorded as grade 3 and none as grade 4. Grade 3 anemia and grade 3 weakness and dizziness were related to pemetrexed and carboplatin toxicity and did not fulfill the criteria for DLTs (Supplementary Table 2). Adverse events related to exemestane and carboplatin pemetrexed combination are reported in Table 2.

Table 1. Patient Characteristics

Age	Ethnicity	Cohort	Smoking/Pack Year	EGFR	Prior TKI	Aromatase IHC	Cycles	Reason Off	Best Response	PFS (mo)	OS (mo)
66	White	1	never smoker	exon 19 del	Yes (Erlotinib)	Positive	9	Progression	PR	5.9	10.9
55	White	1	smoker/15	EGFR WT	No	Positive	29	Progression	PR	20.2	63.6
66	White	1	smoker/14	EGFR WT	No	Negative	12	Progression	SD	8.6	16.1
58	Hispanic	1	never smoker	EGFR WT	No	Negative	3	Clinical Decline	SD	3.0	6.3
60	White	2	missing	EGFR WT[a]	No	Positive	5	Clinical Decline	PR	3.9	4.5
69	White	2	smoker/9.25	EGFR WT	No	Positive	54	Progression	PR	36.6	43.4
78	Pacific Islander	2	never smoker	EGFR WT	No	Positive	10	Progression	PR	7.2	31
64	White	2	smoker/40	EGFR WT	No	Negative	0	Progression	PD	0.2	1.3

[a]confirmatory documentation is unable to be located

EGFR WT, epidermal growth factor receptor wild type; TKI, tyrosine kinase inhibitor; IHC, immunohistochemistry; PFS, Progression Free Survival; OS, Overall Survival; PR, partial response; SD, stable disease; PD, progressive disease.01592447623

Table 2. Adverse Events Related to Exemestane or Chemotherapy

Related to Exemestane

AE Description	Grade 1	Grade 2	Grade 3	Grade 4	Total (Events)	No. of Pts	% of Pts
Hot Flashes	1	0	0	0	1	1	12.5
GERD	2	0	0	0	2	1	12.5
Total (Per Grade)	3	0	0	0	3		

Related to Carboplatin and Pemetrexed Combination

AE Description	Grade 1	Grade 2	Grade 3	Grade 4	Total (Events)	No. of Pts	% of Pts
Fatigue	3	1	0	0	4	2	25
Constipation	2	0	0	0	2	1	12.5
Nausea	2	3	0	0	5	3	37.5
Decreased Appetite	1	0	0	0	1	1	12.5
Abdominal Distension	1	0	0	0	1	1	12.5
Loss of Appetite	1	0	0	0	1	1	12.5
Anemia	3	1	2	0	6	2	25
Anorexia	1	1	0	0	2	1	12.5
Weight Loss	1	1	0	0	2	1	12.5
Leukopenia	0	1	0	0	1	1	12.5
Weakness	0	0	1	0	1	1	12.5
Dizziness	0	0	1	0	1	1	12.5
Thrombocytopenia	1	0	0	0	1	1	12.5
Vomiting	1	0	0	0	1	1	12.5
GERD	1	0	0	0	1	1	12.5
Total (Per Grade)	18	8	4	0	30		

Adverse events according to grade and relation to exemestane or carboplatin with pemetrexed.

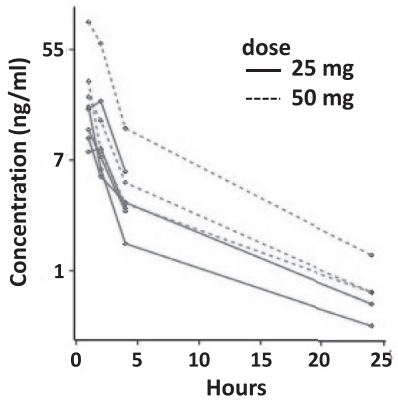
PK and Pharmacodynamic Studies

The mean of the maximum serum concentration (C_{max}) of exemestane for cohort 1 (exemestane 25 mg daily) was 14.68 ng/mL and for cohort 2 (exemestane 50 mg daily) was 48 ng/mL (Fig. 2*A*). The AUC from zero to infinity for the two cohorts was 51.73 and 184.17 ng × h/mL, respectively (Fig. 2*B*). The established MTD was exemestane 50 mg PO daily with pemetrexed (500 mg/m^2 IV q3 wk) and carboplatin (AUC 6 mg × min/mL IV q3 wk).

Clinical outcomes

No patients were removed from the study for adverse events. Clinical outcome, biomarker, and QOL correlates are presented subsequently. Data indicate that the ORR was 62.5% (e.g., five partial response out

A



B

Mean pharmacokinetic parameters for exemestane

	Exemestane (25 mg)		Exemestane (50 mg)	
	Mean	SD	Mean	SD
C_{max} (ng/mL)	14.68	5.32	48.00	36.57
*$AUC_{0\text{-}last}$ (ng × h/mL)	44.48	17.09	177.58	139.77
*$AUC_{0\text{-}inf}$ (ng × h/mL)	51.73	17.39	184.17	140.97
$T_{1/2}$ (h)	4.97	3.09	5.36	1.36

*AUC was calculated using the linear-trapezoidal method.
AUC: area under the curve

Figure 2. (*A*) Exemestane concentrations over time by cohort. Concentration is reported in natural log scale. (*B*) Mean pharmacokinetic parameters for exemestane. AUC, area under the curve; C_{max}, maximum serum concentration.

of eight treated), the clinical benefit rate was 87.5% (e.g., five partial response + two stable disease), and patients exhibited extended PFS, particularly among patients with tumors expressing aromatase enzyme (Table 1). Among the seven patients that completed at least one cycle of therapy, the ORR is 71.4% and the clinical benefit rate is 100%. The ORR was significantly associated with aromatase expression determined by immunohistochemistry using the Fisher's exact test ($p = 0.02$) (Fig. 3*F*). Different levels of aromatase expression are found in Figure 3*B–E*, as compared to a positive control in Figure 3*A*. There was no substantial correlation between ORR and either ERα or PgR, but there was a positive association between ERα and aromatase expression ($p = 0.036$) using Spearman's correlation test (data not found). Although not a primary end point of this trial, the median PFS was 5.9 months (95% confidence interval [CI]: 1.3–10.5). The median OS from initiation of study treatment and that from diagnosis was 10.8 months (95% CI: 0–24.5) and 17.8 months (95% CI: 0–35.7), respectively (Supplementary Fig. 1). Of note, OS based on survival from the time of first treatment on the trial was not significantly associated with aromatase expression ($p = 0.121$) (Table 1). However, OS based on survival from the time of diagnosis was significantly associated with aromatase expression ($p = 0.046$).

Exploratory Tissue and Blood Biomarker Analysis

Exploratory assays of selected biomarkers in tumor biopsy specimens were performed using formalin-fixed, paraffin-embedded tissues.[24,25] We find evidence of aromatase expression by immunohistochemistry assays in five of the eight patients treated on the trial. The three patients that were negative for aromatase expression were two patients with stable disease only and the single patient who completed only partial treatment on cycle 1 of combination therapy before exiting the trial to opt for an alternative therapy.

As expected, circulating estrogen levels (estradiol, estriol and estrone) decrease with exemestane use (Fig. 4*A–C*). The serum levels of androgens, sex hormone binding globulin, and albumin are found in Supplementary Table 3.

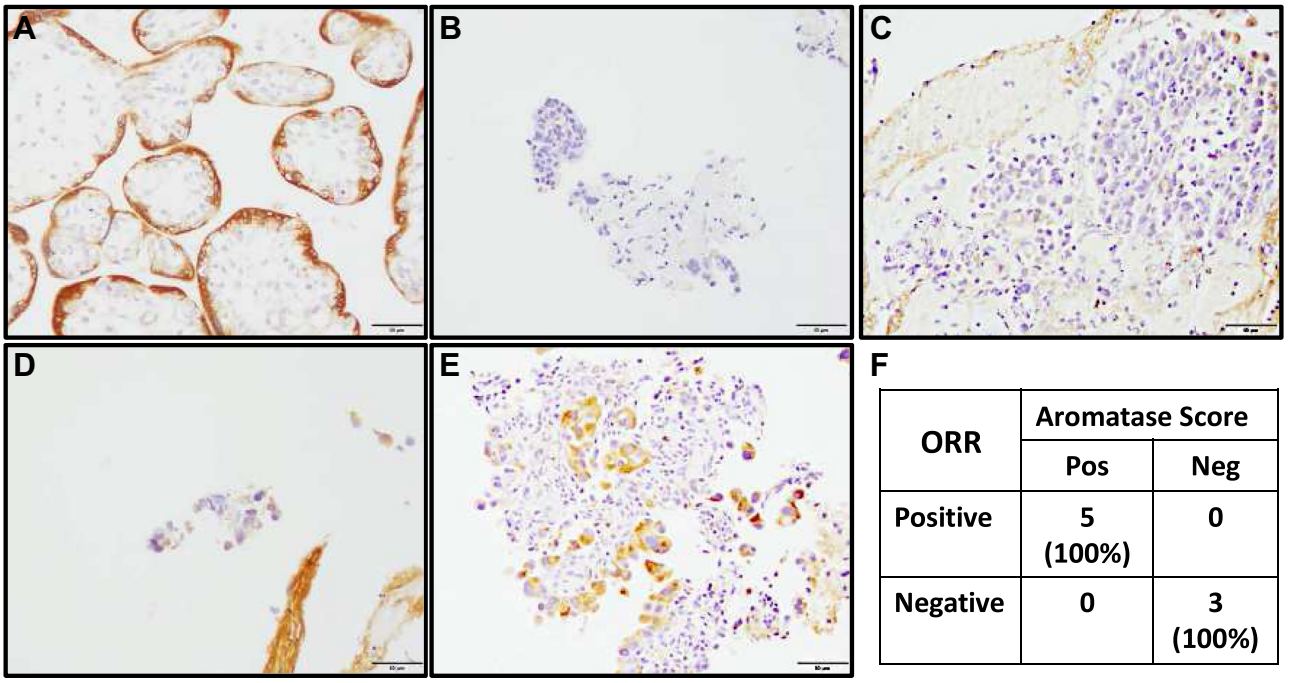
QOL Assessments

There was no significant trend over the cycles in QOL using FACT-G ($p = 0.07$) or FACT-L ($p = 0.19$) in the regression model. The scores for each assessment are found in Supplementary Figure 2.

Discussion

In this unique phase 1 clinical trial, we found that the combination of the aromatase inhibitor, exemestane, with pemetrexed and carboplatin as a first-line treatment in postmenopausal women with advanced NSCLC was very well tolerated with no unexpected toxicity. The established MTD was exemestane 50 mg PO daily with pemetrexed (500 mg/m^2 IV q3 wk) and carboplatin (AUC 6 mg × min/mL IV q3 wk), with good PK parameters. Furthermore, among the secondary objectives of the trial, the exemestane-pemetrexed-carboplatin regimen resulted in an ORR of 62.5%, a clinical benefit rate of 87.5%, and a median survival of 13.5 months. These clinical outcomes compare favorably with those reported previously for patients with NSCLC treated in phase 1 to 3 trials using carboplatin-pemetrexed doublets[28,29] and other historical trials using platinum doublet therapies.[1]

The several patients who received 15 or more cycles of maintenance therapy offer support to the observation



ORR	Aromatase Score	
	Pos	Neg
Positive	5 (100%)	0
Negative	0	3 (100%)

Figure 3. Response rates by aromatase IHC score. Aromatase expression was assessed in tumor samples from patients using antibody 677[24] and appropriate positive and negative controls. (*A*) Strong aromatase staining in trophoblast tissue (positive control), and different levels of aromatase expression in NSCLC specimens: (*B*) negative, (*C*) weak, (*D*) moderate and (*E*) strong. (*F*) Response rates to exemestane were positively correlated with aromatase expression. IHC, immunohistochemistry; ORR, objective response rate.

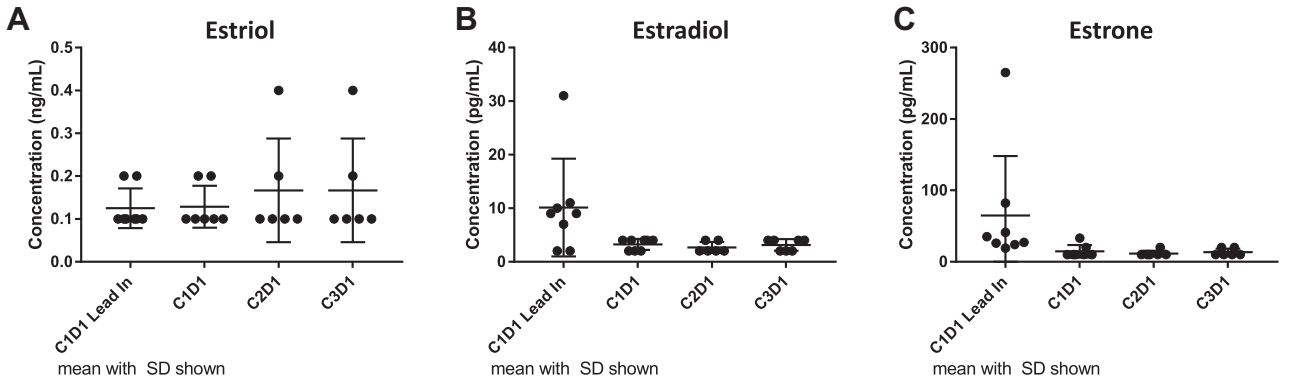


Figure 4. Circulating estrogen levels. (*A*) Estriol, (*B*) estradiol, and (*C*) estrone levels over the cycle with use of exemestane. Mean with SD is found.

that this was a well-tolerated regimen. No patient was removed from the study for adverse events. Hematologic side effects of the exemestane-carboplatin-pemetrexed treatment compared favorably with historical controls managed with pemetrexed-carboplatin.[28,29] No grade 3-4 neutropenia or grade 3-4 thrombocytopenia was noted, and two patients had grade 3 anemia. Likewise, only 25% of the patients in this study had grade 3-4 nonhematologic toxicity (weakness and dizziness) compared with a partial list of toxicities from other platinum doublet studies, such as 6% in carboplatin-pemetrexed trials,[29] 14% and 27% grade 3-4 nausea and vomiting in the carboplatin-gemcitabine and cisplatin-gemcitabine trials, respectively, 24% grade 3-4 asthenia in carboplatin-docetaxel trials, and 23% grade 3 anorexia, 21% fatigue, and 2% neuropathy with carboplatin-paclitaxel trials.[29,30] It is especially notable that neuropathy, a side effect that can continue beyond completion of the therapy, and alopecia, a side effect that often causes substantial emotional distress, were mild, transient, and not cumulative in the exemestane-carboplatin-pemetrexed trial.

Results from trials that include assessment of symptoms and QOL as end points may provide meaningful information in evaluating cancer treatment benefits.[27] The FACT-G is an indicator of patient-related QOL,[27] and the FACT-L is a validated, disease-specific QOL instrument that correlates QOL changes with clinical outcomes in patients with NSCLC.[26] Thus, QOL assessments using FACT-G and FACT-L instruments were recorded over the several cycles of exemestane-carboplatin-pemetrexed therapy, and the cumulative scores of the patients on this trial were stable over the course of the therapy.

Importantly, the ORR in this trial associated with the level of NSCLC aromatase expression as revealed by immunohistochemistry in specimens obtained at diagnosis. As expected, circulating estrogen levels decreased over time in patients treated with exemestane therapy. Of significance to the present trial, biosynthesis of estrogen can occur from aromatization of ovarian and adrenal androgens, and such peripheral aromatization is reported to also occur in lung cancer.[7,9,31] Aromatase is a cytochrome P450 enzyme found in various tissues that directs the conversion of androstenedione and testosterone to estrone and 17β-estradiol, respectively. The aromatase enzyme is expressed in most lung cancer tissues studied, and its expression is found to be considerably higher in metastatic cells compared with primary cancer cells.[22,32] Recent in situ experiments have revealed that estrogens are synthesized locally in clinical lung cancer specimens by the action of aromatase, thus suggesting a potential role of sex steroids in the development of lung carcinoma. The activity of aromatase has also been reported in tumor tissues obtained at surgery from both male and female patients with lung cancer, and this enzyme activity is associated with high intratumoral concentrations of estrogens in lung cancers from the patients with NSCLC.[7,9,31] Weinberg et al.[9] reported aromatase to be predominantly found in the cytoplasm of epithelial cells in NSCLC tissues, with minimal staining in stromal and interstitial tissues. Using immunostaining to detect aromatase in a tumor microarray, Mah et al.[22] confirmed this finding. Similarly, studies using laser-capture microdissection followed by reverse-transcription PCR analysis revealed aromatase in NSCLC tissues from patients to be mostly in the epithelium of tumors, with confirmation by cytoplasmic staining of aromatase using immunohistochemistry.[31] It is notable that tobacco carcinogens elicit marked increments in the intratumoral levels of estrogens, suggesting estrogens may also play a role in tobacco carcinogen-induced lung cancer progression.[33]

The tumor levels of aromatase generally reveal correlation with ER expression and tumor stage.[32,34] It is important to note that the lower levels of aromatase in the lung tumors of patients with NSCLC are associated with a better prognosis for long-term survival, particularly in postmenopausal women, as in the current trial, suggesting an aromatase assay may ultimately be developed as a prognostic tool in lung cancer management.[32] Reverse transcriptase-polymerase chain reaction studies of *ESR1* and *CYP19A1* (aromatase) expression in NSCLC specimens confirm the importance of aromatase in lung cancer prognosis.[35] In vitro, aromatase inhibitors, such as anastrozole and exemestane, are found to decrease tumor cell growth in lung tumor xenografts implanted in nude mice,[9] a finding confirmed by others.[7,22,36] In this trial, we found that aromatase expression was not positively correlated with OS defined from the time of the first treatment but was associated with OS defined from the time of NSCLC diagnosis. Although OS was not a primary end point in this trial, these findings, coupled with independent work, suggest that high tumor aromatase levels and, consequently, high intratumoral estrogen levels in NSCLC may offer a unique opportunity to intervene in tumor progression by targeted inhibition of aromatase to promote patient survival.

On the basis of extensive clinicopathologic studies of tumor biomarkers, Tanaka et al.[37] report that *EGFR* wild-type lung adenocarcinoma is an estrogen-dependent carcinoma, and aromatase expression and ERβ expression are potent prognostic markers for *EGFR* wild-type lung adenocarcinoma, with high aromatase expression significantly ($p = 0.019$) correlated with short survival in women. Because female patients with breast cancer receive antiestrogens, such as tamoxifen or aromatase inhibitors, as part of their clinical treatment, a retrospective study evaluated the incidence and mortality risk of lung cancer among patients with breast cancer managed in several years with or without antiestrogen treatment.[14] Notably, the incidence of lung cancer was found to be lower in women treated with antiestrogens compared with that of women who did not receive antiestrogens, but this value did not reach statistical significance. Nonetheless, lung cancer mortality was significantly ($p < 0.001$) reduced in patients with breast cancer who received antiestrogen therapy.[38] Independent reports confirm that antiestrogen use in patients with breast cancer reduces the risk of subsequent lung cancer[39]; and a long-term follow-up of postmenopausal patients with early stage breast cancer who were randomized to 5 years of adjuvant tamoxifen compared with 2 years of treatment revealed a lower incidence of lung cancer up to 10 years after treatment stopped.[40] In addition, patients with breast cancer who were treated with exemestane after 2 to 3 years of tamoxifen therapy were reported to have reduced incidence of primary lung cancer compared with those who continued using

only tamoxifen.[41] Collectively, these preclinical and clinical findings establish a good argument for the biological role of steroid hormones and their receptors in lung cancer progression.

There was no substancial correlation between ORR and either ERα or PgR expression by immunohistochemistry in this trial. The expression and potential role of steroid hormone receptors in clinical outcome in lung tumors are reviewed elsewhere.[42] In studies highlighted by Miki et al.,[42] investigators applied different criteria to define ERα or ERβ expression in NSCLC cases. For example, results of different studies revealed a high detection (>50%) of ERα-positive cases in NSCLC,[7,42] whereas other discordant studies reported no or low detection (<10%) of ERα-positive cases in NSCLC.[42] Differences in the above-mentioned findings of lung carcinoma cases could well be owing to the use of different anti-ERα antibodies, divergent assay protocols, or small sample sizes. Of note, the relative abundance and immunointensity of ERα were found to be lower in NSCLC compared with those of breast carcinoma, whereas those of the nonclassical ERβ were found to be generally higher in NSCLC.[7] Further investigation is needed to establish standardized guidelines for performance of immunohistochemistry methods in NSCLC tissues particularly for ERβ to obtain consistent and reliable data.[16,42] Using alternate methods to assess ER in lung cancers, microarray data from lung tumor cells extracted by laser-capture microdissection reveal that ERβ expression is associated with alteration of greater than 500 genes, whereas ERα expression is correlated with changes in the activity of less than 20 genes, suggesting a more prominent role for ERβ in the lungs.[43]

Activation of estrogen signaling pathways promotes both tumor cell proliferation and tumor survival in NSCLC. Recent studies have revealed that estrogen plays a key role in suppressing apoptosis in the lungs.[6,17,18,44] Such activity of estrogens can be important in promoting lung cancer progression and possibly in interference with antitumor efficacy of chemotherapeutic agents typically used to treat lung cancer. Chemotherapeutics such as platinum-based and taxane-based agents can induce apoptosis of cancer cells. However, recent findings suggest that this action may be suppressed by estradiol, which acts as a tumor survival factor[45] and a promoter of tumor immune tolerance.[46–48] An independent report based on retrospective clinicopathologic studies offers evidence that ERα is an independent prognostic factor in advanced NSCLC and might also be a predictive factor for response to pemetrexed-carboplatin therapy in women.[49] Hence, combination therapy with exemestane and either cisplatin or carboplatin (standard chemotherapeutic agents in NSCLC) results in markedly increased antitumor activity in lung cancer xenograft studies in vivo.[36,45] Aromatase inhibitors such as anastrozole, letrozole, and exemestane were found to have significant ($p < 0.001$) antitumor effects in NSCLC.[7,9,31] The current phase 1 trial to assess the safety and tolerability of exemestane in combination with a platinum-based chemotherapy regimen presents evidence in support of antitumor efficacy of aromatase inhibitors when combined with standard chemotherapy. Since the initiation of this trial, the standard of care has changed, with pembrolizumab often added to carboplatin and pemetrexed.[50] Future studies should evaluate exemestane along with this regimen, including pembrolizumab, because there is emerging evidence that antiestrogens combined with immunotherapy may have synergistic effects in some cancers.[47] A trial of exemestane in postmenopausal women with NSCLC who have failed immunotherapy seeks to evaluate whether exemestane provides synergy to recent immunotherapy use (NCT02666105). These phase 1 results support future trials to better establish clinical efficacy with combination chemotherapy and immunotherapy.

Acknowledgments

This work is dedicated to the memory of our distinguished colleague and friend Dr. Lee Goodglick (1960–2014) who made major contributions in cancer research during his professional career, particularly in addressing the epidemic of lung cancer among women. This work was supported by funding from the Gateway for Cancer Research and in part by the National Institutes of Health grant numbers P50 CA090440, CTSI UL1 TR 001881, and U54 CA143930, UCLA Jonsson Comprehensive Cancer Center, Stiles Program in Oncology, Hickey Family Foundation, Iris Cantor-UCLA Women's Health Center, and the National Lung Cancer Partnership. Pfizer provided exemestane for use in this trial.

Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at https://doi.org/10.1016/j.jtocrr.2021.100150.

References

1. Schiller JH, Harrington D, Belani CP, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *N Engl J Med*. 2002;346:92–98.
2. Jemal A, Miller KD, Ma J, et al. Higher lung cancer incidence in young women than young men in the United States. *N Engl J Med*. 2018;378:1999–2009.
3. Subramanian J, Govindan R. Lung cancer in never smokers: a review. *J Clin Oncol*. 2007;25:561–570.
4. Sathish V, Martin YN, Prakash YS. Sex steroid signaling: implications for lung diseases. *Pharmacol Ther*. 2015;150:94–108.

5. Verma MK, Miki Y, Sasano H. Sex steroid receptors in human lung diseases. *J Steroid Biochem Mol Biol*. 2011;127:216–222.
6. Marquez-Garban DC, Chen HW, Fishbein MC, Goodglick L, Pietras RJ. Estrogen receptor signaling pathways in human non-small cell lung cancer. *Steroids*. 2007;72:135–143.
7. Niikawa H, Suzuki T, Miki Y, et al. Intratumoral estrogens and estrogen receptors in human non-small cell lung carcinoma. *Clin Cancer Res*. 2008;14:4417–4426.
8. Stabile LP, Davis AL, Gubish CT, et al. Human non-small cell lung tumors and cells derived from normal lung express both estrogen receptor alpha and beta and show biological responses to estrogen. *Cancer Res*. 2002;62:2141–2150.
9. Weinberg OK, Marquez-Garban DC, Fishbein MC, et al. Aromatase inhibitors in human lung cancer therapy. *Cancer Res*. 2005;65:11287–11291.
10. The Coronary Drug Project. Findings leading to discontinuation of the 2.5-mg day estrogen group. The Coronary Drug Project Research Group. *JAMA*. 1973;226:652–657.
11. Chlebowski RT. Menopausal hormone therapy, hormone receptor status, and lung cancer in women. *Semin Oncol*. 2009;36:566–571.
12. Ganti AK, Sahmoun AE, Panwalkar AW, Tendulkar KK, Potti A. Hormone replacement therapy is associated with decreased survival in women with lung cancer. *J Clin Oncol*. 2006;24:59–63.
13. Slatore CG, Chien JW, Au DH, Satia JA, White E. Lung cancer and hormone replacement therapy: association in the vitamins and lifestyle study. *J Clin Oncol*. 2010;28:1540–1546.
14. Chlebowski RT, Wakelee H, Pettinger M, et al. Estrogen plus progestin and lung cancer: follow-up of the Women's Health Initiative randomized trial. *Clin Lung Cancer*. 2016;17:10–17.e1.
15. Dougherty SM, Mazhawidza W, Bohn AR, et al. Gender difference in the activity but not expression of estrogen receptors alpha and beta in human lung adenocarcinoma cells. *Endocr Relat Cancer*. 2006;13:113–134.
16. Kazmi N, Marquez-Garban DC, Aivazyan L, et al. The role of estrogen, progesterone and aromatase in human non-small-cell lung cancer. *Lung Cancer Manag*. 2012;1:259–272.
17. Hammoud Z, Tan B, Badve S, Bigsby RM. Estrogen promotes tumor progression in a genetically defined mouse model of lung adenocarcinoma. *Endocr Relat Cancer*. 2008;15:475–483.
18. Hershberger PA, Stabile LP, Kanterewicz B, et al. Estrogen receptor beta (ERbeta) subtype-specific ligands increase transcription, p44/p42 mitogen activated protein kinase (MAPK) activation and growth in human non-small cell lung cancer cells. *J Steroid Biochem Mol Biol*. 2009;116:102–109.
19. Pietras RJ, Marquez DC, Chen HW, Tsai E, Weinberg O, Fishbein M. Estrogen and growth factor receptor interactions in human breast and non-small cell lung cancer cells. *Steroids*. 2005;70:372–381.
20. Garon EB, Siegfried JM, Stabile LP, et al. Randomized phase II study of fulvestrant and erlotinib compared with erlotinib alone in patients with advanced or metastatic non-small cell lung cancer. *Lung Cancer*. 2018;123:91–98.
21. Mazieres J, Barlesi F, Rouquette I, et al. Randomized phase II trial evaluating treatment with EGFR-TKI associated with antiestrogen in women with nonsquamous advanced-stage NSCLC: IFCT-1003 LADIE trial. *Clin Cancer Res*. 2020;26:3172–3181.
22. Mah V, Seligson DB, Li A, et al. Aromatase expression predicts survival in women with early-stage non small cell lung cancer. *Cancer Res*. 2007;67:10484–10490.
23. Chlebowski RT, Schwartz AG, Wakelee H, et al. Oestrogen plus progestin and lung cancer in postmenopausal women (Women's Health Initiative trial): a post-hoc analysis of a randomised controlled trial. *Lancet*. 2009;374:1243–1251.
24. Geisler J, Suzuki T, Helle H, et al. Breast cancer aromatase expression evaluated by the novel antibody 677: correlations to intra-tumor estrogen levels and hormone receptor status. *J Steroid Biochem Mol Biol*. 2010;118:237–241.
25. Hammond ME, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer [published correction appears in *J Clin Oncol*. 2010;28:3543]. *J Clin Oncol*. 2010;28:2784–2795.
26. Cella DF. Quality of life outcomes: measurement and validation. *Oncol Williston Park*. 1996;10(suppl):233–246.
27. Cella DF, Bonomi AE, Lloyd SR, Tulsky DS, Kaplan E, Bonomi P. Reliability and validity of the Functional Assessment of Cancer Therapy-Lung (FACT-L) quality of life instrument. *Lung Cancer*. 1995;12:199–220.
28. Paz-Ares LG, de Marinis F, Dediu M, et al. PARAMOUNT: final overall survival results of the phase III study of maintenance pemetrexed versus placebo immediately after induction treatment with pemetrexed plus cisplatin for advanced nonsquamous non-small-cell lung cancer. *J Clin Oncol*. 2013;31:2895–2902.
29. Zukin M, Barrios CH, Pereira JR, et al. Randomized phase III trial of single-agent pemetrexed versus carboplatin and pemetrexed in patients with advanced non-small-cell lung cancer and Eastern Cooperative Oncology Group performance status of 2. *J Clin Oncol*. 2013;31:2849–2853.
30. Scagliotti GV, Kortsik C, Dark GG, et al. Pemetrexed combined with oxaliplatin or carboplatin as first-line treatment in advanced non-small cell lung cancer: a multicenter, randomized, phase II trial. *Clin Cancer Res*. 2005;11:690–696.
31. Miki Y, Suzuki T, Abe K, et al. Intratumoral localization of aromatase and interaction between stromal and parenchymal cells in the non-small cell lung carcinoma microenvironment. *Cancer Res*. 2010;70:6659–6669.
32. Mah V, Marquez D, Alavi M, et al. Expression levels of estrogen receptor beta in conjunction with aromatase predict survival in non-small cell lung cancer. *Lung Cancer*. 2011;74:318–325.
33. Meireles SI, Esteves GH, Hirata R Jr, et al. Early changes in gene expression induced by tobacco smoke: evidence for the importance of estrogen within lung tissue. *Cancer Prev Res (Phila)*. 2010;3:707–717.

34. Abe K, Miki Y, Ono K, et al. Highly concordant coexpression of aromatase and estrogen receptor beta in non-small cell lung cancer. *Hum Pathol*. 2010;41:190–198.
35. Aresti U, Carrera S, Iruarrizaga E, et al. Estrogen receptor 1 gene expression and its combination with estrogen receptor 2 or aromatase expression predicts survival in non-small cell lung cancer. *PLoS One*. 2014;9:e109659.
36. Koutras A, Giannopoulou E, Kritikou I, et al. Antiproliferative effect of exemestane in lung cancer cells. *Mol Cancer*. 2009;8:109.
37. Tanaka K, Shimizu K, Kakegawa S, et al. Prognostic significance of aromatase and estrogen receptor beta expression in EGFR wild-type lung adenocarcinoma. *Am J Transl Res*. 2016;8:81–97.
38. Bouchardy C, Benhamou S, Schaffar R, et al. Lung cancer mortality risk among breast cancer patients treated with anti-estrogens. *Cancer*. 2011;117:1288–1295.
39. Chu SC, Hsieh CJ, Wang TF, Hong MK, Chu TY. Antiestrogen use in breast cancer patients reduces the risk of subsequent lung cancer: a population-based study. *Cancer Epidemiol*. 2017;48:22–28.
40. Rosell J, Nordenskjöld B, Bengtsson NO, et al. Long-term effects on the incidence of second primary cancers in a randomized trial of two and five years of adjuvant tamoxifen. *Acta Oncol*. 2017;56:614–617.
41. Coombes RC, Hall E, Gibson LJ, et al. A randomized trial of exemestane after two to three years of tamoxifen therapy in postmenopausal women with primary breast cancer [published correction appears in N Engl J Med. 2006;355:1746]. *N Engl J Med*. 2004;350:1081–1092.
42. Miki Y, Abe K, Suzuki S, Suzuki T, Sasano H. Suppression of estrogen actions in human lung cancer. *Mol Cell Endocrinol*. 2011;340:168–174.
43. Kerr A 2nd, Eliason JF, Wittliff JL. Steroid receptor and growth factor receptor expression in human nonsmall cell lung cancers using cells procured by laser-capture microdissection. *Adv Exp Med Biol*. 2008;617:377–384.
44. Stabile LP, Lyker JS, Gubish CT, Zhang W, Grandis JR, Siegfried JM. Combined targeting of the estrogen receptor and the epidermal growth factor receptor in non-small cell lung cancer shows enhanced antiproliferative effects. *Cancer Res*. 2005;65:1459–1470.
45. Márquez-Garbán DC, Chen HW, Goodglick L, Fishbein MC, Pietras RJ. Targeting aromatase and estrogen signaling in human non-small cell lung cancer. *Ann N Y Acad Sci*. 2009;1155:194–205.
46. Márquez-Garbán DC, Deng G, Comin-Anduix B, et al. Antiestrogens in combination with immune checkpoint inhibitors in breast cancer immunotherapy. *J Steroid Biochem Mol Biol*. 2019;193:105415.
47. Rodriguez-Lara V, Hernandez-Martinez JM, Arrieta O. Influence of estrogen in non-small cell lung cancer and its clinical implications. *J Thorac Dis*. 2018;10:482–497.
48. Rothenberger NJ, Somasundaram A, Stabile LP. The role of the estrogen pathway in the tumor microenvironment. *Int J Mol Sci*. 2018;19:611.
49. Lund-Iversen M, Scott H, Strøm EH, Theiss N, Brustugun OT, Grønberg BH. Expression of estrogen receptor-alpha and survival in advanced-stage non-small cell lung cancer. *Anticancer Res*. 2018;38:2261–2269.
50. Gandhi L, Rodriguez-Abreu D, Gadgeel S, et al. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. *N Engl J Med*. 2018;378:2078–2092.