

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

Alessio Cortellini^{1,2}  | Andrea De Giglio³ | Katia Cannita⁴ | Diego L. Cortinovis⁵ | Robin Cornelissen⁶ | Cinzia Baldessari⁷ | Raffaele Giusti⁸ | Ettore D'Argento⁹ | Francesco Grossi¹⁰ | Matteo Santoni¹¹ | Annamaria Catino¹² | Rossana Berardi¹³ | Vincenzo Sforza¹⁴ | Giovanni Rossi¹⁵ | Lorenzo Antonuzzo¹⁶ | Vincenzo Di Noia¹⁷ | Diego Signorelli¹⁸ | Alain Gelibter¹⁹ | Mario Alberto Occhipinti¹⁹ | Alessandro Follador²⁰ | Francesca Rastelli²¹ | Rita Chiari²² | Luigi Della Grava²³ | Alessandro Inno²⁴ | Michele De Tursi²⁵ | Pietro Di Marino²⁶ | Giovanni Mansueto²⁷ | Federica Zoratto²⁸ | Marco Filetti⁸  | Michele Montrone¹² | Fabrizio Citarella²⁹ | Maria Vittoria Pensieri^{1,2} | Marco Russano²⁹ | Luca Cantini^{6,13} | Olga Nigro³⁰ | Alessandro Leonetti³¹ | Paola Bordi³¹ | Gabriele Minuti³² | Lorenza Landi³ | Alessandro De Toma¹⁸ | Clelia Donisi³³ | Serena Ricciardi³⁴ | Maria Rita Migliorino³⁴ | Valerio Maria Napoli³⁵ | Gianmarco Leone³⁵ | Giulio Metro³⁶ | Giuseppe L. Banna³⁷ | Alex Friedlaender³⁸ | Alfredo Addeo³⁸ | Corrado Ficorella^{2,4} | Giampiero Porzio⁴

¹Department of Surgery and Cancer, Imperial College London, London, United Kingdom

²Department of Biotechnology and Applied Clinical Sciences, University of L'Aquila, L'Aquila, Italy

³Division of Medical Oncology, S.Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy

⁴Medical Oncology, St. Salvatore Hospital, L'Aquila, Italy

⁵Medical Oncology, Ospedale San Gerardo, Monza, Italy

⁶Department of Pulmonary Diseases, Erasmus Medical Center, Rotterdam, The Netherlands

⁷Department of Oncology and Hematology, Modena University Hospital, Modena, Italy

⁸Medical Oncology, St. Andrea Hospital, Rome, Italy

⁹Comprehensive Cancer Center, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Rome, Italy

¹⁰Medical Oncology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

¹¹Medical Oncology, Hospital of Macerata, Macerata, Italy

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

¹³Oncology Clinic, Università Politecnica Delle Marche, Ospedali Riuniti Di Ancona, Ancona, Italy

¹⁴Thoracic Medical Oncology, Istituto Nazionale Tumori "Fondazione G. Pascale", IRCCS, Naples, Italy

¹⁵Lung Cancer Unit, IRCCS Ospedale Policlinico San Martino, Genoa, Italy

¹⁶Department 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 6th author's surname was corrected from 'Baldezarri' to 'Baldessari'.]

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- ¹⁷Unità di Oncologia medica e Terapia Biomolecolare, Azienda Ospedaliero-Universitaria Ospedali Riuniti di Foggia, Foggia, Italy
- ¹⁸Department of Medical Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy
- ¹⁹Medical Oncology (B), Policlinico Umberto I, "Sapienza" University of Rome, Rome, Italy
- ²⁰Department of Oncology, University Hospital Santa Maria Della Misericordia, Udine, Italy
- ²¹Medical Oncology, Fermo Area Vasta 4, Fermo, Italy
- ²²Medical Oncology, Ospedali Riuniti Padova Sud "Madre Teresa Di Calcutta", Monselice, Italy
- ²³Pneumo-Oncology Unit, Monaldi Hospital, Naples, Italy
- ²⁴Oncology Unit, IRCCS Ospedale Sacro Cuore Don Calabria, Negrar, Italy
- ²⁵Department of Medical, Oral & Biotechnological Sciences, University G. D'Annunzio, Chieti-Pescara, Chieti, Italy
- ²⁶Clinical Oncology Unit, S.S. Annunziata Hospital, Chieti, Italy
- ²⁷Medical Oncology, F. Spaziani Hospital, Frosinone, Italy
- ²⁸Medical Oncology, Santa Maria Goretti Hospital, Latina, Italy
- ²⁹Medical Oncology, Campus Bio-Medico University, Rome, Italy
- ³⁰Medical Oncology, ASST-Sette Laghi, Varese, Italy
- ³¹Medical Oncology Unit, University Hospital of Parma, Parma, Italy
- ³²Department of Oncology and Hematology, AUSL Romagna, Ravenna, Italy
- ³³Medical Oncology Unit, University Hospital and University of Cagliari, Cagliari, Italy
- ³⁴Pneumo-Oncology Unit, St. Camillo-Forlanini Hospital, Rome, Italy
- ³⁵Department of Oncology, University of Turin, San Luigi Hospital, Orbassano, Italy
- ³⁶Department of Medical Oncology, Santa Maria della Misericordia Hospital, Azienda Ospedaliera di Perugia, Perugia, Italy
- ³⁷Oncology Department, Queen Alexandra University Hospital, Portsmouth Hospitals NHS Trust, Portsmouth, UK
- ³⁸Oncology 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.¹ 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.² 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.³ Intriguingly, a meta-analysis has also suggested that checkpoint inhibitors significantly improve survival over chemotherapy in smoker patients only.⁴

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.^{5,6} 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.⁵ 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.⁷

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.^{5–7} 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.^{5–7}

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.^{8–10} The key covariates were: age (<70 vs. ≥ 70 years old),¹¹ gender (male vs. female), Eastern Cooperative Oncology Group–Performance Status (ECOG-PS) (0–1 vs. ≥ 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. ≥ 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 ≥ 30).⁷

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. χ^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.¹² 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,⁷ 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	Pembrolizumab cohort			Chemotherapy cohort		
	Response/ratio	ORR (95% CI)	χ^2 test	Response/ratio	ORR (95% CI)	χ^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)		26/47	55.3% (36.1–81.1)	
	PFS (95% CI) (events)		HR (95% CI)	PFS (95% CI) (events)		HR (95% CI)
Former/current 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); $p = 0.0296$
Never smokers	4.1 months (2.7–5.7) (73)			7.5 months (4.7–10.8) (43)		
	OS (95% CI) (censored)		HR (95% CI); p - value	OS (95% CI) (censored)		HR (95% CI)
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)		0.66 (0.45–0.96); $p = 0.0339$
Never smokers	9.4 months (6.9–15.0) [45]			20.0 months (11.8–31.8) (17)		

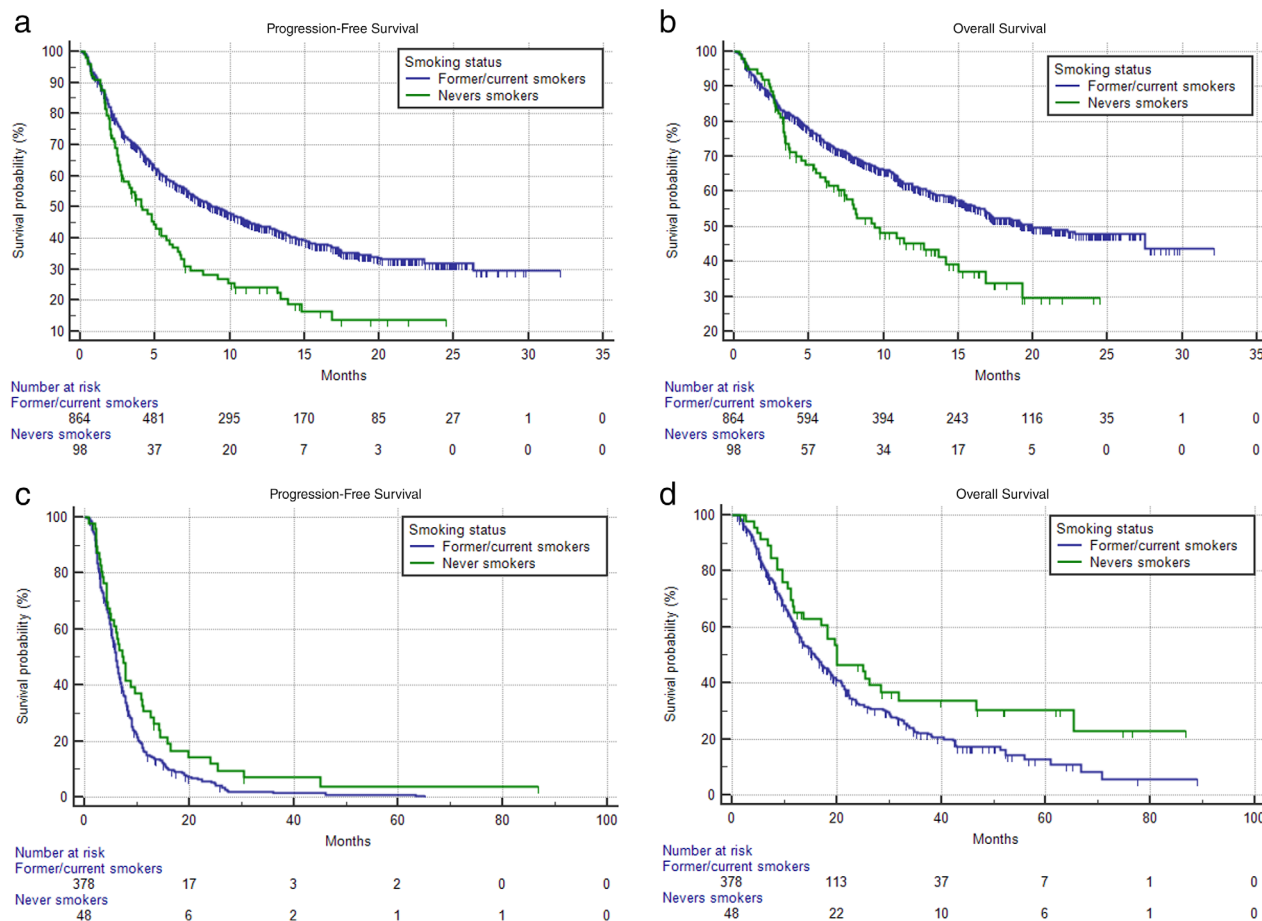
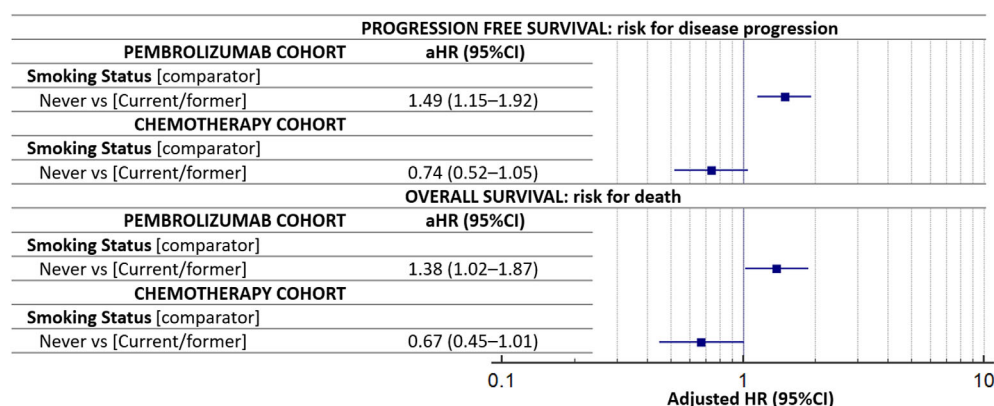


FIGURE 1 Kaplan–Meier survival curves according to smoking status. Pembrolizumab cohort (a) progression-free survival (PFS); and (b) overall survival (OS); chemotherapy cohort (c) PFS and (d) OS. See Table 1 for survival estimations

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) compared to current/former smoker patients within the matched chemotherapy cohort.

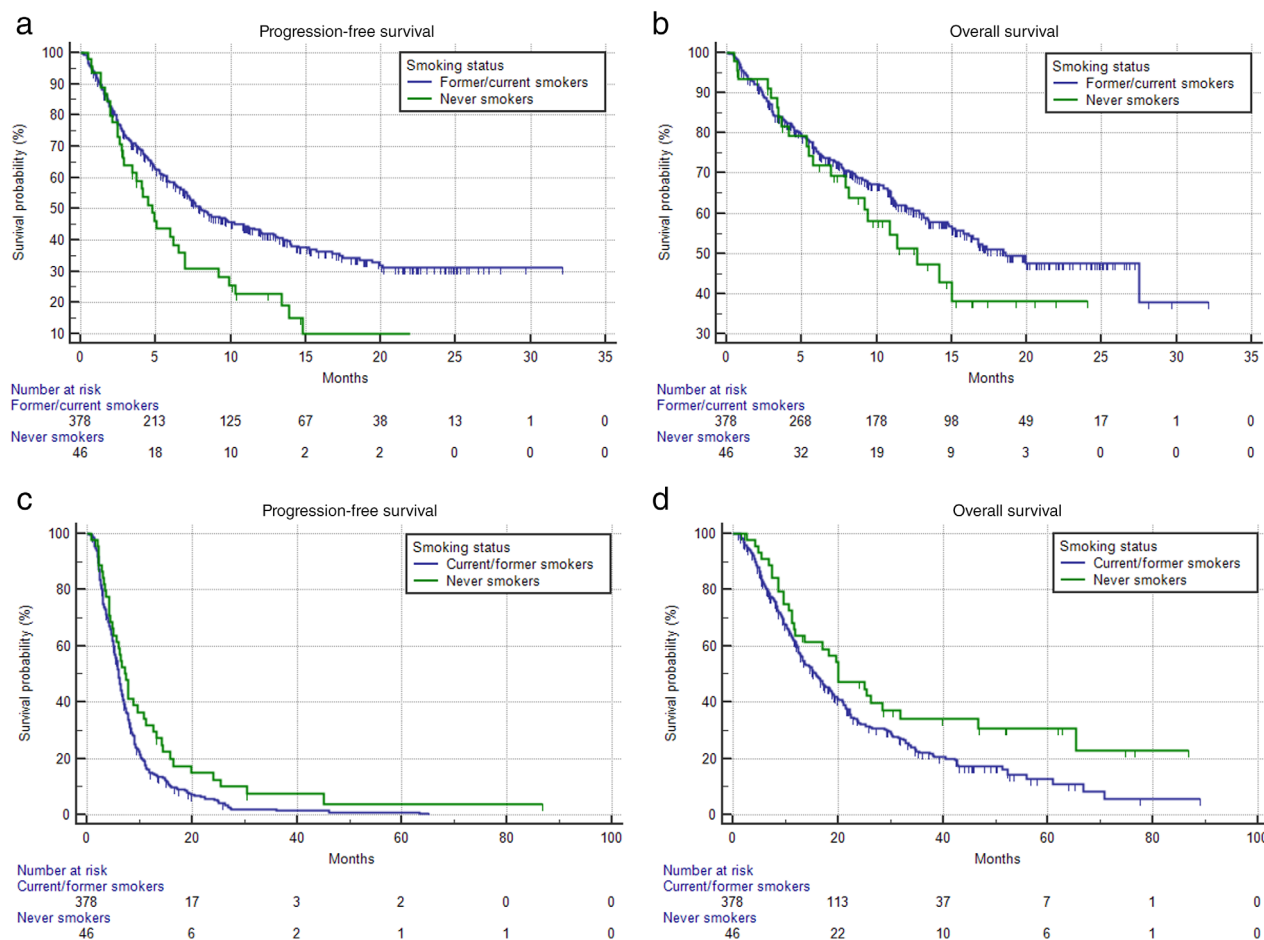


FIGURE 3 Kaplan–Meier survival curves according to smoking status within the randomly matched cohorts; Pembrolizumab cohort PFS. (a) Never smokers 4.7 months (95% CI: 2.8–6.9; 35 progression events), current/former smokers 8.0 months (95% CI: 8.9–10.8; 217 progression events) ($p = 0.0045$). OS. (b) 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 ≥ 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

	Pembrolizumab cohort Progression-free survival	Chemotherapy cohort Progression-free survival
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

	Pembrolizumab cohort Overall survival	Chemotherapy cohort Overall survival
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

	Objective response rate	Progression-free survival	Overall survival
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
BMI			
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
Histology			
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.^{13–15} Nevertheless, the TMB could have its own complementary and independent role from PD-L1 immunohistochemical evaluation.^{16,17} It has been reported that smoking-induced carcinogenesis is associated with a higher TMB,¹⁸ to such an extent that it has been assumed that smoking-related lung cancer is more likely to be immunogenic.¹⁹ 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.²⁰ 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.²¹ 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.²¹ 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.²² 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.⁶

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.^{23,24} 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.²⁵ 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.²⁶ Intriguingly, the TMB was not significantly associated with efficacy in both arms of the same trial population.²⁷

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 combinational 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,⁶ 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.²¹ 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 $\geq 50\%$.²⁸ 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.²⁹ 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.

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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>

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SUPPORTING INFORMATION

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

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