

# Smoking status during first-line immunotherapy and chemotherapy in NSCLC patients: A case-control matched analysis from a large multicenter study

Alessio Cortellini<sup>1,2</sup>  | Andrea De Giglio<sup>3</sup> | Katia Cannita<sup>4</sup> | Diego L. Cortinovis<sup>5</sup> | Robin Cornelissen<sup>6</sup> | Cinzia Baldessari<sup>7</sup> | Raffaele Giusti<sup>8</sup> | Ettore D'Argento<sup>9</sup> | Francesco Grossi<sup>10</sup> | Matteo Santoni<sup>11</sup> | Annamaria Catino<sup>12</sup> | Rossana Berardi<sup>13</sup> | Vincenzo Sforza<sup>14</sup> | Giovanni Rossi<sup>15</sup> | Lorenzo Antonuzzo<sup>16</sup> | Vincenzo Di Noia<sup>17</sup> | Diego Signorelli<sup>18</sup> | Alain Gelibter<sup>19</sup> | Mario Alberto Occhipinti<sup>19</sup> | Alessandro Follador<sup>20</sup> | Francesca Rastelli<sup>21</sup> | Rita Chiari<sup>22</sup> | Luigi Della Gravara<sup>23</sup> | Alessandro Inno<sup>24</sup> | Michele De Tursi<sup>25</sup> | Pietro Di Marino<sup>26</sup> | Giovanni Mansueto<sup>27</sup> | Federica Zoratto<sup>28</sup> | Marco Filetti<sup>8</sup>  | Michele Montrone<sup>12</sup> | Fabrizio Citarella<sup>29</sup> | Maria Vittoria Pensieri<sup>1,2</sup> | Marco Russano<sup>29</sup> | Luca Cantini<sup>6,13</sup> | Olga Nigro<sup>30</sup> | Alessandro Leonetti<sup>31</sup> | Paola Bordi<sup>31</sup> | Gabriele Minuti<sup>32</sup> | Lorenza Landi<sup>3</sup> | Alessandro De Toma<sup>18</sup> | Clelia Donisi<sup>33</sup> | Serena Ricciardi<sup>34</sup> | Maria Rita Migliorino<sup>34</sup> | Valerio Maria Napoli<sup>35</sup> | Gianmarco Leone<sup>35</sup> | Giulio Metro<sup>36</sup> | Giuseppe L. Banna<sup>37</sup> | Alex Friedlaender<sup>38</sup> | Alfredo Addeo<sup>38</sup> | Corrado Ficorella<sup>2,4</sup> | Giampiero Porzio<sup>4</sup>

<sup>1</sup>Department of Surgery and Cancer, Imperial College London, London, United Kingdom

<sup>2</sup>Department of Biotechnology and Applied Clinical Sciences, University of L'Aquila, L'Aquila, Italy

<sup>3</sup>Division of Medical Oncology, S.Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy

<sup>4</sup>Medical Oncology, St. Salvatore Hospital, L'Aquila, Italy

<sup>5</sup>Medical Oncology, Ospedale San Gerardo, Monza, Italy

<sup>6</sup>Department of Pulmonary Diseases, Erasmus Medical Center, Rotterdam, The Netherlands

<sup>7</sup>Department of Oncology and Hematology, Modena University Hospital, Modena, Italy

<sup>8</sup>Medical Oncology, St. Andrea Hospital, Rome, Italy

<sup>9</sup>Comprehensive Cancer Center, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Rome, Italy

<sup>10</sup>Medical Oncology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

<sup>11</sup>Medical Oncology, Hospital of Macerata, Macerata, Italy

<sup>12</sup>Thoracic Oncology Unit, Clinical Cancer Center IRCCS Istituto Tumori "Giovanni Paolo II", Bari, Italy

<sup>13</sup>Oncology Clinic, Università Politecnica Delle Marche, Ospedali Riuniti Di Ancona, Ancona, Italy

<sup>14</sup>Thoracic Medical Oncology, Istituto Nazionale Tumori 'Fondazione G Pascale', IRCCS, Naples, Italy

<sup>15</sup>Lung Cancer Unit, IRCCS Ospedale Policlinico San Martino, Genoa, Italy

<sup>16</sup>Department of Oncology, Careggi University Hospital, Florence, Italy

Alessio Cortellini and Andrea De Giglio equally contributed.

[Correction added on 10 February 2021, after initial publication on 2 February 2021: the 6<sup>th</sup> author's surname was corrected from 'Baldesarri' to 'Baldessari'.]

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2021 The Authors. *Thoracic Cancer* published by China Lung Oncology Group and John Wiley & Sons Australia, Ltd.

- <sup>17</sup>Unità di Oncologia medica e Terapia Biomolecolare, Azienda Ospedaliero-Universitaria Ospedali Riuniti di Foggia, Foggia, Italy
- <sup>18</sup>Department of Medical Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy
- <sup>19</sup>Medical Oncology (B), Policlinico Umberto I, "Sapienza" University of Rome, Rome, Italy
- <sup>20</sup>Department of Oncology, University Hospital Santa Maria Della Misericordia, Udine, Italy
- <sup>21</sup>Medical Oncology, Fermo Area Vasta 4, Fermo, Italy
- <sup>22</sup>Medical Oncology, Ospedali Riuniti Padova Sud "Madre Teresa Di Calcutta", Monselice, Italy
- <sup>23</sup>Pneumo-Oncology Unit, Monaldi Hospital, Naples, Italy
- <sup>24</sup>Oncology Unit, IRCCS Ospedale Sacro Cuore Don Calabria, Negrar, Italy
- <sup>25</sup>Department of Medical, Oral & Biotechnological Sciences, University G. D'Annunzio, Chieti-Pescara, Chieti, Italy
- <sup>26</sup>Clinical Oncology Unit, S.S. Annunziata Hospital, Chieti, Italy
- <sup>27</sup>Medical Oncology, F. Spaziani Hospital, Frosinone, Italy
- <sup>28</sup>Medical Oncology, Santa Maria Goretti Hospital, Latina, Italy
- <sup>29</sup>Medical Oncology, Campus Bio-Medico University, Rome, Italy
- <sup>30</sup>Medical Oncology, ASST-Sette Laghi, Varese, Italy
- <sup>31</sup>Medical Oncology Unit, University Hospital of Parma, Parma, Italy
- <sup>32</sup>Department of Oncology and Hematology, AUSL Romagna, Ravenna, Italy
- <sup>33</sup>Medical Oncology Unit, University Hospital and University of Cagliari, Cagliari, Italy
- <sup>34</sup>Pneumo-Oncology Unit, St. Camillo-Forlanini Hospital, Rome, Italy
- <sup>35</sup>Department of Oncology, University of Turin, San Luigi Hospital, Orbassano, Italy
- <sup>36</sup>Department of Medical Oncology, Santa Maria della Misericordia Hospital, Azienda Ospedaliera di Perugia, Perugia, Italy
- <sup>37</sup>Oncology Department, Queen Alexandra University Hospital, Portsmouth Hospitals NHS Trust, Portsmouth, UK
- <sup>38</sup>Oncology Department, University Hospital of Geneva, Geneva, Switzerland

#### Correspondence

Alessio Cortellini, Imperial College London,  
Department of Surgery & Cancer, Hammersmith  
Hospital, Du Cane Road, W12 0HS London,  
United Kingdom.  
Email: a.cortellini@imperial.ac.uk;  
alessiocortellini@gmail.com

#### Abstract

**Background:** Improved outcome in tobacco smoking patients with non-small cell lung cancer (NSCLC) following immunotherapy has previously been reported. However, little is known regarding this association during first-line immunotherapy in patients with high PD-L1 expression. In this study we compared clinical outcomes according to the smoking status of two large multicenter cohorts.

**Methods:** We compared clinical outcomes according to the smoking status (never smokers vs. current/former smokers) of two retrospective multicenter cohorts of metastatic NSCLC patients, treated with first-line pembrolizumab and platinum-based chemotherapy.

**Results:** A total of 962 NSCLC patients with PD-L1 expression  $\geq 50\%$  who received first-line pembrolizumab and 462 NSCLC patients who received first-line platinum-based chemotherapy were included in the study. Never smokers were confirmed to have a significantly higher risk of disease progression (hazard ratio [HR] = 1.49 [95% CI: 1.15–1.92],  $p = 0.0022$ ) and death (HR = 1.38 [95% CI: 1.02–1.87],  $p = 0.0348$ ) within the pembrolizumab cohort. On the contrary, a nonsignificant trend towards a reduced risk of disease progression (HR = 0.74 [95% CI: 0.52–1.05],  $p = 0.1003$ ) and death (HR = 0.67 [95% CI: 0.45–1.01],  $p = 0.0593$ ) were reported for never smokers within the chemotherapy cohort. After a random case-control matching, 424 patients from both cohorts were paired. Within the matched pembrolizumab cohort, never smokers had a significantly shorter progression-free survival (PFS) (HR = 1.68 [95% CI: 1.17–2.40],  $p = 0.0045$ ) and a nonsignificant trend towards a shortened overall survival (OS) (HR = 1.32 [95% CI: 0.84–2.07],  $p = 0.2205$ ). On the contrary, never smokers had a significantly longer PFS (HR = 0.68 [95% CI: 0.49–0.95],  $p = 0.0255$ ) and OS (HR = 0.66 [95% CI: 0.45–0.97],  $p = 0.0356$ ) compared to current/former smoker patients within the matched chemotherapy cohort. On pooled multivariable analysis, the interaction term between smoking status and treatment modality was concordantly statistically significant with respect to ORR ( $p = 0.0074$ ), PFS ( $p = 0.0001$ ) and OS ( $p = 0.0020$ ), confirming the significantly different impact of smoking status across the two cohorts.

**Conclusions:** Among metastatic NSCLC patients with PD-L1 expression  $\geq 50\%$  receiving first-line pembrolizumab, current/former smokers experienced improved PFS

and OS. On the contrary, worse outcomes were reported among current/former smokers receiving first-line chemotherapy.

#### KEYWORDS

immunotherapy, non-small cell lung cancer, pembrolizumab, smoking, tobacco

## INTRODUCTION

Programmed death 1 (PD-1) checkpoint inhibitors have become the backbone of the treatment algorithm of non-oncogene addicted non-small cell lung cancer (NSCLC) patients.<sup>1</sup> Tobacco use is known to be the main risk factor for lung cancer development and is related to a high all-cause morbidity and mortality overall.<sup>2</sup> Nevertheless, smoking of tobacco has been associated with improved outcomes in NSCLC patients receiving checkpoint inhibitors across different lines and regardless of programmed death-ligand 1 (PD-L1) tumor expression.<sup>3</sup> Intriguingly, a meta-analysis has also suggested that checkpoint inhibitors significantly improve survival over chemotherapy in smoker patients only.<sup>4</sup>

We recently published a large (1016 patients) real-world multicenter study of patients with metastatic NSCLC with PD-L1 expression  $\geq 50\%$  who received first-line single agent pembrolizumab at 34 European institutions, aimed at investigating the clinicopathological correlates of efficacy.<sup>5,6</sup> Multivariable analysis determined that former smokers (but not current smokers) experienced significantly prolonged progression-free survival (PFS) and overall-survival (OS) compared to never smokers.<sup>5</sup> We subsequently gathered a cohort of metastatic NSCLC patient treated with first-line platinum-based doublet chemotherapy for the external validation of the role of BMI in the same study population.<sup>7</sup>

In order to further assess the role of the baseline smoking status during first-line single agent immunotherapy in NSCLC patients with high PD-L1 tumor expression, we compared the clinical outcomes analyses according to the smoking status between the above mentioned two cohorts.

## METHODS

### Study design

We compared the clinical outcomes analyses according to the smoking status (never vs. current/former smokers) of two real-world retrospective multicenter cohorts: a cohort of metastatic NSCLC patients with PD-L1 expression  $\geq 50\%$ , consecutively treated with first-line pembrolizumab monotherapy, from January 2017 to October 2019, at 34 institutions (Supplementary file 1), and a cohort of metastatic epidermal growth factor receptor (EGFR) wild-type NSCLC patients treated with platinum-based doublet chemotherapy in clinical practice from January 2013 to January 2020, at 10 institutions among the abovementioned.<sup>5-7</sup> The measured clinical outcomes were objective response rate (ORR),

PFS and OS. Methods regarding clinical outcomes estimation in the two cohorts have been previously reported.<sup>5-7</sup>

A fixed multivariable regression model was used to estimate clinical outcomes (ORR, PFS and OS) according to the smoking status (current/former smokers vs. never smokers) in both pembrolizumab and chemotherapy cohorts.<sup>8-10</sup> The key covariates were: age ( $<70$  vs.  $\geq 70$  years old),<sup>11</sup> gender (male vs. female), Eastern Cooperative Oncology Group-Performance Status (ECOG-PS) (0-1 vs.  $\geq 2$ ), central nervous system (CNS) metastases (yes vs. no), bone metastases (yes vs. no) and liver metastases (yes vs. no).

Considering the different sample size, a random case-control matching was also performed to better compare the results across the cohorts. All the cases from the chemotherapy cohort and controls from the pembrolizumab cohort were randomly paired on the basis of the smoking status (current/former smokers vs. never smokers) and those characteristics which were significantly unbalanced between the cohorts: ECOG-PS (0-1 vs. 2), age ( $< 70$  vs.  $\geq 70$  years old), and baseline BMI according to the World Health Organization categories (underweight, BMI  $< 18.5$ ; normal-weight,  $18.5 \leq \text{BMI} \leq 24.9$ ; overweight,  $25 \leq \text{BMI} \leq 29.9$ ; obese, BMI  $\geq 30$ ).<sup>7</sup>

Lastly, to take into account the potential role of all baseline characteristics, we performed a pooled analysis, using a multivariable regression model (inclusive of the previously selected covariates plus primary tumor histology [squamous vs. nonsquamous] and baseline BMI) including the interaction term between the smoking status and the treatment modality (pembrolizumab vs. chemotherapy), used as covariates.

All patients provided their written, informed consent to treatment with immunotherapy. The procedures followed were in accordance with the precepts of Good Clinical Practice and the declaration of Helsinki. The study was approved by the respective local ethical committees on human experimentation of each institution, after previous approval by the coordinating center (Comitato Etico per le province di L'Aquila e Teramo, verbale N.15 del 28 Novembre 2019).

Median PFS and median OS were evaluated using the Kaplan-Meier method. Median period of follow-up was calculated according to the reverse Kaplan-Meier method.  $\chi^2$  test was used for the univariable analysis of ORR, logistic regression was used for the fixed multivariable analyses of ORR. Cox proportional hazards regression was used for the univariable analysis of PFS and OS and for the fixed multivariable analyses. The alpha level for all analyses was set to  $p < 0.05$ . Adjusted hazard ratios (HRs) and adjusted odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. Forest plot graphs were used to compare HRs between

the pembrolizumab and chemotherapy cohorts. After the random case-control matching, clinical outcomes of the two cohort were compared with univariable analyses. Considering the sample size of the pembrolizumab cohort (more than twice the chemotherapy cohort) a caliper width of <1 for the standard deviation was used for the random case-control matching.<sup>12</sup> All statistical analyses were performed using MedCalc Statistical Software version 18.11.3 (MedCalc Software bvba, Ostend, Belgium; <http://www.medcalc.org>; 2019).

## RESULTS

A total of 962 patients and 426 patients were included in the pembrolizumab and chemotherapy cohorts, respectively. Patient characteristics of the two cohorts have already been previously reported,<sup>7</sup> a summary of which is available in Table S2. A total of 864 patients (89.8%) and 378 patients (88.7%) were former/current smokers in the pembrolizumab and chemotherapy cohorts, respectively, and 249 patients (58.5%) within the chemotherapy cohort had received a further treatment with either PD-1 or PD-L1 checkpoint inhibitors at the data cutoff.

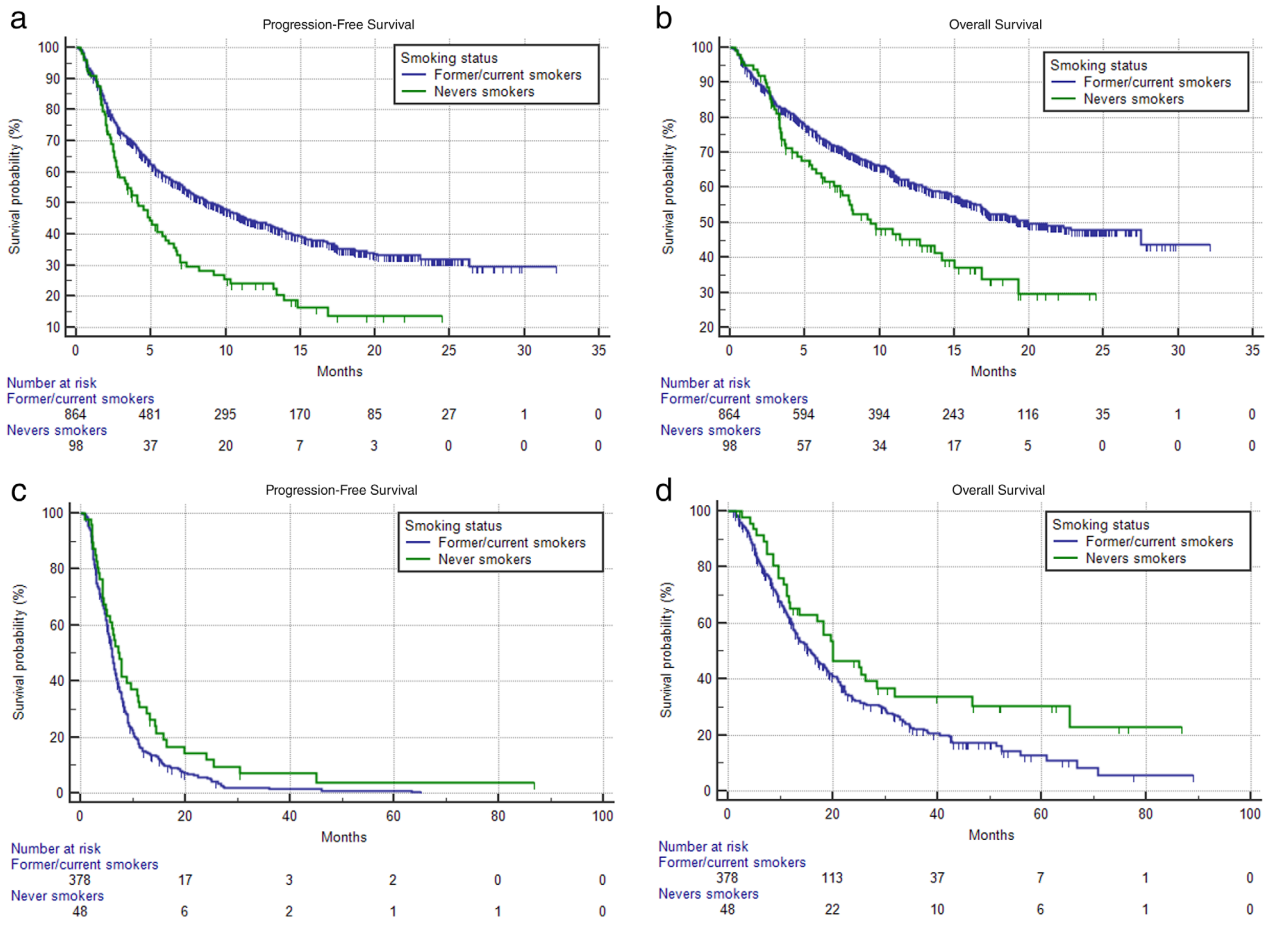
Table 1 summarizes the univariable analysis of ORR, PFS and OS. Never smokers had a significantly lower ORR ( $p = 0.0367$ ), significantly shorter PFS (HR = 1.74 [95% CI: 1.36–2.23],  $p < 0.0001$ ) (Figure 1(a)) and OS (HR = 1.59 [95% CI: 1.19–2.13],  $p = 0.0015$ ) (Figure 1(b)) compared to former/current smokers within the pembrolizumab cohort. In the chemotherapy cohort the smoking status was not significantly related to the ORR ( $p = 0.0919$ ), whilst significantly longer PFS (HR = 0.70 [95% CI: 0.51–0.96],  $p = 0.0296$ ) and OS (HR = 0.66 [95% CI: 0.45–0.96],  $p = 0.0339$ ) were reported for never smokers.

Table 2 summarizes the multivariable analysis of ORR. The smoking status was not confirmed to be associated with ORR in both the pembrolizumab (OR = 0.66 [95% CI: 0.40–1.09],  $p = 0.1070$ ), and chemotherapy (OR = 1.83 [95% CI: 0.94–3.70],  $p = 0.0751$ ) cohorts. Table 3 summarizes the multivariable analysis of PFS. Never smokers were confirmed to have a significantly shorter PFS compared to current/former smokers in the pembrolizumab cohort (HR = 1.49 [95% CI: 1.15–1.92],  $p = 0.0022$ ). On the other hand, the opposite association was not confirmed within the chemotherapy cohort (HR = 0.74 [95% CI: 0.52–1.05],  $p = 0.1003$ ) (Figure 2). Similarly, never smokers were confirmed to have a significantly shorter OS compared to current/former smokers in the pembrolizumab cohort (HR = 1.38 [95% CI: 1.02–1.87],  $p = 0.0348$ ), while a nonsignificant trend of a prolonged OS was reported for never smokers within the chemotherapy cohort (HR = 0.67 [95% CI: 0.45–1.01],  $p = 0.0593$ ) (Table 4) (Figure 2).

After the case-control random matching, 424 patients from the pembrolizumab and chemotherapy cohorts were perfectly paired, with no statistically significant differences between the characteristics of matched subjects; 378 (89.2%) current/former smoker patients were included in both

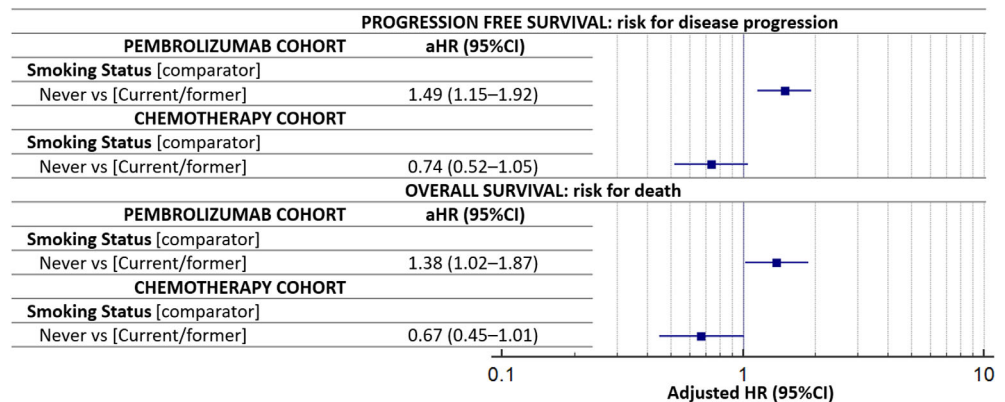
**TABLE 1** Univariate analyses of objective response rate (ORR), progression-free survival (PFS) and overall survival (OS) according to smoking status

Smoking status	Pembrolizumabcohort			Chemotherapycohort		
	Response/ratio	ORR (95% CI)	$\chi^2$ test	Response/ratio	ORR (95% CI)	$\chi^2$ test
Former/current smokers	344/760	45.3% (40.6–50.3)	$p = 0.0367$	158/373	42.4% (36.0–49.5)	$p = 0.0919$
Never smokers	28/84	33.3% (22.1–48.2)	HR (95%CI)	26/47	55.3% (36.1–81.1)	HR (95% CI)
Former/current smokers	PFS (95% CI) (events)			PFS (95% CI) (events)		
Never smokers	9.1 months (7.5–10.7) (486)		1.74 (1.36–2.23); $p < 0.0001$	6.0 months (5.6–6.4) (344)		0.70 (0.51–0.96); $p = 0.0296$
Former/current smokers	4.1 months (2.7–5.7) (73)		HR (95%CI); p - value	7.5 months (4.7–10.8) (43)		HR (95% CI)
Never smokers	OS (95% CI) (censored)			OS (95%CI) (censored)		0.66 (0.45–0.96); $p = 0.0339$
Former/current smokers	19.9 months (16.9–27.5) (522)		1.59 (1.19–2.13); $p = 0.0015$	15.8 months (13.2–18.3) (119)		
Never smokers	9.4 months (6.9–15.0) [45]			20.0 months (11.8–31.8) (17)		



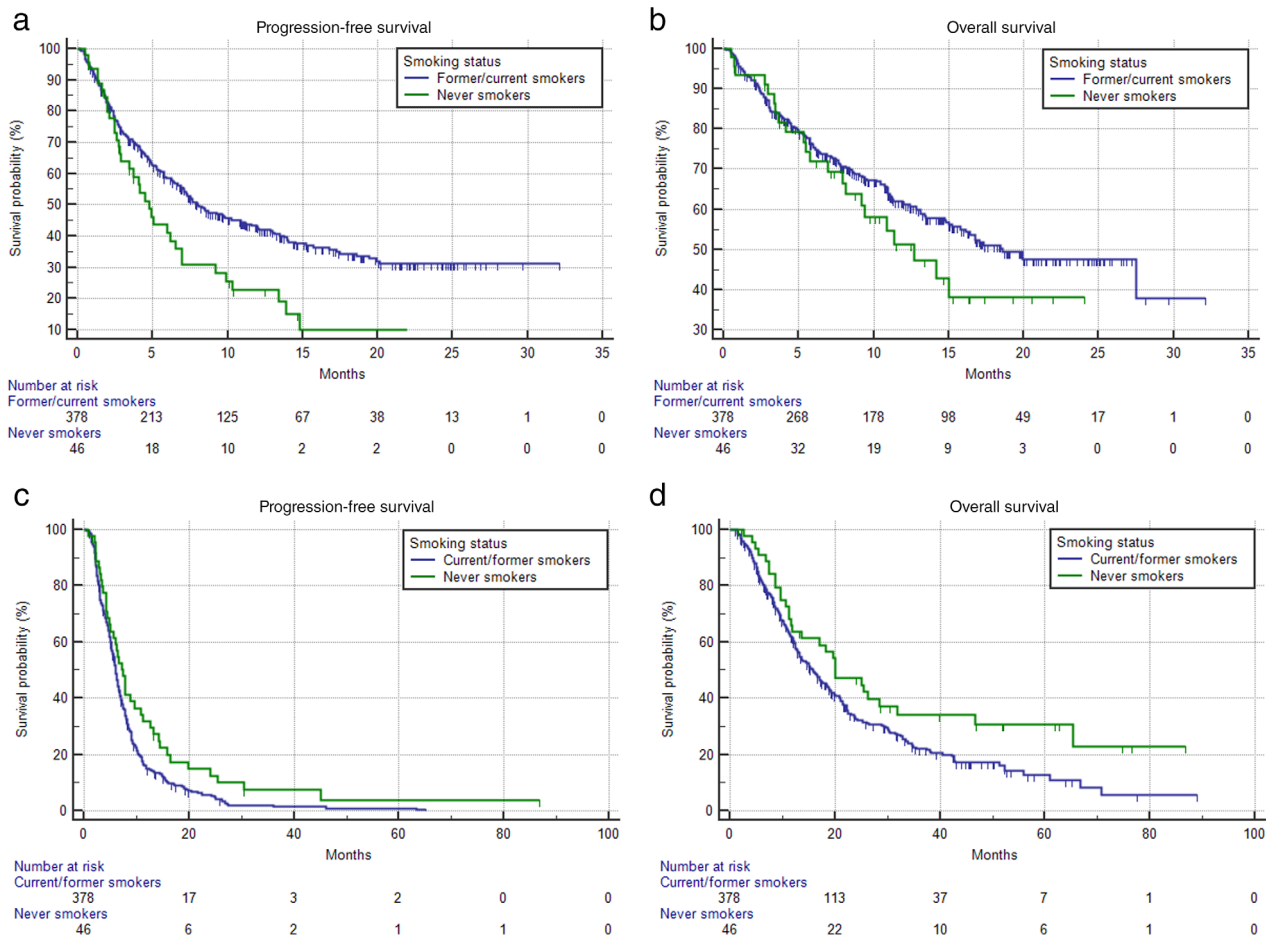
**FIGURE 1** Kaplan–Meier survival curves according to smoking status. Pembrolizumab cohort (a) progression-free survival (PFS); and (b) overall survival (OS); chemotherapy cohort (c) PFS and (d) OS. See Table 1 for survival estimations

**FIGURE 2** Forest plot graph for adjusted hazard ratios (aHRs) for disease progression (progression-free survival [PFS]) and death (overall survival [OS]) according to smoking status



matched cohorts. In the matched pembrolizumab cohort, the ORR for current/former smokers and never smokers was 33.2% (95% CI: 27.5–39.8) and 30.9% (95% CI: 16.5–52.9) ( $p = 0.7658$ ), respectively; among the matched chemotherapy cohort the ORR for current/former smokers and never smokers was 42.4% (95% CI: 36.0–49.5) and 55.6% (95% CI: 35.9–82.0) ( $p = 0.0923$ ), respectively. Never smokers had a significantly shorter PFS (HR = 1.68 [95% CI: 1.17–2.40],

$p = 0.0045$ ) (Figure 3a) and a nonsignificant trend towards a shortened OS (HR = 1.32 [95% CI: 0.84–2.07],  $p = 0.2205$ ) within the matched pembrolizumab cohort (Figure 3b). On the contrary, never smokers had a significantly longer PFS (HR = 0.68 [95% CI: 0.49–0.95],  $p = 0.0255$ ) (Figure 3c) and OS (HR = 0.66 [95% CI: 0.45–0.97],  $p = 0.0356$ ) (Figure 3d) I' compared to current/former smoker patients within the matched chemotherapy cohort.



**FIGURE 3** Kaplan–Meier survival curves according to smoking status within the randomly matched cohorts; Pembrolizumab cohort PFS. (a) Never smokers 4.7 months (95% CI: 2.8–6.9; 35 progression events), current/former smokers 8.0 months (95% CI: 8.9–10.8; 217 progression events) ( $p = 0.0045$ ). OS. (b) Never smokers 12.7 months (95% CI: 7.9–15.0; 24 censored patients), current/former smokers 18.6 months (95% CI: 15.2–27.4; 227 censored patients) ( $p = 0.2205$ ); PFS. (c) Never smokers 7.4 months (95% CI: 5.1–10.8; 41 progression events), current/former smokers 6.0 months (95% CI: 5.6–6.4; 344 progression events) ( $p = 0.0255$ ). OS. (d) Never smokers 20.1 months (95% CI: 11.6–31.8; 16 censored patients), current/former smokers 15.8 months (95% CI: 13.2–18.4; 119 censored patients) ( $p = 0.0255$ ). PFS, progression-free survival; OS, overall survival

**TABLE 2** Summary of the objective response rate (ORR) multivariable analysis in the pembrolizumab and chemotherapy cohorts

Variable (comparator)	Pembrolizumab cohort Objective response rate			Chemotherapy cohort Objective response rate		
	Coefficient	Standard error	OR (95% CI); $p$ -value	Coefficient	Standard error	OR (95% CI); $p$ -value
Smoking status (never vs. current/former)	0.411	0.255	0.66 (0.40–1.09); $p = 0.1070$	−0.606	0.340	1.83 (0.94–3.57); $p = 0.0751$
Gender (male vs. female)	0.006	0.155	0.99 (0.73–1.34); $p = 0.9651$	0.131	0.229	0.88 (0.56–1.37); $p = 0.5672$
Age (elderly vs. non-elderly)	0.034	0.145	0.96 (0.72–1.28); $p = 0.8108$	0.547	0.210	0.58 (0.38–0.87); $p = 0.0093$
CNS metastases (yes vs. no)	0.031	0.188	0.97 (0.67–1.40); $p = 0.8665$	−0.015	0.279	1.02 (0.58–1.75); $p = 0.9545$
Bone metastases (yes vs. no)	0.662	0.161	0.51 (0.37–0.71); $p < 0.0001$	0.683	0.244	0.50 (0.31–0.81); $p = 0.0050$
Liver metastases (yes vs. no)	0.364	0.211	0.69 (0.45–1.05); $p = 0.0853$	0.593	0.317	0.55 (0.29–1.03); $p = 0.0616$
ECOG PS $\geq 2$ vs. (0–1)	0.942	0.216	0.39 (0.26–0.59); $p = 0.0038$	0.176	0.405	0.83 (0.37–1.85); $p = 0.6632$

Abbreviations: CNS, central nervous system; ECOG, Eastern Cooperative Oncology Group; OR, odds ratio.

**TABLE 3** Summary of the progression-free survival (PFS) multivariable analysis in the pembrolizumab and chemotherapy cohorts

	<b>Pembrolizumab cohort Progression-free survival</b>	<b>Chemotherapy cohort Progression-free survival</b>
Variable (comparator)	HR (95% CI); <i>p</i> -value	HR (95% CI); <i>p</i> -value
Smoking status (never vs. current/former)	1.49 (1.15–1.92); <i>p</i> = 0.0022	0.74 (0.52–1.05); <i>p</i> = 0.1003
Gender (male vs. female)	0.99 (0.83–1.19); <i>p</i> = 0.9574	1.21 (0.96–1.54); <i>p</i> = 0.1018
Age (elderly vs. nonelderly)	1.07 (0.90–1.27); <i>p</i> = 0.4282	1.17 (0.95–1.44); <i>p</i> = 0.1345
CNS metastases (yes vs. no)	1.21 (0.98–1.50); <i>p</i> = 0.0733	1.08 (0.81–1.44); <i>p</i> = 0.5611
Bone metastases (yes vs. no)	1.60 (1.33–1.91); <i>p</i> < 0.0001	1.32 (1.05–1.65); <i>p</i> = 0.0160
Liver metastases (yes vs. no)	1.75 (1.41–2.16); <i>p</i> < 0.0001	1.37 (1.02–1.83); <i>p</i> = 0.0338
ECOG PS ≥2 vs (0–1)	2.42 (1.98–2.94); <i>p</i> < 0.0001	2.16 (1.46–3.21); <i>p</i> = 0.0001

Abbreviations: CNS, central nervous system; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio.

**TABLE 4** Summary of the overall survival (OS) multivariable analysis in the pembrolizumab and chemotherapy cohorts

	<b>Pembrolizumab cohort Overall survival</b>	<b>Chemotherapy cohort Overall survival</b>
Variable (comparator)	HR (95% CI); <i>p</i> -value	HR (95% CI); <i>p</i> -value
Smoking status (never vs. current/former)	1.38 (1.02–1.87); <i>p</i> = 0.0348	0.67 (0.45–1.01); <i>p</i> = 0.0593
Gender (male vs. female)	1.11 (0.89–1.39); <i>p</i> = 0.3131	1.05 (0.80–1.39); <i>p</i> = 0.6918
Age (elderly vs. nonelderly)	1.10 (0.90–1.35); <i>p</i> = 0.3298	1.22 (0.96–1.55); <i>p</i> = 0.1005
CNS metastases (yes vs. no)	1.15 (0.89–1.48); <i>p</i> = 0.2743	1.27 (0.92–1.76); <i>p</i> = 0.1396
Bone metastases (yes vs. no)	1.68 (1.36–2.07); <i>p</i> < 0.0001	1.38 (1.06–1.80); <i>p</i> = 0.0144
Liver metastases (yes vs. no)	1.69 (1.32–2.16); <i>p</i> < 0.0001	1.23 (0.86–1.75); <i>p</i> = 0.2427
ECOG PS ≥2 vs (0–1)	2.95 (2.36–6.69); <i>p</i> < 0.0001	2.44 (1.65–3.63); <i>p</i> < 0.0001

Abbreviations: CNS, central nervous system; ECOG, Eastern Cooperative Oncology Group.

**TABLE 5** Pooled multivariable analysis including the interaction term between treatment modality and smoking status

	<b>Objective response rate</b>	<b>Progression-free survival</b>	<b>Overall survival</b>
Variable (comparator)	OR (95% CI); <i>p</i> -value	HR (95% CI); <i>p</i> -value	HR (95% CI); <i>p</i> -value
Treatment modality (chemotherapy vs. pembrolizumab)	0.79 (0.61–1.03); <i>p</i> = 0.0799	1.93 (1.67–2.23); <i>p</i> < 0.0001	1.27 (1.07–1.51); <i>p</i> = 0.0055
Smoking status (never vs. current/former)	0.68 (0.41–1.12); <i>p</i> = 0.1236	1.71 (1.32–2.25); <i>p</i> < 0.0001	1.51 (1.12–2.04); <i>p</i> = 0.0060
Interaction smoking status*treatment modality	<i>p</i> = 0.0074	<i>p</i> = 0.0001	<i>p</i> = 0.0020
ECOG PS (≥ 2 vs. 0–1)	0.46 (0.31–0.67); <i>p</i> = 0.0001	2.39 (2.01–2.85); <i>p</i> < 0.0001	2.88 (2.37–3.49); <i>p</i> < 0.0001
Gender (male vs. female)	0.98 (0.75–1.26); <i>p</i> = 0.8317	1.04 (0.90–1.21); <i>p</i> = 0.5111	1.12 (0.94–1.33); <i>p</i> = 0.1966
Age (elderly vs. nonelderly)	0.83 (0.66–1.06); <i>p</i> = 0.1295	1.08 (0.94–1.23); <i>p</i> = 0.2531	1.15 (0.99–1.35); <i>p</i> = 0.0650
CNS metastases (yes vs. no)	0.99 (0.72–1.35); <i>p</i> = 0.9193	1.17 (0.99–1.39); <i>p</i> = 0.0611	1.19 (0.97–1.45); <i>p</i> = 0.0861
Liver metastases (yes vs. no)	0.64 (0.45–0.91); <i>p</i> = 0.0124	1.63 (1.37–1.93); <i>p</i> < 0.0001	1.51 (1.24–1.85); <i>p</i> < 0.0001
Bone metastases (yes vs. no)	0.51 (0.38–0.66); <i>p</i> < 0.0001	1.53 (1.33–1.77); <i>p</i> < 0.0001	1.57 (1.33–1.85); <i>p</i> < 0.0001
<b>BMI</b>			
Normal weight (comparator)			
Underweight	0.53 (0.27–1.01); <i>p</i> = 0.0520	1.26 (0.91–1.74); <i>p</i> = 0.1619	0.97 (0.65–1.44); <i>p</i> = 0.9062
Overweight	0.78 (0.59–1.02); <i>p</i> = 0.0612	0.98 (0.85–1.14); <i>p</i> = 0.8728	0.91 (0.76–1.08); <i>p</i> = 0.3176
Obese	1.41 (0.98–2.04); <i>p</i> = 0.0665	0.81 (0.66–1.01); <i>p</i> = 0.0620	0.89 (0.69–1.14); <i>p</i> = 0.3776
<b>Histology</b>			
Nonsquamous vs. squamous	1.07 (0.81–1.42); <i>p</i> = 0.6202	0.85 (0.73–0.99); <i>p</i> = 0.0483	0.94 (0.78–1.14); <i>p</i> = 0.5765

Abbreviations: CNS, central nervous system; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio.

Table 5 summarizes the multivariable regression analyses from the pooled population for ORR, PFS and OS including all the baseline patient characteristics. At the pooled analysis, the interaction term between the smoking status and treatment modality was concordantly statistically significant with respect to ORR ( $p = 0.0074$ ), PFS ( $p = 0.0001$ ) and OS ( $p = 0.0020$ ), confirming the significantly different impact of smoking status across the two cohorts.

## DISCUSSION

The primary aim of this analysis was to further evaluate the opposite role of the smoking status according to the first-line treatment modality in NSCLC patients. The fixed multivariable analyses confirmed that never smokers had significantly shortened PFS and OS compared to current/former smokers among NSCLC patients with PD-L1 expression  $\geq 50\%$  receiving first-line pembrolizumab. On the contrary, a trend towards prolonged PFS and OS was reported for never smoker patients receiving first-line platinum-based chemotherapy. Of note, never smokers achieved a prolonged OS within the chemotherapy cohort, despite 58.5% of patients receiving PD-1/PD-L1 checkpoint inhibitors as a later line of treatment. Even though a significantly lower ORR was reported for never smokers on the univariable analysis in the pembrolizumab cohort, no further significant associations between smoking habit and ORR were found.

The random case-control matching strengthened our findings with regard to PFS. Never smokers had a significantly shorter PFS and a trend towards a shortened OS within the matched pembrolizumab cohort. Conversely, significantly longer PFS and OS were reported for never smokers, compared to current/former smokers, within the matched chemotherapy cohort. Finally, the concordantly statistically significant interaction term between the treatment modality (pembrolizumab vs. chemotherapy) and smoking status with respect to ORR, PFS and OS at the pooled analysis, further confirmed the differential role of the smoking status between the cohorts, regardless of any other baseline characteristics.

The tumor mutational burden (TMB) has been already proposed as an agnostic predictive biomarker for PD-1 checkpoint inhibitors across different malignancies, even though its applicability in the real-life context is still controversial.<sup>13-15</sup> Nevertheless, the TMB could have its own complementary and independent role from PD-L1 immunohistochemical evaluation.<sup>16,17</sup> It has been reported that smoking-induced carcinogenesis is associated with a higher TMB,<sup>18</sup> to such an extent that it has been assumed that smoking-related lung cancer is more likely to be immunogenic.<sup>19</sup> Interestingly, Rizvi et al. reported that a smoking-associated genomic signature, characterized by high frequency of transversion, was significantly associated to improved ORR and PFS among 34 advanced NSCLC patients treated with pembrolizumab, whilst the self-reported

smoking history did not significantly predict the clinical outcome within the same population.<sup>20</sup> Recently, Gainor et al. reported that among NSCLC patients with PD-L1 expression  $\geq 50\%$  receiving first-line single agent pembrolizumab, heavy smokers experienced numerically better outcomes compared to never/light smokers.<sup>21</sup> Moreover, they confirmed that the TMB was higher within heavy smoker patients, compared to light/never smokers, while no significant differences were found between light and never smokers.<sup>21</sup> In addition, we have to consider that tobacco smoking exposure has been also associated with increasing *in vivo* and *in vitro* intratumoral PD-L1 expression.<sup>22</sup> Concordantly, we previously reported a significant trend towards an increased PD-L1 expression according to the smoking status (never, former and current smokers) within our study population.<sup>6</sup>

In the context of single-agent pembrolizumab, current/former smokers have already been confirmed to experience improved ORR and prolonged survival within the phase I Keynote 001 trial population.<sup>23,24</sup> Similarly, the subgroup analysis of the Keynote 024 trial revealed that the survival benefit for single agent pembrolizumab over chemotherapy in NSCLC patients with high PD-L1 expression was greater for former smokers, compared to current and never smokers.<sup>25</sup> On the contrary, in the Keynote 189 trial, the subgroup analysis showed no significant differences according to smoking status. However, the survival benefit for the experimental arm (chemotherapy/pembrolizumab) over the control arm (chemotherapy/placebo) was greater for never smokers (HR for death 0.23), compared to current/former smokers (HR for death 0.54), appearing that the addition of chemotherapy had flattened the smoking-related effects on immunotherapy.<sup>26</sup> Intriguingly, the TMB was not significantly associated with efficacy in both arms of the same trial population.<sup>27</sup>

From this perspective, considering the smoking status as an easily available surrogate for the underlying TMB, it might be used to assist clinicians in the decision-making process for first-line treatment. With that in mind, a combination approach, rather than single agent pembrolizumab, might be taken into consideration with greater solicitude in never smoker patients with high PD-L1 expression, compared to former/current smokers.

Certainly, we are a long way from minimizing the strong negative role of smoking overall. In fact, in this study population we already confirmed that former smokers experienced the best outcome, compared to current and never smokers,<sup>6</sup> suggesting the presence of an underlying global/functional benefit from smoking cessation, without impairing the TMB-gain related to the smoking habit.

Several study limitations have to be acknowledged beyond the retrospective design and consequent selection biases. The biggest flaw in the study was the lack of information regarding quantification of the smoking status. For a proper estimation of its effect, it should have been classified in a more quantitative way (e.g., pack per year), as has already been determined in other studies.<sup>21</sup> Moreover, we were not able to separately assess former/current smokers within the chemotherapy cohort because this analysis was



not preplanned. Additionally, the chemotherapy cohort was not powered to detect significant findings according to smoking categories and being a historic cohort we did not have data regarding PD-L1 expression. However, considering the real-world prevalence of PD-L1 expression in NSCLC, we assumed that one third of the patients in the chemotherapy cohort had a PD-L1 expression  $\geq 50\%$ .<sup>28</sup> TMB is not routinely assessed in clinical practice in Europe, and therefore we were unable to perform a correlation analyses. Moreover, we should also consider the true incidence of oncogene addiction in NSCLC beyond *EGFR*, *ALK* and *ROS-1*, which are regularly evaluated as it is known that oncogene addiction is inversely related with smoking status and immunotherapy efficacy.<sup>29</sup> Additional limitations include the lack of available data regarding comorbidities which might have been affected by smoking habit.

In conclusion, our study confirmed that current/former smoker NSCLC patients with PD-L1 expression  $\geq 50\%$  receiving first-line single agent pembrolizumab experienced improved PFS and OS compared to never smokers, whilst the opposite trend was found within NSCLC patients treated with first-line platinum-based chemotherapy. The random case-control matching and the pooled analysis further strengthened our results on the opposite role of smoking during immunotherapy and chemotherapy. The putative predictive role of the smoking status in this setting needs to be assessed in prospective controlled trials.

## ACKNOWLEDGMENTS

A special thanks to the “Consorzio Interuniversitario Nazionale per la Bio-Oncologia” for their support in this study.

## CONFLICT OF INTEREST

Dr Alessio Cortellini received speaker fees and grant consultancies by Astrazeneca, MSD, BMS, Roche, Novartis, Istituto Gentili and Astellas. Dr Alessandro Leonetti received speaker fees by Astrazeneca. Dr Raffaele Giusti received speaker fees and grant consultancies by Astrazeneca and Roche. Dr Alex Friedlaender received grant consultancies by Roche, Pfizer, Astellas and BMS. Dr Alfredo Addeo received grant consultancies by Takeda, MSD, BMJ, Astrazeneca, Roche and Pfizer. Dr Rita Chiari received speaker fees by BMS, MSD, Takeda, Pfizer, Roche and Astrazeneca. Dr Carlo Genova received speaker fees/grant consultancies by Astrazeneca, BMS, Boehringer-Ingelheim, Roche and MSD.

## ORCID

Alessio Cortellini  <https://orcid.org/0000-0002-1209-5735>

Marco Filetti  <https://orcid.org/0000-0002-4734-7541>

## REFERENCES

1. Remon J, Passiglia F, Ahn MJ, Barlesi F, Forde PM, Garon EB, et al. Immune checkpoint inhibitors in thoracic malignancies: review of the existing evidence by an IASLC expert panel and recommendations. *J Thorac Oncol*. 2020;15(6):914–947. <https://doi.org/10.1016/j.jtho.2020.03.006>.
2. Ashraf-Uz-Zaman M, Bhalerao A, Mikelis CM, Cucullo L, German NA. Assessing the current state of lung cancer chemoprevention: a comprehensive overview. *Cancers (Basel)*. 2020;12(5):1265. <https://doi.org/10.3390/cancers12051265>.
3. El-Osta H, Jafri S. Predictors for clinical benefit of immune checkpoint inhibitors in advanced non-small-cell lung cancer: a meta-analysis. *Immunotherapy*. 2019;11(3):189–99.
4. Kim JH, Kim HS, Kim BJ. Prognostic value of smoking status in non-small-cell lung cancer patients treated with immune checkpoint inhibitors: a metaanalysis. *Oncotarget*. 2017;8(54):93149–55.
5. Cortellini A, Tiseo M, Banna GL, Cappuzzo F, Aerts JGTV, Barbieri F, et al. Clinicopathologic correlates of first-line pembrolizumab effectiveness in patients with advanced NSCLC and a PD-L1 expression of  $\geq 50\%$ . *Cancer Immunol Immunother*. 2020;69:2209–2221. <https://doi.org/10.1007/s00262-020-02613-9>.
6. Cortellini A, Friedlaender A, Banna GL, Porzio G, Bersanelli M, Cappuzzo F, et al. Immune-related adverse events of Pembrolizumab in a large real-world cohort of patients with NSCLC with a PD-L1 expression  $\geq 50\%$  and their relationship with clinical outcomes. *Clin Lung Cancer*. 2020;21(6):498–508. <https://doi.org/10.1016/j.clcc.2020.06.010>.
7. Cortellini A, Ricciuti B, Tiseo M, Bria E, Banna GL, Aerts JGTV, et al. Baseline BMI and BMI variation during first line pembrolizumab in NSCLC patients with a PD-L1 expression  $\geq 50\%$ : a multicenter study with external validation. *J Immunother Cancer*. 2020;8(2):e001403. <https://doi.org/10.1136/jitc-2020-001403>.
8. Woolley KK. How variables uncorrelated with the dependent variable can actually make excellent predictors: the important suppressor variable case. Southwest Educational Research Association Annual Meeting proceedings. 1997. <https://eric.ed.gov/?id=ED407420>. Accessed April 20, 2020.
9. Thompson FT, Levine DU. Examples of easily explainable suppressor variables in multiple regression research. *Multi Lin Regres Viewpoints*. 1997;24:11–3.
10. “Stopping stepwise: Why stepwise selection is bad and what you should use instead”. On towardsdatascience.com website. Available at: <https://towardsdatascience.com/stopping-stepwise-why-stepwise-selection-is-bad-and-what-you-should-use-instead-90818b3f52df>. Accessed March 29, 2020.
11. Gridelli C, Balducci L, Ciardiello F, di Maio M, Felip E, Langer C, et al. Treatment of elderly patients with non-small-cell lung cancer: results of an international expert panel meeting of the Italian Association of Thoracic Oncology. *Clin Lung Cancer*. 2015;16(5):325–33.
12. Austin PC. Optimal caliper widths for propensity-score matching when estimating differences in means and differences in proportions in observational studies. *Pharm Stat*. 2011;10(2):150–61. <https://doi.org/10.1002/pst.433>.
13. Willis C, Fiander M, Tran D, Korytowsky B, Thomas J-M, Calderon F, et al. Tumor mutational burden in lung cancer: a systematic literature review. *Oncotarget*. 2019;10(61):6604–22.
14. Subbiah V, Solit DB, Chan TA, Kurzrock R. The FDA approval of pembrolizumab for adult and pediatric patients with tumor mutational burden (TMB)  $\geq 10$ : a decision centered on empowering patients and their physicians. *Ann Oncol*. 2020;31(9):1115–8. <https://doi.org/10.1016/j.annonc.2020.07.002>.
15. Prasad V, Addeo A. The FDA approval of pembrolizumab for patients with TMB  $>10$  Mut/Mb: was it a wise decision? No. *Ann Oncol*. 2020;31(9):1112–4.
16. Ricciuti B, Mahadevan N, Umeton R. 246 Clinicopathologic and genomic correlates of tumor mutational burden and its impact on PD-(L)1 inhibition efficacy in non-small cell lung cancer according to different PD-L1 expression subgroups. *Journal for ImmunoTherapy of Cancer*. 2020;8. <https://doi.org/10.1136/jitc-2020-SITC2020.0246>.
17. Hellmann MD, Nathanson T, Rizvi H, Creelan BC, Sanchez-Vega F, Ahuja A, et al. Genomic features of response to combination immunotherapy in patients with advanced non-small-cell lung cancer. *Cancer Cell*. 2018;33(5):843–52.e4.

18. Hellmann MD, Ciuleanu TE, Pluzanski A, Lee JS, Otterson GA, Audigier-Valette C, et al. Nivolumab plus Ipilimumab in lung cancer with a high tumor mutational burden. *N Engl J Med*. 2018;378(22):2093–104.
19. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SAJR, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. *Nature*. 2013;500(7463):415–21.
20. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science (New York, NY)*. 2015;348(6230):124–8.
21. Gainor JF, Rizvi H, Jimenez Aguilar E, Skoulidis F, Yeap BY, Naidoo J, et al. Clinical activity of programmed cell death 1 (PD-1) blockade in never, light, and heavy smokers with non-small-cell lung cancer and PD-L1 expression  $\geq 50$ . *Ann Oncol*. 2020;31(3):404–11.
22. Wang GZ, Zhang L, Zhao XC, Gao SH, Qu LW, Yu H, et al. The aryl hydrocarbon receptor mediates tobacco-induced PD-L1 expression and is associated with response to immunotherapy. *Nat Commun*. 2019;10(1):1–13.
23. Leighl NB, Hellmann MD, Hui R, Carcereny E, Felip E, Ahn MJ, et al. Pembrolizumab in patients with advanced non-small-cell lung cancer (KEYNOTE-001): 3-year results from an open-label, phase 1 study. *Lancet Respir Med*. 2019;7(4):347–57.
24. Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med*. 2015;372(21):2018–28.
25. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. *N Engl J Med*. 2016;375(19):1823–33.
26. Gandhi L, Rodríguez-Abreu D, Gadgeel S, Esteban E, Felip E, de Angelis F, et al. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. *N Engl J Med*. 2018;378(22):2078–92.
27. Garassino M, Rodríguez-Abreu D, Gadgeel S, Esteban E, Felip E, Speranza G, et al. OA04.06 evaluation of TMB in KEYNOTE-189: Pembrolizumab plus chemotherapy vs placebo plus chemotherapy for nonsquamous NSCLC. *J Thorac Oncol*. 2019;14(10):S216–7.
28. Dietel M, Savelov N, Salanova R, Micke P, Bigras G, Hida T, et al. Real-world prevalence of programmed death ligand 1 expression in locally advanced or metastatic non-small-cell lung cancer: the global, multicenter EXPRESS study. *Lung Cancer*. 2019;134:174–9.
29. Smolle E, Leithner K, Olschewski H. Oncogene addiction and tumor mutational burden in non-small-cell lung cancer: clinical significance and limitations. *Thorac Cancer*. 2020;11(2):205–15.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Cortellini A, De Giglio A, Cannita K, et al. Smoking status during first-line immunotherapy and chemotherapy in NSCLC patients: A case-control matched analysis from a large multicenter study. *Thoracic Cancer*. 2021;12: 880–889. <https://doi.org/10.1111/1759-7714.13852>