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Research Paper

Smoking and Prognostic Factors in an Observational Setting in Patients with Advanced Non-Small Cell Lung Carcinoma

Chien-Te Li[1], Magdalena Marek[2], Salih Z Guclu[3], Younseup Kim[4], Mohamed Meshref[5], Shukui Qin[6], Zbigniew Kadziola[7], Kurt Krejcy[7], Sedat Altug[8] ✉

1. Chest Department, Changhua Christian Hospital, Changhua City, and the Institute of Medical and Molecular Toxicology, Chung Shan Medical University, Taichung City, Taiwan.
2. Department of Lung Cancer Chemotherapy, Bystra Hospital, Bystra, Poland.
3. Department of Chest Diseases, Izmir Chest Diseases Research Hospital, Izmir, Turkey.
4. Department of Pulmonology, Dankook University Hospital, Cheonan Choong Nam, Republic of Korea.
5. Department of Clinical Oncology, Faculty of Medicine, Cairo University, Cairo, and Eli Lilly, Egypt.
6. Chinese PLA Cancer Center, Nanjing Bayi Hospital, Nanjing, China.
7. Eli Lilly Regional Operations, Vienna, Austria.
8. Medical Department, Eli Lilly Turkey, Istanbul, Turkey.

✉ Corresponding author: Sedat Altug, MD, Medical Department, Eli Lilly Turkey, Istanbul, Turkey. altug_sedat@lilly.com.

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Abstract

Background: This prospective observational study estimated the effect of prognostic factors, particularly continued smoking during therapy, on survival in advanced non-small cell lung cancer (NSCLC) patients receiving gemcitabine-platinum. Further, prognostic factors were used to build a survival model to improve prognosis prediction in naturalistic clinical settings.

Methods: Eligibility criteria included: Stage IIIB/IV NSCLC, no prior chemotherapy, and Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1. A Cox regression model was constructed and validated by randomizing patients into two datasets (Construction [C]:Validation [V]; 3:1 ratio). Country, disease stage, hypercalcemia, "N" factor, weight reduction, performance status, and superior vena cava obstruction were pre-defined variables forced into the model. Continued smoking was tested with adjustment for these variables.

Results: One thousand two hundred and fourteen patients (C=891 and V=323) were enrolled. The final predictive model, established in the Construction dataset, identified four significant ($p \leq 0.05$) and independent predictors of survival, which were disease stage, performance status, gemcitabine-platinum regimen, and T-stage. Smoking during therapy was not significantly associated with survival (Hazard Ratio [95% CI]: 0.955 [0.572, 1.596], p=0.8618; versus never smokers).

Conclusions: Although continued smoking during therapy was not significantly associated with shorter survival, the model developed in this study forms an evidence-based approach to assessing prognosis in advanced stage NSCLC.

Key words: smoking; observational; NSCLC; prognostic factors; predictive modeling.

Background

Lung cancer is one of the leading causes of cancer-related deaths worldwide [1-2], with non-small cell lung cancer (NSCLC) representing approximately 75%-85% of all types of lung cancer [3]. In the majority of cases, patients with NSCLC present with locally advanced (Stage III) or metastatic disease (Stage IV) [4].

Smoking is the single most important cause of NSCLC [5-8], with approximately 85% of human lung cancers arising in current or former smokers. No

prospective study has been published that evaluated the effect of smoking on survival in advanced NSCLC treated with chemotherapy. Furthermore, limited data is available on the effect of smoking on chemotherapy toxicity. Considering the high incidence of advanced stage NSCLC and the common use of chemotherapy in these patients, these questions appear to be of major clinical relevance.

Numerous factors have been shown to influence survival and toxicity in patients with advanced NSCLC, such as disease stage, performance status, smoking, age, weight loss, and gender [5,9-12]. Additionally, molecular markers such as *p53* and *ras* mutations, and expression of ERCC1, beta-tubulin III and RRM1, have been found to influence treatment outcome [5,13-15].

The main aim of this prospective, observational study was to estimate the effect of prognostic factors, in particular, continued smoking during therapy, on survival in patients with advanced NSCLC receiving gemcitabine-platinum as first-line therapy. Further, prognostic factors identified in previous studies were used to build a survival model with the aim of improving prognosis prediction, in naturalistic clinical settings, in patients with advanced NSCLC who are receiving gemcitabine-platinum as first-line therapy.

Methods

Study Design

This prospective, non-interventional, international, observational study (B9E-AA-B004) was designed to estimate the effect of prognostic factors, including continued smoking during therapy, on treatment outcomes in patients with advanced NSCLC receiving gemcitabine-platinum as first-line therapy as part of their routine care. To ensure this study reflected real-life clinical practice, all care provided to the patients (including visit frequency, procedures performed at visits, and advice regarding smoking behavior) was at the discretion of the participating oncologist. Patients were recruited between June 2004 and October 2005 from nine countries (China, Egypt, Israel, Pakistan, Poland, Romania, South Korea, Taiwan, and Turkey) and were followed for survival until death, 18 months after the start of treatment, or lost to follow-up. The study was conducted in accordance with the ethics and regulatory requirements of each country and all participants provided written informed consent prior to enrollment in the study.

Participants

Patients were eligible if they: (a) were diagnosed with Stage IIIB or IV NSCLC; (b) were chemonaive; (c) received gemcitabine in combination with platinum (carboplatin or cisplatin) as part of their routine care; (d) had an Eastern Cooperative Oncology Group (ECOG) performance status ≤1; and (e) not simultaneously participating in a gemcitabine-platinum interventional study. The dosing schedule of gemcitabine-platinum therapy, use of concomitant medications, supportive care measures, and all subsequent lines of tumor therapy were at the discretion of the treating oncologist.

Effectiveness and Safety Measures

Eligible participants who received at least one dose of gemcitabine-platinum were evaluated for effectiveness and safety. Effectiveness was measured by survival, defined as the time from start of gemcitabine-platinum therapy to the date of death due to any cause. The effect of prognostic factors on an occurrence of any selected adverse events (AEs; neutropenia, thrombocytopenia, reduced haemoglobin, infection requiring hospitalisation or intravenous antibiotics, respiratory distress syndrome, dyspnea, death, or life-threatening toxicity) and methylation of *p16* and *RASSF1A* were assessed.

Statistical Analyses

Statistical analyses were performed using the SAS program (Version 9, SAS Institute, Cary, NC). A total of 3000 patients were planned to be enrolled with patients allocated in a 3:1 ratio to the Construction:Validation datasets to provide 80% power to draw the conclusion that continued smoking during chemotherapy reduces median survival time by 15% after balancing for clinical characteristics that have a statistically significant effect on survival. To identify potential prognostic factors associated with survival in advanced NSCLC patients treated with first-line gemcitabine-platinum, a total of 42 prognostic factors were analyzed. Some of the factors analyzed in this study were previously identified as potential prognostic factors in NSCLC [17] and include: continued smoking during therapy; number of cigarettes/day during therapy; baseline smoking level; continuing smoker versus ex-smoker; heavy smoker at baseline; never smoked; race; country; disease stage; hypercalcemia; TNM staging (T, N, and M); weight loss >10%; performance status; superior vena cava obstruction present; age <70 years; gemcitabine-platinum regimen; largest tumor >5cm; gender; metastatic disease (extra-thoracic; liver; bone; brain); diagnosis (histology); dyspnea present; cough present; hemoptysis present; pain present; expectoration present; chronic obstructive pulmonary disease present; pleural effusion present; *p16* status; *RASSF1A* status; albumin

(normal range indicator); hemoglobin; aspartate aminotransferase (AST/SGOT); alanine aminotransferase (ALT/SGPT); bilirubin (total); albumin; lactic dehydrogenase; calcium. Univariate Cox regression analysis was used to first assess the association between each variable and survival, followed by multivariate stepwise Cox regression analysis for variable selection (with entry and stay cutoff levels of 0.1). If information on any baseline or treatment variable was missing in >10% of patients, that variable was not used to build the primary model. Results are reported as hazard ratios (HR) with 95% confidence intervals.

Additional sensitivity analyses were performed to examine the potential impact of missing data. This involved effect of smoking variables adjusted in multivariate models, also for variables where >10% of the data was missing using the same model selection process described above.

Validation of the final predictive model was assessed using a Cox regression on the Validation dataset with the values of the linear predictor calculated from the coefficients estimated in the Construction dataset [16]. Also, predicted one-year survival was directly compared with the actual one-year survival for patients in the Validation dataset.

The occurrence of AEs and association of *p16* and *RASSF1A* methylation to baseline prognostic factors was analyzed using a univariate and multivariate (stepwise) logistic regression.

Results

Participant Characteristics and Treatments

Baseline clinical and demographic characteristics, including potentially important prognostic factors, are outlined in Table 1. Of 1214 patients enrolled from nine countries/regions, 891 (73.4%) were assigned to the Construction dataset and 323 (26.6%) to the Validation dataset. Patients were on average 60.5 years of age, with 75.1% being male. Approximately half of the patients had NSCLC of adenocarcinoma origin (48.9%), while approximately two-thirds of patients (69.4%) were assigned to gemcitabine-cisplatin treatment. The mean number of gemcitabine-platinum cycles received was 3.76 (95% CI: 3.66, 3.86), with 34.1% of patients receiving no therapy post gemcitabine-platinum treatment. Post gemcitabine-platinum treatment therapies are outlined in Table 2, with the most common second-line treatment approaches being docetaxel (18.3%) or radiotherapy (17.1%). Of the 1214 patients enrolled, 253 (20.8%) were alive at study completion, 637 (52.5%) had died, 310 (25.5 %) were lost to follow-up, while data was not available for 14 (1.2%). Three hundred and nineteen (26.3%) patients discontinued study therapy due to inadequate response, while 48 (4.0%) discontinued study therapy due to AEs.

Originally, 3000 patients were planned to be enrolled in this study; however, based on slower than expected recruitment, a decision was made to stop accrual after 17 months. At the time, a total of 1214 qualified patients had been enrolled in this study.

Table 1. Baseline Characteristics of Potential Prognostic Factors (Construction and Validation Datasets)

Characteristic	Construction Dataset N=891		Validation Dataset N=323		Total N=1214	
Country of Treatment, n(%)						
China	208	(23.3)	92	(28.5)	300	(24.7)
Egypt	158	(17.7)	42	(13.0)	200	(16.5)
Israel	19	(2.1)	20	(6.2)	39	(3.2)
Pakistan	64	(7.2)	13	(4.0)	77	(6.3)
Poland+Romania	50	(5.6)	44	(13.6)	94	(7.7)
South Korea	147	(16.5)	46	(14.2)	193	(15.9)
Taiwan	179	(20.1)	16	(5.0)	195	(16.1)
Turkey	66	(7.4)	50	(15.5)	116	(9.6)
Performance Status (ECOG), n(%)						
0	294	(33.0)	115	(35.6)	409	(33.7)
1	596	(66.9)	208	(64.4)	804	(66.2)
Missing data	1	(0.1)	-		1	(0.1)
Tumor Stage, n(%)						
Stage IIIB	385	(43.2)	129	(39.9)	514	(42.3)
Stage IV	501	(56.2)	191	(59.1)	692	(57.0)
Missing data	5	(0.6)	3	(0.9)	8	(0.7)
Age, years						
Mean (SD)	61.0 (10.8)		59.2 (10.6)		60.5 (10.8)	
Missing data	9 (1.0)		4 (1.2)		13 (1.1)	
Gender, n(%)						
Female	222	(24.9)	78	(24.1)	300	(24.7)

	Male	667	(74.9)	245	(75.9)	912	(75.1)
	Missing data	2	(0.2)	-		2	(0.2)
Brain Metastasis, n(%)							
	No	832	(93.4)	298	(92.3)	1130	(93.1)
	Yes	59	(6.6)	25	(7.7)	84	(6.9)
Tumor Type, n(%)							
	Adenocarcinoma	434	(48.7)	160	(49.5)	594	(48.9)
	Large Cell Lung Carcinoma	41	(4.6)	10	(3.1)	51	(4.2)
	Mixed Cell Carcinoma, Lung	10	(1.1)	8	(2.5)	18	(1.5)
	Non-small Cell Carcinoma	122	(13.7)	45	(13.9)	167	(13.8)
	Squamous Cell Carcinoma of Lung	284	(31.9)	100	(31.0)	384	(31.6)
Hypercalcemia (Calcium >2.75 mmol/L), n(%)							
	No	581	(65.2)	235	(72.8)	816	(67.2)
	Yes	25	(2.8)	5	(1.5)	30	(2.5)
	Missing data	285	(32.0)	83	(25.7)	368	(30.3)
Weight Loss >10% During the Last 6 Months, n(%)							
	No	602	(67.6)	241	(74.6)	843	(69.4)
	Yes	230	(25.8)	65	(20.1)	295	(24.3)
	Missing data	59	(6.6)	17	(5.3)	76	(6.3)
Superior Vena Cava Obstruction at Start of Therapy, n(%)							
	No	850	(95.4)	310	(96.0)	1160	(95.6)
	Yes	25	(2.8)	5	(1.5)	30	(2.5)
	Missing data	16	(1.8)	8	(2.5)	24	(2.0)
Chronic Obstructive Pulmonary Disease at Start of Therapy, n(%)							
	No	725	(81.4)	253	(78.3)	978	(80.6)
	Yes	146	(16.4)	60	(18.6)	206	(17.0)
	Missing data	20	(2.2)	10	(3.1)	30	(2.5)
Prescribed NSCLC Treatment, n(%)							
	Gemcitabine-carboplatin	306	(34.3)	63	(19.5)	369	(30.4)
	Gemcitabine-cisplatin	583	(65.4)	260	(80.5)	843	(69.4)
	Missing data	2	(0.2)	-		2	(0.2)

Abbreviations: ECOG = Eastern Cooperative Oncology Group; N = total number of patients; n = number of patient in specified category; NSCLC = non-small cell lung cancer; SD = standard deviation.

Table 2. Post Gemcitabine-Platinum Treatment Approaches

Post Gemcitabine-Platinum Therapy, n(%)	Construction Dataset N=891		Validation Dataset N=323		Total N=1214	
Died on Gemcitabine-Platinum Therapy	96	(10.8)	18	(5.6)	114	(9.4)
No Post Gemcitabine-Platinum Therapy	309	(34.7)	105	(32.5)	414	(34.1)
Post Gemcitabine-Platinum Therapy*						
Docetaxel	169	(19.0)	53	(16.4)	222	(18.3)
Paclitaxel	57	(6.4)	11	(3.4)	68	(5.6)
Vinorelbine	39	(4.4)	14	(4.3)	53	(4.4)
Gefitinib	59	(6.6)	29	(9.0)	88	(7.2)
Radiotherapy	140	(15.7)	67	(20.7)	207	(17.1)
Other	95	(10.7)	34	(10.5)	129	(10.6)

* Patients may have received more than one post gemcitabine-platinum therapy.

Effectiveness and Toxicity

The median overall survival time was 12.7 months (95% CI: 11.5, 13.7; n=1213). On completion of study therapy, of the 1214 patients enrolled, 594 (48.9%) had no reported progression of disease. Of the 637 (52.5%) that died during the study, the majority (95.6%), in the opinion of the investigator, died as a result of study disease.

Of the 1214 patients enrolled, 266 (21.9%) reported at least one AE. The most frequently reported AEs were low hemoglobin count (<8 g/dL; n=161, 13.3%), low neutrophil count (<1.0x10^9/L associated with fever of ≥38.5°C or documented infection; n=99, 8.2%), and thrombocytopenia (<50x10^9/L with bleeding; n=73, 6.0%).

Univariate Analyses of Survival

Of 42 prognostic factors analyzed, 16 were found by univariate Cox regression analysis to be significantly (p≤0.05) associated with survival, including baseline smoking level; heavy smoker at baseline; race; country; disease stage; performance status; superior vena cava obstruction; age <70 years; TNM staging; largest tumor >5cm; gender; metastatic liver, bone and brain disease; dyspnea present; chronic obstructive pulmonary disease; albumin (normal range indicator); and lactic dehydrogenase.

Development and Validation of the Predictive Model for Survival

Firstly, all variables (excluding smoking variables) with <10% missing values were included in the initial Cox's model. A stepwise Cox regression was then performed with essential factors identified by Brundage et al (2002) [17]. Country, disease stage, hypercalcemia, "N" factor, weight reduction, performance status, and superior vena cava obstruction were forced into the model. As a result, 6 variables were additionally selected by the regression (gemcitabine-platinum regimen, T-stage, chronic obstructive pulmonary disease, metastatic brain disease, gender, and diagnosis). The final predictive model (Figure 1) identified four significant (p≤0.05) and independent predictors of survival, which were disease stage, performance status, gemcitabine-platinum regimen, and T-stage. No smoking variables were represented in the final predictive model.

Cox regression performed on the Validation dataset showed high significance (p=0.0008) of linear predictor calculated using the coefficients estimated in the Construction dataset. Patients in the Validation dataset (n=323) were also classified into three groups based on the predicted one-year survival probabilities forecast from the final predictive model: (1) predicted probability <0.2; (2) predicted probability ranging from ≥0.2 to <0.5; and (3) predicted probability ≥0.5. The predicted and observed proportions surviving were calculated for each of the above groups (Figure 2). These analyses support the predictive model being strongly associated with actual survival in the Validation dataset.

Development of the Predictive Model for Adverse Events

Of 42 prognostic factors analyzed, 6 were found by univariate logistic regression analysis to be significantly (p≤0.05) associated with AEs, which were country, gemcitabine-platinum regimen, largest tumor >5cm, presence of hemoptysis, presence of pain, and albumin.

The final predictive model, as established in the Construction dataset using multivariate stepwise logistic regression (with gender forced into the model, and excluding smoking variables from the selection process), identified 5 significant (p≤0.05) and independent predictors of AEs, that being disease stage (IIIB versus IV), country, weight loss >10%, age <70 years, and the presence of pain. The smoking variables added to the established model did not reach significance (p>0.05).

Effect of Continued Smoking during Therapy on Survival and Adverse Events

Smoking characteristics at baseline and during the study are outlined in Table 3. Overall, 70.8% of patients had smoked at some point prior to therapy (i.e. ever smokers). Approximately half of ever smokers (53.7%) had ceased smoking at initiation of therapy or within 6 months prior to treatment start, while 11.2% of smokers continued to smoke during therapy. Of those patients continuing to smoke during therapy, the mean number of cigarettes smoked per day was 16.57 (95% CI: 13.0, 20.1). There was no statistically significant difference in survival observed between "never smokers" and "ever smokers", with an unadjusted HR (versus never smokers) of 1.143 (95% CI: 0.925, 1.441; p=0.2155). None of the smoking variables forced into the established multivariate model were significantly associated with survival, with an adjusted HR of 0.955 (95% CI: 0.572, 1.596) observed for continued smoking during therapy (versus never smokers; p=0.8618), and an adjusted HR of 0.905 (95% CI: 0.648, 1.263) observed for ex-smokers (versus never smokers; p=0.5579). No statistically significant association was observed between AEs and continued smoking during therapy (adjusted OR=1.297 [95% CI: 0.716, 2.350]; p=0.3912).

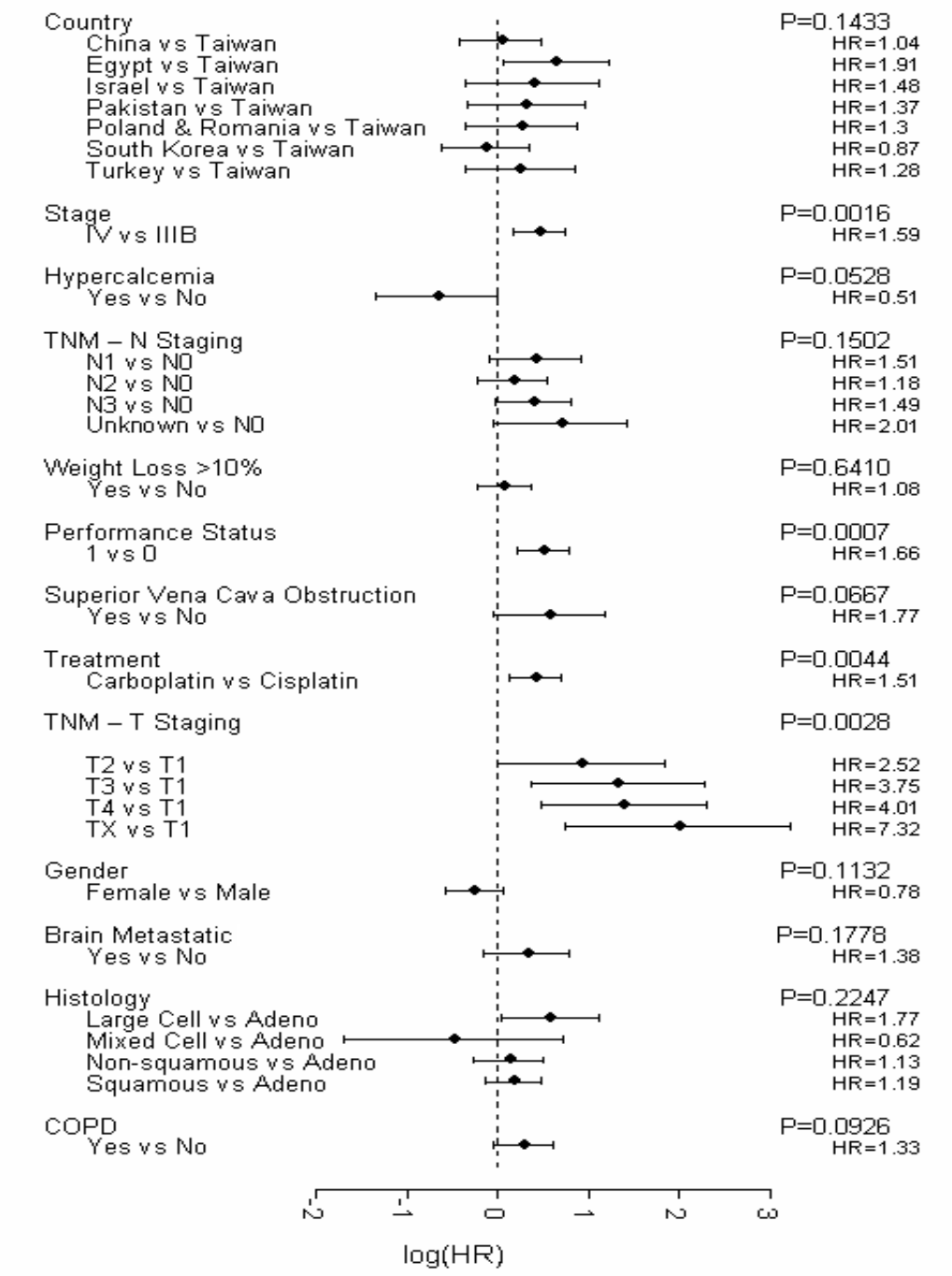

Figure 1. Final Predictive Model for Survival (Construction Dataset). Abbreviations: adeno = adenocarcinoma; COPD = chronic obstructive pulmonary disease; HR = hazard ratio; TNM = tumor, node, metastasis; vs = versus.

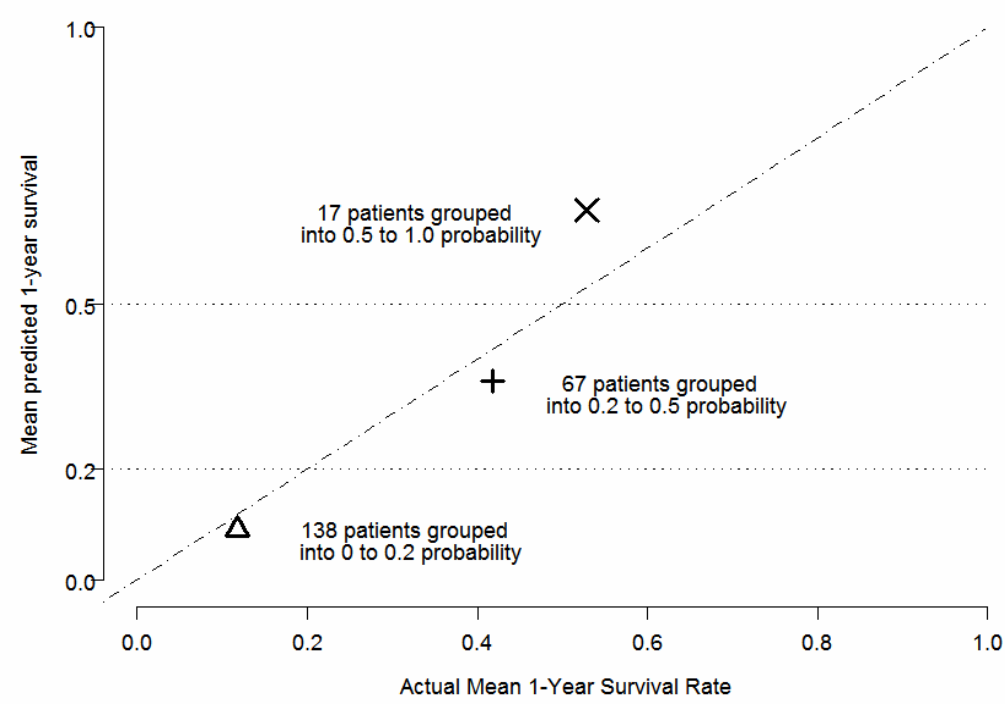

Figure 2. Validation of the Predictive Model: Predicted versus Actual One-Year Survival (Validation Dataset).

Table 3. Smoking Characteristics at Baseline and During Study (Construction and Validation Datasets)

Smoking Characteristic, n(%)	Construction Dataset N=891	Validation Dataset N=323	Total N=1214
Ever Smoked Prior to Therapy			
No	224 (25.1)	88 (27.2)	312 (25.7)
Yes	629 (70.6)	230 (71.2)	859 (70.8)
Missing data	38 (4.3)	5 (1.5)	43 (3.5)
Pack-Years Smoked Prior to Therapy			
n	620	229	849
Mean (95% CI)	45.06 (42.6, 47.6)	49.71 (44.5, 54.9)	46.31 (44.0, 48.6)
Time from Smoking Cessation to Treatment Start for Patients who Ever Smoked Prior to Therapy†			
No cessation	47 (7.5)	32 (13.9)	79 (9.2)
Restarted after cessation	36 (5.7)	20 (8.7)	56 (6.5)
0 (cessation at Treatment Start)†	46 (7.3)	20 (8.7)	66 (7.7)
>0 & <=1mo	129 (20.5)	51 (22.2)	180 (21.0)
>1mo & <=6mo	168 (26.7)	47 (20.4)	215 (25.0)
>6mo & <=1yr	38 (6.0)	1 (0.4)	39 (4.5)
>1yr & <=5yr	75 (11.9)	29 (12.6)	104 (12.1)
>5yr & <=10yr	30 (4.8)	10 (4.3)	40 (4.7)
>10yr	60 (9.5)	20 (8.7)	80 (9.3)
Mean (95% CI), years‡	2.96 (2.38, 3.53)	3.33 (2.17, 4.48)	3.05 (2.53, 3.57)
Smoking During Therapy§			
No	801 (89.9)	269 (83.3)	1070 (88.1)
Yes#	83 (9.3)	53 (16.4)	136 (11.2)
Missing data	7 (0.8)	1 (0.3)	8 (0.7)
Cigarettes Per Day During Therapy			
n	83	53	136
Mean (95% CI)	15.05 (10.6, 19.5)	18.94 (12.9, 25.0)	16.57 (13.0, 20.1)
Heavy Smoker as a Proportion of All Patients			
No	397 (44.6)	155 (48.0)	552 (45.5)

	Yes	447 (50.2)	162 (50.2)	609 (50.2)
	Missing data	47 (5.3)	6 (1.9)	53 (4.4)

† Patients who stopped smoking but without giving a stop date should be considered to have stopped just before the start of therapy.

‡ Patients who stopped & restarted are not included.

§ Patients who have the cessation date > therapy start date and their 'No. cigarettes during therapy' is entered as zero or blank are considered not smoking during therapy.

One patient, who had never smoked prior to therapy, started smoking during therapy.

Association of *p16* and *RASSF1A* Methylation Status to Baseline Prognostic Factors and Adverse Events

Methylation status of *p16* and *RASSF1A* was analyzed in 86 patients, with hypermethylation observed in 15 (17.4%, *p16*) and 8 (9.3%, *RASSF1A*) patients, respectively. Thirty-nine prognostic factors were analyzed by univariate and multivariate (stepwise) logistic regression. In those with *RASSF1A* hypermethylation, no factors were identified as significant following univariate (n=86) and stepwise logistic regression (n=65). In those with *p16* hypermethylation, the following factors were found to be significant in univariate analyses (n=86): performance status (1 versus 0, unadjusted OR=10.240 [95% CI: 1.276, 82.162] p=0.0286), extra-thoracic metastatic disease (yes versus no, unadjusted OR=6.891 [95% CI: 1.968, 24.124], p=0.0025), and total bilirubin (1μmol/L versus 0, unadjusted OR=1.240 [95% CI: 1.037, 1.482], p=0.0181). Following stepwise regression (n=65), extra-thoracic metastatic disease was identified as a significant factor (yes versus no, OR=5.595 [95% CI: 1.342, 23.333], p<0.0181).

Neither *p16* nor *RASSF1A* were significant when added to the established predictive model for AEs. Additionally, the univariate logistic regression did not identify methylation factors as predictive of AEs.

Discussion

In this prospective, observational study, the effect of prognostic factors, in particular continued smoking during therapy, on survival and other treatment outcomes, was assessed in patients with advanced NSCLC receiving gemcitabine-platinum as first-line therapy. Continued smoking during gemcitabine-platinum therapy was not associated with shorter survival in patients with advanced NSCLC. Construction and validation of a predictive model identified four independent prognostic factors associated with survival: disease stage, performance status, gemcitabine-platinum regimen, and T-stage.

The greatest risk factor associated with lung cancer is cigarette smoking [18-19], with approximately 85% of all lung cancer cases in men and 47% in women attributed to tobacco smoking [1]. Previous studies have identified an association towards longer survival [19], as well as statistically significant differences in survival [20], between "never smokers" and "ever smokers" who have undergone chemotherapy. Our study had a similar proportion of never smokers (25.7%) compared to previous studies (14.5% [21]; 16% [20]; 36.3% [19]). However, unlike these other studies, there was no statistically significant difference in survival observed between "never smokers" and "ever smokers" receiving gemcitabine-platinum as first-line therapy. A retrospective study by Nguyen et al (2006) in which exploratory subgroup analyses were performed, reported similar findings to this study, in which no significant difference in median survival time between "ever smokers" and "never smokers" was observed in patients receiving gemcitabine-cisplatin therapy [22]. However, gemcitabine-cisplatin was also a therapy utilized in the study by Scagliotti et al (2008) and an association towards longer survival was observed in "never smokers" (15.3 months) compared to "ever smokers" (10.3 months) [21]. In terms of continued smoking during therapy, in our study, fewer patients (11.2%) continued smoking during therapy when compared to a previous study (48%) [20]. It is interesting to note that the retrospective study of Tsao et al (2006) encompassed patients treated with first-line chemotherapy between 1993 and 2002 [20], while our study reflects patient treatment during 2004-2005. It is therefore possible that the lower proportion of patients continuing to smoke during therapy in our study reflects the increased influence that oncologists are having on their patients as well as a greater public awareness of the association between smoking and lung cancer. Despite differences in the proportion of patients continuing smoking during therapy, similar findings in terms of the impact of continued smoking during therapy on survival were noted between our study and Tsao et al (2006), with no statistically significant difference in survival observed between those patients continuing to smoke during therapy and those who discontinued prior to therapy initiation [20]. This suggests the detrimental effects of cigarette

smoking may occur earlier in disease progression. Another explanation may be that the effects of continued smoking during therapy are not evident in patients treated with chemotherapy in late stage disease as their duration of survival is limited. Additionally, bias as a result of the non-randomized, unblinded design of the study and confounding are inherently associated with observational studies and should be considered when interpreting the results. The effect of continued smoking during therapy on toxicity was also analyzed in this study. Although an association towards a greater rate of AEs was observed in patients who continued to smoke during therapy, this was not statistically significant.

In addition to smoking, numerous factors have been shown to influence survival and toxicity in patients with advanced NSCLC, such as disease stage, performance status, age, weight loss, and gender [5,9-12,19-20]. In this study, development of a predictive model in a Construction dataset, and subsequent validation with a Validation dataset, led to the identification of four significant ($p \leq 0.05$) and independent predictors of survival, which were disease stage (IV versus IIIB), performance status (ECOG 1 versus 0), gemcitabine-platinum regimen (carboplatin versus cisplatin), and T-stage (versus T1). Performance status has been shown in a number of trials to be a powerful predictor of survival [9,11,19-20,23-25]. Similarly, in this study, performance status was identified as an independent prognostic factor, with improved survival associated with a better baseline performance status. In this study, disease stage was also identified as an independent prognostic factor, with improved survival noted in those patients with Grade IIIB NSCLC when compared with Grade IV disease. Similar findings have been shown in other studies [19,24]. T-stage was also shown to be an independent prognostic factor in our study, with improved survival observed in patients with T1 stage compared with T2-TX. Hence, in relation to TNM the prognostic value in terms of survival prediction, in our study, can be based on T-stage alone. This is in alignment with ASCO guidelines [26]. Although platinum combinations with a third-generation chemotherapy agent are widely recognized as standard of care for first-line treatment of patients with advanced NSCLC, the choice of platinum agent (carboplatin or cisplatin) varies. In our study, the choice of platinum agent was shown to influence survival, with cisplatin proving to be more effective than carboplatin. This finding is consistent with a meta-analysis, in which carboplatin combinations with third-generation chemotherapy were shown to be inferior to cisplatin combinations with third-generation chemotherapy [27].

Genetic factors are also involved in lung cancer development, with aberrant promoter methylation playing an important role. Previous studies of NSCLC have reported varied frequencies of *p16* methylation, with Wang et al (2008) reporting *p16* methylation in 38% of samples [28], while Guzman et al (2007) observed *p16* methylation in 79.7% of samples [29]. In this study, the *p16* methylation observed was low (17.6%) compared to observations in previous studies. Additionally, *RASSF1A* methylation has been implicated in NSCLC, with *RASSF1A* methylation reported in 21% [28] and 40% [30] of samples, while in the current study *RASSF1A* was observed in only 8.2% of samples. Possible explanations for the different methylation frequencies include techniques to study methylation status and study population. Wang et al (2008) [28] utilized microarray technology, while Guzman et al (2007) [29], Li et al (2003) [30] and the current study utilized PCR-based technology. These studies focused on samples from China [28], Chile [29] and the US [30], respectively, whereas the current study analyzed samples from nine different countries (China, Egypt, Israel, Pakistan, Poland, Romania, South Korea, Taiwan, and Turkey). Moreover, different frequencies of methylation have been observed in different NSCLC histological types [29], thus different proportions of histological types may be present in each of the different studies.

The naturalistic setting of this study provides insight into the use of gemcitabine-platinum first-line therapy and treatment outcomes. In addition to the strengths of this study, we acknowledge several limitations which should be taken into account when interpreting the results. Firstly, as the number of patients enrolled in this study was reduced (n=1214) compared with the planned number (n=3000) the study was not adequately powered to test the hypothesis that smoking during chemotherapy may be associated with shorter survival in patients with advanced NSCLC who receive first-line chemotherapy with gemcitabine-platinum. Secondly, there was a higher than anticipated decrease in smoking during therapy. Thirdly, the observational nature of the study can lead to bias and confounding.

Conclusions

This observational study shows that continued smoking during gemcitabine-platinum therapy was not associated with shorter survival in patients with advanced NSCLC. Additionally, construction and validation of a predictive model identified four independent prognostic factors that were associated with survival - disease stage, performance status, gemcitabine-platinum regimen, and T-stage.

Authors' contributions

KK conceived the study design. SA, C-TL, MMarek, SZG, YK, MMeshref and SQ acquired the data. KK, ZK, MMeshref and SA analyzed and interpreted the data. SA and MMeshref contributed to the drafting of the manuscript. C-TL, MMarek, SZG, YK, SQ, ZK, KK and SA critically revised the manuscript drafts. All authors read and approved the final manuscript.

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Conflict of Interests

Chien-Te Li, Magdalena Marek, Salih Z Guclu, Younseup Kim and Shukui Qin were investigators for trial B9E-AA-B004 and have no other competing interests. Mohamed Meshref was a clinical investigator for this trial while the trial was active and is now an employee of Eli Lilly and Company. Sedat Altug is an employee of Eli Lilly and Company. Zbigniew Kadziola and Kurt Krejcy are employees of, and hold stock/stock options in, Eli Lilly and Company.

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