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Expression of Estrogen Receptor-α and Survival in Advanced-stage Non-small Cell Lung Cancer

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Abstract. *Background/Aim: The favorable prognosis of women with non-small-cell lung cancer (NSCLC) compared to men might be explained by sex hormone-related mechanisms. We investigated whether this observation could be explained by the expression of estrogen receptor-alpha (ER-α) in tumor tissue. Materials and Methods: Archived, formalin fixed, paraffin embedded tumor tissue samples were retrospectively analyzed for nuclear expression of ER-α with immunohistochemistry. Results: Biopsies from 222 patients were analyzed. Twenty-three percent were ER-α positive. Fifty-four percent of the patients were men and 46% of the tumors were adenocarcinomas. One hundred-nine (49%) patients received pemetrexed and carboplatin and 113 (51%) received gemcitabine and carboplatin. Females with ER-α positive tumors who received PC had a substantial survival benefit over all other groups (20 vs. 4.6 months; p=0.003). Conclusion: ER-α is an independent prognostic factor in advanced NSCLC and might also be a predictive factor for response to pemetrexed/carboplatin in women.*

Several studies have shown that women with non-small-cell lung cancer (NSCLC) have more favorable outcomes than men in all stages of the disease (1-3). The reason is unclear, but the role of sex hormones has been proposed as a possible explanation. Estrogen receptors (ER) are expressed in many lung tumors (4-6), and studies indicate that estrogen influences the development of lung cancer (7, 8). Hormone replacement therapy appears to increase mortality in NSCLC among women (8), and there are data suggesting that estrogen activates lung adenocarcinoma cell lines derived from women, but not from men (9, 10). Expression of estrogen receptors has been recognized as a prognostic factor in NSCLC (11), but the results are not consistent. Some studies have shown positive and negative prognostic values of ER depending on gender and stage of disease (5, 6, 12-14). Possible reasons for the inconsistent results are differences in the study design and patient selection. Some authors analyzed ER-α (6, 15-17) and others ER-β (5, 12-15, 17, 18). There are also several isoforms of ER-β (11) and some have analyzed expression of ER in the cytoplasm (6, 14, 15, 18) while others have analyzed nuclear expression (6, 12, 13, 17). Most authors have analyzed patients with lower stage disease treated with either surgery or curative radiotherapy (6, 13-16, 18). Only few patients with advanced disease have been included (12, 15, 18).

Most patients with advanced NSCLC will receive platinum-doublet chemotherapy (19). Pemetrexed in combination with a platinum-compound is one of the recommended regimens for patients with non-squamous NSCLC. Previous reports have shown a survival benefit among women treated with pemetrexed combined with carboplatin (PC) compared to gemcitabine combined with carboplatin (GC) (20, 21). In this study we aimed to investigate the relations between ER-α expression, gender, treatment and survival in advanced NSCLC.

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Key Words: Prognostic factor, immunohistochemistry, first-line chemotherapy, gender difference, sex hormones, estrogen receptor, palliative chemotherapy.

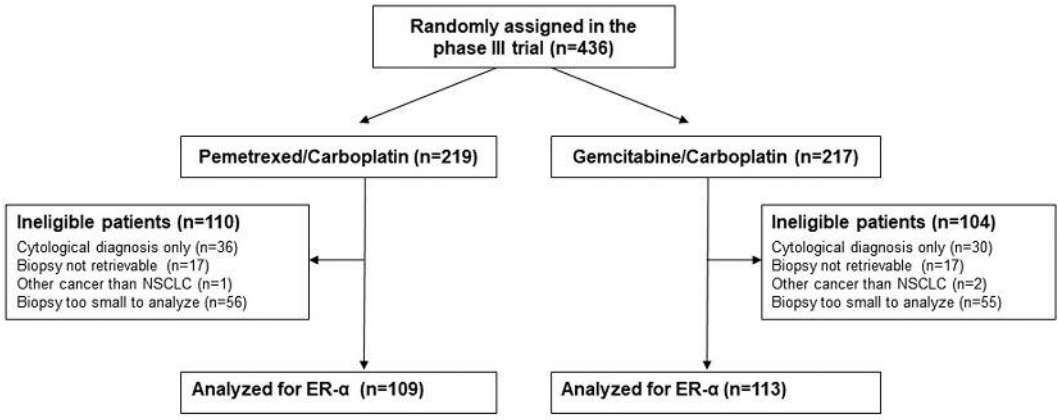

Figure 1. *Patient selection scheme*.

Materials and Methods

Materials. In the period May 2005 – July 2006 the Norwegian lung cancer study group conducted a phase III trial in advanced NSCLC where carboplatin was combined with either pemetrexed or gemcitabine (20). Tissue blocks from 222 participants in this study were eligible for ER-α investigation (Figure 1). The baseline characteristics, study chemotherapy and the post-study systemic therapy administered are listed in Table I.

All tumor material was derived from core biopsies or forceps biopsies. The area of the biopsies ranged from 0.8 to 1.2 mm^2.

Immunohistochemical assays. 4 μm sections were cut and placed on Superfrost Plus slides (Menzel-Glaser, Braunschweig, Germany). The slides were pretreated with Cell Conditioning 1 (Ventana Medical Systems, Inc, (VMSI), catalog number 950-124). Staining was performed on Ventana Bench Mark XT automated slide strainer with anti-Estrogen receptor (clone SP1) rabbit monoclonal primary antibody (VMSI, catalog number 790-4324) with ultra-view universal DAB detection kit (VMSI, catalog number 760-500). The SP1 clone is directed against an epitope on ER-α located in the nucleus. A previously confirmed ER positive carcinoma was used as a positive control. Other specimens were incubated with rabbit immunoglobulin under the same conditions as negative controls.

Assessment of ER-α status. The authors MLI, HS and EHS examined 10% of the slides individually and after establishment of inter-rater reliability MLI examined the remaining slides. ER-α was chosen in order to be able to compare our results with those of other studies (6, 13, 15-17, 22), and because there was experience with this clone from routine diagnostics. Based on previous studies on breast cancer (23) and common practice in breast carcinoma, nuclear staining was evaluated as present or absent. Tumors with >1% positive nuclei were considered positive. Scoring of cytoplasmic staining was not performed. Photomicrographs with typical staining patterns are shown in Figure 2.

Ethics approval and consent to participate. Approval was granted by the Regional committee for medical and health research ethics in Central Norway. Reference number: 196-04. The committee granted us permission to conduct the biomarker study without asking patients for consent since almost all patients had deceased by the time we conducted the study.

Statistical analyses. Survival was calculated using the Kaplan-Meier method and the data were compared using the log-rank test. Hazard ratio (HR) was calculated with the cox proportional hazard method. To confirm subgroup analyses (as appropriate) we used interaction tests and for group comparisons we used the Chi-square test. $p<0.05$ was defined as the statistical significance level.

Results

ER-α status and patient characteristics. Immunohistochemical staining of nuclear ER-α in >1% of tumor cells was identified in 50/222 patients (23%). There were no statistically significant differences in the proportion of ER-α positive tumors depending on gender (men: 20%, women: 25%; $p=0.37$); stage of disease (IIIB: 22%; IV: 23%; $p=0.88$); performance status (PS 0-1: 25%, PS 2: 15%; $p=0.12$); smoking history (never-smokers: 12%, smokers: 23%; $p=0.27$); or between squamous cell carcinomas and adenocarcinomas (SCC: 18%, ADC: 26%; $p=0.25$). There were no significant differences in baseline characteristics between patients with ER-α negative and those with ER-α positive tumors (Table I).

Among males with ER-α positive tumors, we found more patients with PS 0 in the pemetrexed and carboplatin arm (55% *vs*. 0%; $p=0.007$). There were no significant differences in baseline characteristics between the ER-α positive tumors in males *versus* females (Table II).

Survival analyses. Final survival data for the present study were collected five years after study enrolment was completed. At that time, 7 (3%) patients were alive.

Survival curves and results from univariate analyses are shown in Figure 3 and Table III respectively. Patients with

Table I. *Baseline characteristics and treatment*.

	ER-α negative (n=172)		ER-α positive (n=50)		All patients analyzed for ER status (n=222)		Excluded patients (n=214)	
	n	%	n	%	n	%	n	%
Age								
Median (Range)	63 (35-83)		64 (43-85)		64 (35-85)		66 (25-90)	
Gender								
Men	95	55%	24	48%	119	54%	132	62%
Women	77	45%	26	52%	103	46%	82	38%
Stage of disease								
IIIB	57	33%	16	32%	73	33%	51	24%
IV	115	67%	34	68%	149	67%	163	76%
Performance status								
0	38	22%	11	22%	49	22%	42	20%
1	88	51%	31	62%	119	54%	130	61%
2	46	27%	8	16%	54	24%	42	20%
Appetite loss	94	55%	24	48%	118	53%	111	52%
Histology								
Adenocarcinoma	76	44%	26	52%	102	46%	112	52%
Squamous cell carcinoma	51	30%	11	22%	62	28%	41	19%
Large cell carcinoma	9	5%	2	4%	11	5%	13	6%
Other	36	21%	11	22%	47	21%	48	22%
Smoking history								
Never smoker	15	9%	2	4%	17	8%	16	8%
Former or current smoker	157	91%	48	96%	205	92%	196	92%
Treatments								
Pemetrexed/carboplatin	85	49%	24	48%	109	49%	110	51%
Gemcitabine/carboplatin	87	51%	26	51%	113	51%	104	49%
Cycles of chemotherapy								
Mean	3.1		3.4		3.2		3.3	
Systemic second line therapy								
Any	51	30%	19	38%	70	32%	65	30%
Docetaxel	16	9%	7	14%	23	10%	29	14%
Erlotinib	12	7%	7	14%	19	9%	12	6%
Re-induction	7	4%	2	4%	9	4%	4	2%
Carboplatin/vinorelbine	7	4%	2	4%	9	4%	10	5%
Pemetrexed	5	3%	-	-	5	2%	4	2%
Other	4	2%	1	2%	5	2%	6	3%
Systemic third line therapy	10	6%	2	4%	12	5%	18	8%

ER-α positive tumors had a significantly longer median survival than those with ER-α negative tumors (ER-α positive: 10.8 months, ER-α negative: 6.4 months; p=0.008). There was no significant difference in median overall survival between the treatment arms in the ER-α positive patients (PC: 11.2 months, GC: 8.8 months; p=0.38), but an interaction test revealed significantly different associations between treatment arms and survival for men and women in this subgroup (p=0.004). Among women with ER-α positive tumors there was a substantial and highly significant difference (PC (n=13): 20.0 months, GC (n=13): 4.6 months; p=0.003). The survival difference was also significant when using cut-off values of 5% positive tumor cells (PC (n=11): 20.0, GC (n=13): 4.6; p=0.001) and 10% positive tumor cells (PC (n=8): 12.6 months, GC (n=13): 4.6 months; p=0.026). Furthermore, the survival difference remained significant when excluding patients who received second line EGFR-TKI therapy (PC (n=11): 20.0 months, GC (n=11): 4.5 months; p=0.002) or all patients who received any second line systemic therapy (PC (n=7): 20.0 months, GC (n=8): 3.5 months; p=0.003).

Multivariate analyses adjusting for baseline characteristics previously identified as significant prognostic factors in the phase III study (20, 24) revealed that ER-α status (HR=0.72; 95%CI=0.51-1.006; p=0.047) remained a significant positive prognostic factor in the overall population.

Discussion

We found that 23% of the tumors expressed ER-α and this was a significant positive prognostic factor, mainly due to

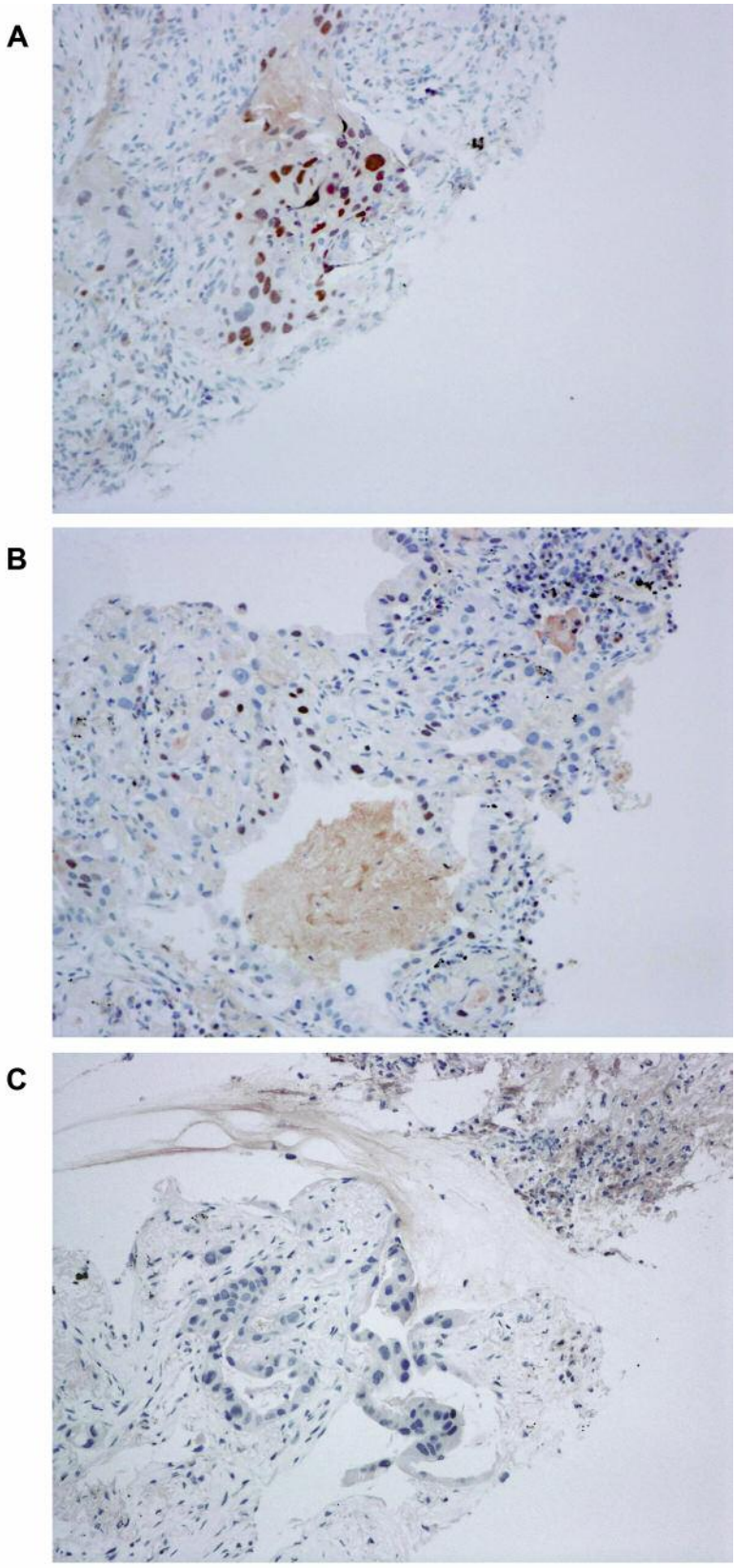

Figure 2. *Patterns of nuclear staining of ER-α on 200× magnification. A and B: Brown staining indicates positive nuclei. C: Negative staining.*

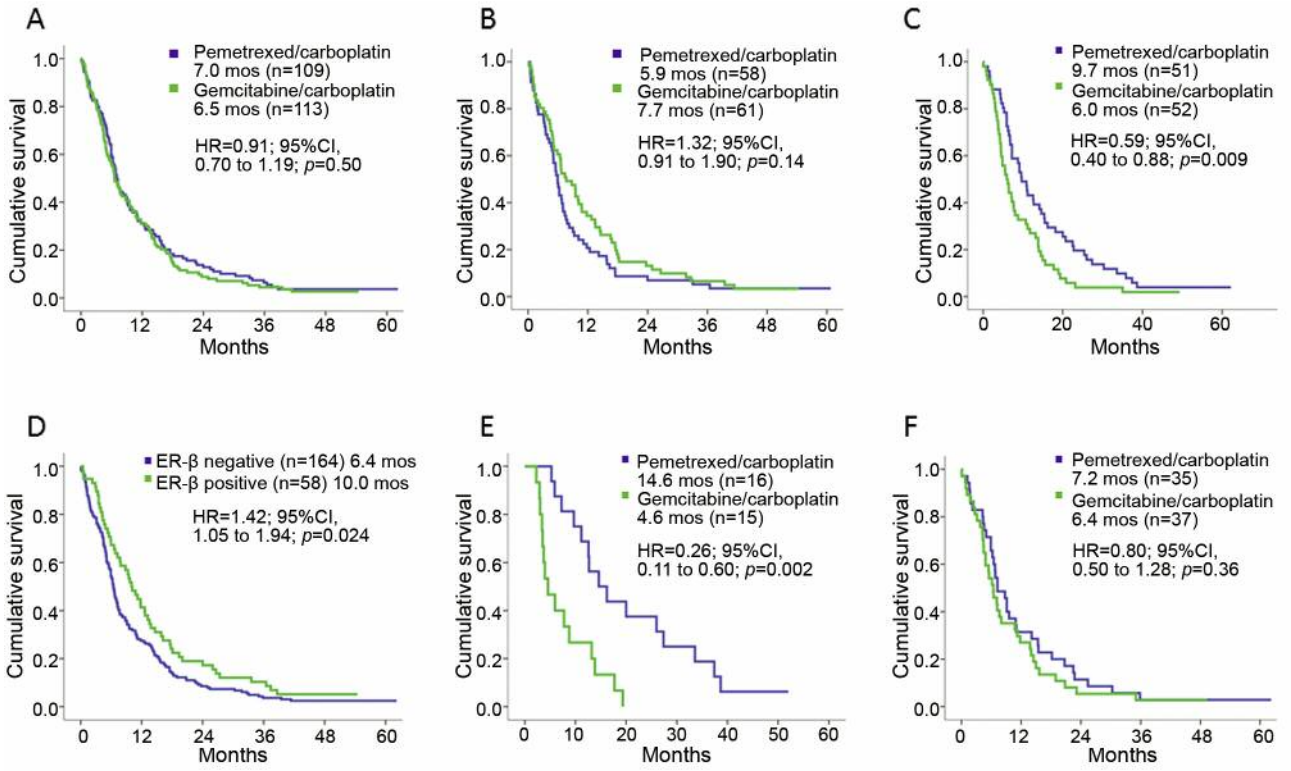

Figure 3. *Survival depending on (A) study treatment among all patients, (B) study treatment among men, (C) study treatment among women, (D) ER-α status among all patients, (E) study treatment among ER-α positive women, and (F) study treatment among ER-α negative women.*

the significant survival benefit of the pemetrexed-regimen among women with ER-α positive tumors.

We are not aware of other studies exploring associations between ER-α status, chemotherapy regimens and survival, but there are reports with varying results elaborating the incidence and prognostic role of ER-α in NSCLC (6, 15-18). One reason for the diverse results between studies might be the use of different antibody clones, lack of a standard method for assessment of ER-α and patient selection.

Our study is hampered by the absence of data regarding hormone replacement therapy. In addition, 26% of the cases were either classified as large cell carcinoma or "other" (Table I). According to the present diagnostic standards this is too high, but when the study was conducted (2005-2006), the clinical implications of sub-classification of NSCLC were not established. Unfortunately, there was not enough remaining tissue to reclassify the tumors with immunohistochemistry (IHC), nor test for EGFR, ALK, ROS1 or PD-L1 status.

Furthermore, most available tumor samples were small. Analyses of the few surgical resection specimens in our cohort revealed that IHC staining was not evenly expressed in all tumor cells, thus the expression of ER-α in biopsies might not be representative of the whole tumor, but the results remained significant when cutoff-values for ER-positivity of 5% and 10% were applied.

Pemetrexed is approved for first- and second-line treatment of non-squamous NSCLC, and recently also as maintenance therapy (20, 2, 25-27). Despite the favorable toxicity profile and appealing survival benefit, there are concerns about providing maintenance therapy to all patients with advanced NSCLC. Not all patients benefit from such therapy; side effects might have a negative impact on quality of life; it is time-consuming, tiring and increases costs (28, 29). Thus, identifying biomarkers predicting pemetrexed sensitivity would have large clinical implications. Currently, no such biomarkers are routinely used. Inhibition of thymidylate synthase (TS) is the main mechanism of action of pemetrexed, and several studies show that pemetrexed is more effective in patients with low TS-levels than those with higher TS-level (30, 31), but also patients with high TS-levels respond to pemetrexed. In a previous study of the present cohort, we found that there was no difference in survival between patients receiving PC and those receiving GC depending on TS-level, possibly indicating that TS is a prognostic rather than a predictive marker in advanced NSCLC (32).

Table II. *Baseline characteristics of ER-α positive patients.*

	All ER-α positive women (n=26)		ER-α positive women receiving PC (n=13)		ER-α positive women receiving GC (n=13)		All ER-α positive men (n=24)		ER-α positive men receiving PC (n=11)		ER-α positive men receiving GC (n=13)	
	n	%	n	%	n	%	n	%	n	%	n	%
Age												
Median (Range)	62 (43-85)		64 (43-85)		60 (50-71)		68 (50-81)		68 (50-77)		72 (52-81)	
Stage												
IIIB	6	23%	3	23%	3	23%	10	42%	5	46%	5	39%
IV	20	77%	10	77%	10	77%	14	58%	6	55%	8	62%
Performance status												
0	5	19%	3	23%	2	15%	6	25%	6	55%	-	-
1	18	69%	10	77%	8	62%	13	54%	3	27%	10	77%
2	3	12%	-	-	3	23%	5	21%	2	18%	3	23%
Appetite loss	13	50%	6	46%	7	54%	11	46%	6	55%	5	39%
Histology												
Adenocarcinoma	13	50%	6	46%	7	54%	13	54%	7	64%	6	46%
Squamous cell carcinoma	5	19%	2	15%	3	23%	6	25%	1	9%	5	39%
Large cell carcinoma	3	12%	2	15%	1	8%	2	8%	1	9%	1	8%
Other	5	19%	3	23%	2	15%	3	13%	2	18%	1	8%
Smoking history												
Never smoker	2	8%	1	8%	1	8%	-	-	-	-	-	-
Former or current smoker	24	92%	12	92%	12	92%	24	100%	11	100%	13	100%
Cycles of chemotherapy												
Mean	3.2	3.8	2.7	3.5	3.5	3.6						
Any second line therapy	11	42%	6	46%	5	62%	8	33%	3	27%	5	39%
Docetaxel	5	19%	2	15%	3	23%	2	8%	2	18%	-	-
Erlotinib	4	15%	2	15%	2	15%	3	13%	-	-	3	23%
Any third line therapy	2	8%	1	8%	1	8%	-	-	-	-	-	-

PC: Pemetrexed plus carboplatin; GC: gemcitabine plus carboplatin.

The results of this study should be interpreted with caution. The sample size was limited, especially in the subgroup analyses. We are not aware of any convincing explanations for our observations, although one can speculate that the folate-dependent enzyme, aminoimidazole-carboxamide ribonucleotide formyl-transferase (AICART), which is inhibited by pemetrexed, might have a role. Reduced AICART leads to elevated levels of AICAR monophosphate (ZMP) followed by activation of cAMP-dependent protein kinase (AMPK) (33). AMPK is a key factor in estrogen-dependent cell proliferation, and an increase of AMPK may lead to an increased progression of the cell cycle – which might increase sensitivity to pemetrexed and/or carboplatin in ER-α positive tumors. Another presumable explanation for the favorable outcome of the ER-α group is the affinity for platinum compounds in cells expressing ER-α (34). Neither of these theories explain the gender differences we found and based on our material we are not able to give any reasonable explanation for these differences.

Hsu *et al*. have reviewed several studies with ER as a prognostic marker in lung cancer (35), and found that there were referring to a variety of isoforms and scorings. These differences might partly explain conflicting results from different studies.

To the best of our knowledge, this study is the first to demonstrate a possible association between expression of ER-α, gender, and survival outcomes of different chemotherapy regimens in advanced NSCLC. With the known diversity among studies on ER receptors in lung carcinomas further research with standardized study design is crucial before the final clinical benefit of assessing ER-α can be established.

Conclusion

In conclusion, we found that ER-α was a significant prognostic factor for survival in advanced NSCLC. A survival benefit was seen in women treated with pemetrexed/carboplatin compared with gemcitabine/ carboplatin, mainly explained by a substantial survival advantage of the pemetrexed-regimen among the women with tumors expressing ER-α.

Conflicts of Interest

Noah Theiss is an employee of Ventana Medical Systems (Roche). Bjørn H. Grønberg, Marius Lund-Iversen and Odd Terje Brustugun have received honoraria for lectures at meetings arranged by Eli Lilly and Company and Roche, and their travel expenses for

Table III. *Survival analyses*.

	Median (months)	95%CI		Median (months)	95%CI	HR (95%CI)	*p*-Value
Age							
<75 years (n=184)	6.8	5.8-7.8	≥75 years (n=38)	6.6	4.0-9.2	0.91 (0.64-1.29)	0.59
Gender							
Women (n=103)	7.2	5.4-9.1	Men (n=119)	6.6	5.7-7.5	0.89 (0.68-1.16)	0.37
Stage of disease							
IIIB (n=73)	6.4	5.4-7.5	IV (n=149)	7.4	7.8-9.0	1.18 (0.89-1.57)	0.25
Performance status							
0-1 (n=168)	7.4	5.8-9.0	2 (n=54)	5.1	3.4-6.8	0.62 (0.45-0.84)	0.002
Appetite loss							
No appetite loss (n=104)	10.0	8.5-11.5	Appetite loss (n=118)	5.6	4.6-6.5	0.59 (0.45-0.78)	<0.001
Histology							
Squamous cell carcinoma (n=62)	7.2	5.8-8.7	Adeno- and large cell carcinoma (113)	7.1	5.7-8.5	1.18 (0.86-1.62)	0.31
Smoking history							
Never-smoker (n=17)	7.2	3.7-10.7	Ever-smoker (n=205)	6.8	6.0-7.7	1.12 (0.67-1.86)	0.67
ER-α status							
ER-α negative (n=172)	6.4	5.7-7.2	ER-α positive (n=50)	10.8	8.1-13.5	1.55 (1.12-2.15)	0.008
Chemotherapy							
Pemetrexed/carboplatin (n=109)	7.0	6.0-8.0	Gemcitabine/carboplatin (n=113)	6.6	5.2-8.0	0.91 (0.70-1.19)	0.50
Men							
Pemetrexed/carboplatin (n=58)	5.9	5.1-6.7	Gemcitabine/carboplatin (n=61)	7.7	4.9-10.5	1.32 (0.91-1.90)	0.14
Women							
Pemetrexed/carboplatin (n=51)	9.7	7.3-12.1	Gemcitabine/carboplatin (n=52)	6.0	4.1-7.9	0.59 (0.40-0.88)	0.009
ER-α positive women							
Pemetrexed/carboplatin (n=13)	20.0	4.2-35.8	Gemcitabine/carboplatin (n=13)	4.6	2.0-7.2	0.22 (0.08-0.59)	0.003
ER-α positive men							
Pemetrexed/carboplatin (n=11)	7.8	2.9-12.8	Gemcitabine/carboplatin (n=13)	11.7	7.6-15.9	1.66 (0.72-3.85)	0.24
ER-α negative women							
Pemetrexed/carboplatin (n=38)	8.8	6.0-11.5	Gemcitabine/carboplatin (n=39)	6.4	4.5-8.4	0.77 (0.49-1.21)	0.26
ER-α negative men							
Pemetrexed/carboplatin (n=47)	5.6	4.5-6.8	Gemcitabine/carboplatin (n=48)	6.6	4.1-9.1	1.19 (0.79-1.79)	0.42
No second-line therapy (n=152)	5.1	4.3-5.9	Any second-line therapy (n=152)	14.0	10.5-17.5	1.98 (1.48-2.66)	<0.001

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