European Journal of Cancer 148 (2021) 24–35

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Original Research

Post-progression outcomes of NSCLC patients with PD-L1 expression ≥ 50% receiving first-line single-agent pembrolizumab in a large multicentre real-world study

Alessio Cortellini [a,b,*], Katia Cannita [c], Marcello Tiseo [d,e], Diego L. Cortinovis [f], Joachim G.J.V. Aerts [g], Cinzia Baldessari [h], Raffaele Giusti [i], Miriam G. Ferrara [j,k], Ettore D'Argento [j], Francesco Grossi [l], Annalisa Guida [m], Rossana Berardi [n], Alessandro Morabito [o], Carlo Genova [p], Lorenzo Antonuzzo [q], Francesca Mazzoni [q], Alessandro De Toma [r], Diego Signorelli [r,s], Alain Gelibter [t], Giada Targato [u], Francesca Rastelli [v], Rita Chiari [w], Danilo Rocco [x], Stefania Gori [y], Michele De Tursi [z], Giovanni Mansueto [aa], Federica Zoratto [ab], Marco Filetti [i], Sergio Bracarda [ac], Fabrizio Citarella [ad], Marco Russano [ad], Luca Cantini [g,n], Olga Nigro [ae], Sebastiano Buti [e], Gabriele Minuti [af], Lorenza Landi [af], Serena Ricciardi [ag], Maria R. Migliorino [ag], Salvatore Natalizio [ah], Carnio Simona [ai], Marco De Filippis [ai], Giulio Metro [aj], Vincenzo Adamo [ak], Alessandro Russo [ak], Gian P. Spinelli [al], Massimo Di Maio [am], Giuseppe L. Banna [an], Alex Friedlaender [ao], Alfredo Addeo [ao], David J. Pinato [b,ap], Corrado Ficorella [a,c], Giampiero Porzio [c]

[a] *Department of Biotechnology and Applied Clinical Sciences, University of L'Aquila, L'Aquila, Italy*
[b] *Division of Cancer, Department of Surgery and Cancer, Imperial College London, Hammersmith Hospital, London, UK*
[c] *Medical Oncology, St. Salvatore Hospital, L'Aquila, Italy*
[d] *Department of Medicine and Surgery, University of Parma, Parma, Italy*
[e] *Medical Oncology Unit, University Hospital of Parma, Parma, Italy*
[f] *Medical Oncology, Ospedale San Gerardo, Monza, Italy*
[g] *Department of Pulmonary Diseases, Erasmus Medical Center, Rotterdam, the Netherlands*
[h] *Dipartimeto di Oncologia Ed Ematologia, AOU Policlinico Modena, Modena, Italy*
[i] *Medical Oncolgy, St. Andrea Hospital, Rome, Italy*
[j] *Comprehensive Cancer Center, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Rome, Italy*

* *Corresponding author*: Imperial College London, Department of Surgery & Cancer, Room 138 ICTEM Building Level 1, Hammersmith Hospital Du Cane Road, London, W12 0HS, UK.
E-mail addresses: a.cortellini@imperial.ac.uk, alessiocortellini@gmail.com (A. Cortellini)

https://doi.org/10.1016/j.ejca.2021.02.005

[k] Department of Translational Medicine and Surgery, Università Cattolica Del Sacro Cuore, Romae, Lazio, Italy
[l] Medical Oncology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[m] Department of Oncology, Azienda Ospedaliera Santa Maria, Terni, Italy
[n] Oncology Clinic, Università Politecnica Delle Marche, Ospedali Riuniti Di Ancona, Ancona, Italy
[o] Thoracic Medical Oncology, Istituto Nazionale Tumori 'Fondazione G Pascale', IRCCS, Napoli, Italy
[p] Lung Cancer Unit, IRCCS Ospedale Policlinico San Martino, Genova, Italy
[q] Department of Oncology, Careggi University Hospital, Florence, Italy
[r] Department of Medical Oncology, Fondazione IRCCS Istituto Nazionale Dei Tumori, Milan, Italy
[s] Niguarda Cancer Center, Grande Ospedale Metropolitano Niguarda, Milan, Italy
[t] Medical Oncology (B), Policlinico Umberto I, "Sapienza" University of Rome, Rome, Italy
[u] Department of Oncology, University Hospital Santa Maria Della Misericordia, Udine, Italy
[v] Medical Oncology, Fermo Area Vasta 4, Fermo, Italy
[w] Medical Oncology, Ospedali Riuniti Padova Sud "Madre Teresa Di Calcutta", Monselice, Italy
[x] Pneumo-Oncology Unit, Monaldi Hospital, Naples, Italy
[y] Oncology Unit, IRCCS Ospedale Sacro Cuore Don Calabria, Negrar, VR, Italy
[z] Department of Medical, Oral & Biotechnological Sciences University G. D'Annunzio, Chieti-Pescara, Chieti, Italy
[aa] Medical Oncology, F. Spaziani Hospital, Frosinone, Italy
[ab] Medical Oncology, Santa Maria Goretti Hospital, Latina, Italy
[ac] Struttura Complessa di Oncologia Medica e Traslazionale, Azienda Ospedaliera Santa Maria di Terni, Italy
[ad] Medical Oncology, Campus Bio-Medico University, Rome, Italy
[ae] Medical Oncology, ASST-Sette Laghi, Varese, Italy
[af] Department of Oncology and Hematology, AUSL Romagna, Ravenna, Italy
[ag] Pneumo-Oncology Unit, St. Camillo-Forlanini Hospital, Rome, Italy
[ah] Dipartimento di Oncologia Ed Ematologia, Universitá Modena e Raggio Emilia, Modena, Italy
[ai] Department of Oncology, University of Turin, San Luigi Hospital, Orbassano, TO, Italy
[aj] Department of Medical Oncology, Santa Maria Della Misericordia Hospital, Azienda Ospedaliera di Perugia, Perugia, Italy
[ak] Medical Oncology, A.O. Papardo & Department of Human Pathology, University of Messina, Italy
[al] UOC Territorial Oncology of Aprilia, AUSL Latina, University of Rome Sapienza, Aprilia, Italy
[am] Department of Oncology, University of Turin and Medical Oncology, AO Ordine Mauriziano, Turin, Italy
[an] Portsmouth Hospitals University NHS Trust, Portsmouth, UK
[ao] Oncology Department, University Hospital of Geneva, Geneva, Switzerland
[ap] Department of Translational Medicine, Università Del Piemonte Orientale "A. Avogadro", Novara, Italy

Received 27 November 2020; received in revised form 6 February 2021; accepted 11 February 2021
Available online 12 March 2021

KEYWORDS
Non-small cell lung cancer;
Immunotherapy;
PD-L1;
Pembrolizumab;
Performance status;
Post-progression;
Radiotherapy;
Radiation therapy

Abstract ***Background:*** Treatment sequencing with first-line immunotherapy, followed by second-line chemotherapy, is still a viable option for NSCLC patients with PD-L1 expression ≥50%.

Methods: We evaluated post-progression treatment pathways in a large real-world cohort of metastatic NSCLC patients with PD-L1 expression ≥ 50% treated with first-line pembrolizumab monotherapy.

Results: Overall, 974 patients were included. With a median follow-up of 22.7 months (95%CI: 21.6–38.2), the median overall survival (OS) of the entire population was 15.8 months (95% CI: 13.5–17.5; 548 events). At the data cutoff, among the 678 patients who experienced disease progression, 379 (55.9%) had not received any further treatment, and 359 patients (52.9%) had died. Patients who did not receive post-progression therapies were older ($p = 0.0011$), with a worse ECOG-PS ($p < 0.0001$) and were on corticosteroids prior to pembrolizumab ($p = 0.0024$). At disease progression, 198 patients (29.2%) received a switched approach and 101 (14.9%) received pembrolizumab ByPD either alone (64 [9.4%]) or in combination with local ablative treatments (37 [5.5%]) (LATs). After a random-case control matching according to ECOG-PS, CNS metastases, bone metastases, and (previous) best response to pembrolizumab, patients receiving pembrolizumab ByPD plus LATs were confirmed to have a significantly longer post-progression OS compared to patients receiving pembrolizumab ByPD alone 13.9 months versus 7.8 months ($p = 0.0179$) 241 patients (35.5%) among the 678 who had experienced PD, received a second-line systemic treatment (regardless of previous treatment beyond PD). As compared to first-line treatment commencement, patients' features at the moment of second-line initiation showed a significantly higher proportion of patients aged under 70 years ($p = 0.0244$), with a poorer ECOG-PS ($p < 0.0001$) and having CNS ($p = 0.0001$), bone ($p = 0.0266$) and liver metastases ($p = 0.0148$).

Conclusions: In the real-world scenario NSCLC patients with PD-L1 expression ≥50% treated with first-line single-agent pembrolizumab achieve worse outcomes as compared to the Keynote-024 trial. Poor post-progression outcomes are major determinants of the global results that should be considered when counselling patients for first-line treatment choices.

1. Introduction

The Keynote-024 trial established single-agent pembrolizumab as the standard of care for advanced non-small-cell lung cancer (NSCLC) patients with programmed cell death-ligand1 (PD-L1) expression ≥50% [1,2]. However, since the Keynote-189 and Keynote-407 trials, this has been challenged by chemo-immunotherapy combinations [3,4], as no head-to-head randomised controlled trial (RCT) has compared the two strategies in the PD-L1 high subgroup.

Although some metanalyses suggested that in patients with high PD-L1 expression, there is an incremental benefit from the addition of chemotherapy to first-line immunotherapy, with respect to response rate and progression-free survival (PFS) [5–7], the increased toxicity of a triplet regimen compared to a single-agent immune-checkpoint inhibitor (ICI) should be considered in weighting oncological benefit against toxicity.

In this scenario, treatment sequencing with first-line immunotherapy, followed by second-line chemotherapy, might still be a viable option for patients with PD-L1 expression ≥50%. Post-progression analyses of RCTs revealed conflicting results on this subject. Among the 154 patients of the experimental arm of the Keynote-024 trial, 51.9% received a further treatment line at the last data-analysis [8], while 38% of the 637 patients of the experimental arm of the Keynote-042 trial received subsequent anticancer therapy [9].

In clinical practice, a non-negligible proportion of NSCLC patients experiences life-threatening progressive disease (PD), without reaching the subsequent treatment line. This is true in all treatment settings, including immunotherapy [10,11]. Recently, we published a large real-world multicentre study of metastatic NSCLC patients with PD-L1 expression ≥50%, receiving first-line single-agent pembrolizumab at 34 European institutions, aimed at investigating the clinicopathologic correlates of efficacy [12–15].

To provide further insights into clinical outcomes of NSCLC patients with high PD-L1 expression after disease progression, we performed an updated analysis of the aforementioned cohort, with a particular focus on post-progression outcomes.

2. Materials and methods

2.1. Study design

Following a request for data updating of the cohort of metastatic NSCLC patients with PD-L1 expression ≥50%, treated with first-line pembrolizumab monotherapy, from January 2017 to May 2020, 31 institutions participated (Supplementary file 1).

The aim of this analysis was to evaluate post-progression clinical outcomes, including both treatment beyond PD and further treatment lines. The measured clinical outcomes were post-progression overall survival (ppOS), second-line PFS (II line PFS), and second-line overall survival (II line OS). Methods regarding clinical outcomes estimation have already been detailed [12–15]. In order to be closer to the real-life scenario, both patients who experienced radiological progressive disease and those with clinical progression according to the investigators have been included.

PpOS was defined as the length of time between the first occurrence of PD during pembrolizumab and death (resulting from any cause), or to the last contact; ppOS was evaluated with univariable analyses, according to the therapeutic strategies chosen by clinicians at the moment of disease progression, categorised as patients who received pembrolizumab beyond PD (ByPD), (with or without local ablative treatments - LATs) and patients who received other post-progression systemic treatments (switched approach).

By considering the possible positive selection bias associated with oligo-progressive disease [16], investigators were also asked to clarify whether patients who received pembrolizumab ByPD had experienced oligo-progression (defined as progression of a single metastasis already present and/or progression that can be safely treated with ablative treatments).

The possible relationship between baseline patients' features and post-progression pathways (categorised as no post-progression treatments, pembrolizumab ByPD and switched approach) was evaluated. The following clinicopathologic characteristics were evaluated: age (<70 versus ≥70 years old) [17], gender (male versus female), Eastern Cooperative Oncology Group—PS (ECOG-PS) (0 versus 1 versus ≥2), central nervous system (CNS) metastases (yes versus no), bone

metastases (yes versus no), liver metastases (yes versus no), Body Mass Index (BMI) according to the World Health Organisation (WHO) categories [18,19], PD-L1 tumour expression (<90% versus ≥ 90%) [12], smoking status (current versus former versus never smoker) [12,15], and corticosteroids administration within the 30 days before treatment commencement (dose equivalent or higher to 10 mg prednisone per day) (yes versus no) [12].

Additionally, considering the limited sample size of the subgroups, a random case-control matching was also performed to better compare clinical outcomes of patients receiving pembrolizumab ByPD alone and those who received pembrolizumab ByPD plus LATs. Considering the retrospective design and data lack availability regarding patients characteristics at the moment of LATs delivery, all the cases (from the ByPD plus LATs group) and controls (from the ByPD alone group), were randomly paired on the basis of those baseline characteristics that might have influenced clinicians' choice at the moment of disease progression, including ECOG-PS (0–1 versus 2), CNS metastases (yes versus no), bone metastases (yes versus no), and (previous) best response to pembrolizumab (partial/complete response versus stable/progressive disease) [data not shown].

Further analyses were performed only among patients who received a second-line systemic treatment (regardless of previous treatments with pembrolizumab beyond PD). II line PFS was defined as the time from second-line treatment initiation, to disease progression/death (whichever occurred first) or to the last contact. II line OS was defined as the time from second-line treatment initiation, to death or to the last contact.

Second-line treatments were categorised as platinum-based doublet chemotherapy, single-agent chemotherapy and other regimens. Those patients' characteristics that could have changed over time, including ECOG-PS, age, CNS metastases, bone metastases and liver metastases, were re-assessed at the second line treatment commencement. All patients' features were then compared to their baseline distribution. For evaluating whether some of the clinical characteristics affected clinical outcomes, univariable and multivariable analyses of II line PFS and II line OS were performed (using a stepwise selection of covariates, with an entry significance level of 0.05). Having received previous pembrolizumab ByPD (yes versus no) was also considered as a covariate. Patients without events were considered to be censored at the time of the last follow-up. The data cutoff period was September 2020.

2.2. PD-L1 expression evaluation

PD-L1 expression analysis among the entire population has already been reported [12]. Considering that tumour proportion score (TPS) for PD-L1 expression has been validated with the 22C3 antibody only, we referred to 'PD-L1 expression' throughout the study [12,20]. All the immunohistochemical (IHC) analyses were performed locally at each participating institution, using a different antibodies and platforms according to their respective clinical practice (including 22C3 [60.4%], SP263 [32.1%], E1L3N [0.9%], 28-8 [1.7%], not available [4.9%]) [12]. Considering that in some institutions, the PD-L1 expression level is reported only as '≥50%', and not as a discrete value, only patients with data available regarding the absolute value of PD-L1 tumour staining have been included in the clinical outcome analysis according to PD-L1 expression [12]. Nevertheless, each of the recruited patients had a PD-L1 expression of ≥50%. We previously verified that 90% was the optimal threshold for clinical outcomes estimation according to PD-L1 tumour expression in the whole population [12], confirming its significant role in identifying patients with improved responses and survival, as also reported by Aguilar *et al.* [21]. Therefore, 90% was set as the cutoff for the present analysis.

2.3. Statistical analysis

Descriptive statistics were used to report patients' characteristics. Median ppOS, II line PFS and II line OS were evaluated using the Kaplan–Meier method. The median period of follow-up was calculated according to the reverse Kaplan–Meier method. $\chi 2$ test was used for the correlation analyses. The log-rank test was used for univariable analyses and Cox regression models were used for multivariable analyses and for the estimation of hazard ratios (HRs) with 95% confidence intervals (CIs). A caliper width of <1 for the standard deviation was used for the random case-control matching. All statistical analyses were performed using MedCalc Statistical Software version 18.11.3 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2019).

3. Results

3.1. Post-progression overall survival analysis

The entire cohort consisted of 974 metastatic NSCLC patients with PD-L1 expression ≥50%. With a median follow-up of 22.7 months (95%CI: 21.6–38.2), the median PFS and OS of the entire population were 7.0 months (95%CI: 6.1–8.2; 678 events) and 15.8 months (95%CI: 13.5–17.5; 548 events), respectively (Supplementary Fig. 1). At the data cut-off, 678 patients (69.6%) experienced disease progression; the post-progression median follow-up was 14.4 months (95% CI: 11.9–33.1). The absolute PD-L1 expression value was available for 488 (71.9%) out of 678 patients. Fig. 1 reports the study's flow diagram. Baseline characteristics

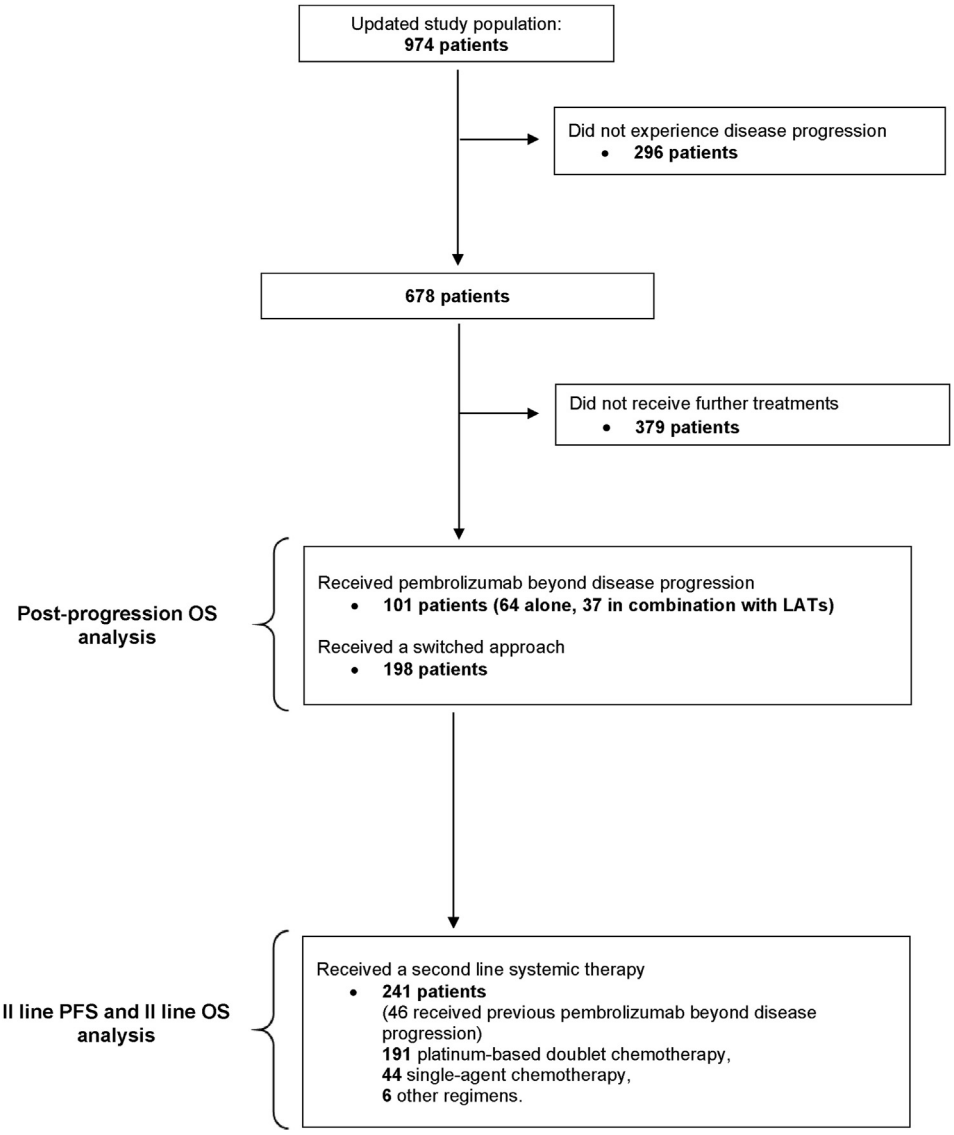

Fig. 1. Study's flow diagram.

of patients who experienced disease progression are summarised in Table 1.

At the data cut-off, among the 678 patients who experienced disease progression, 379 (55.9%) had not received any further treatment, and 359 patients (52.9%) had died. 198 patients (29.2%) received a switched approach and 101 (14.9%) received pembrolizumab ByPD either alone (64 [9.4%]) or in combination with LATs (37 [5.5%]) (Supplementary Fig. 2). Table 1 also reports the correlation analysis between baseline clinicopathologic characteristics and post-progression pathways. There was a significant association between older age ($p = 0.0011$), higher ECOG-PS ($p < 0.0001$), baseline corticosteroid administration ($p = 0.0024$) and not having received any post-progression treatment.

One patient (2.7%) received surgery, one patient (2.7%) received radiation therapy (RT) plus surgery and 35 patients (94.6%) received RT. Eighteen patients (28.1%) among those who received pembrolizumab ByPD alone, and 28 patients (75.7%) among those who received pembrolizumab ByPD in combination with LATs, were marked as oligo-progressive patients ($p < 0.0001$).

The median ppOS of patients who received a switched approach was 8.2 months (95%CI: 7.1–9.1; 131 events), while the median ppOS of those who received pembrolizumab ByPD alone and with the addition of LATs was 8.0 months (95%CI: 5.4–11.8; events) and 13.9 months (95%CI: 6.1–14.3; 18 events), respectively (log-rank test: $p = 0.0958$) (Fig. 2A).

3.2. Random case-control matching

After the case-control random matching, 35 patients from the pembrolizumab ByPD plus LATs and 35 patients from the pembrolizumab ByPD alone were

Table 1
Patients' characteristics.

	N° (%)				χ2 test
	678 patients	Post-progression outcome			
		No treatments 379	Pembrolizumab ByPD 101	Switched Approach 198	
AGE, (years)					
Median	70.2	71.4	69.6	67.2	P = 0.0011
Range	28–92	31–92	38–86	28–86	
Elderly (≥70)	347 (51.2)	216 (62.2)	50 (14.4)	81 (23.3)	
Smoking status					
Never smokers	85 (12.5)	48 (56.5)	14 (16.5)	23 (27.1)	P = 0.8182
Former smokers	370 (56.6)	201 (54.1)	54 (14.6)	115 (31.1)	
Current smokers	223 (32.9)	130 (58.3)	33 (14.8)	60 (26.9)	
SEX					
Male	449 (66.2)	246 (54.8)	68 (15.1)	135 (30.1)	P = 0.7090
Female	229 (33.8)	133 (58.1)	33 (14.4)	63 (27.5)	
ECOG PS					
0	180 (26.5)	61 (33.9)	47 (26.1)	72 (40.0)	P < 0.0001
1	353 (52.1)	199 (54.6)	45 (12.7)	109 (30.0)	
≥2	145 (21.4)	119 (82.1)	9 (6.2)	17 (11.7)	
Histology					
Squamous	156 (23.0)	97 (62.2)	18 (11.5)	41 (26.3)	P = 0.1690
Non-squamous	522 (77.0)	282 (54.0)	83 (15.9)	157 (30.1)	
PD-L1 expression [a]					
<90%	408 (83.6)	243 (59.6)	54 (13.2)	111 (27.2)	P = 0.6327
≥90%	80 (16.4)	52 (59.6)	10 (12.5)	18 (22.5)	
CNS metastases					
Yes	133 (19.6)	77 (57.9)	19 (14.3)	37 (27.8)	P = 0.8747
No	545 (80.4)	302 (55.4)	82 (15.0)	161 (29.5)	
Bone metastases					
Yes	257 (37.9)	144 (56.0)	38 (14.8)	75 (29.2)	P = 0.9976
No	421 (62.1)	235 (55.8)	63 (15.0)	123 (29.2)	
Liver metastases					
Yes	127 (18.7)	81 (63.8)	11 (8.7)	35 (27.6)	P = 0.0508
No	551 (81.3)	298 (54.1)	90 (16.3)	163 (29.6)	
Baseline corticosteroids					
Yes	190 (28.0)	126 (66.3)	24 (12.6)	40 (21.1)	P = 0.0024
No	488 (72.0)	253 (51.8)	77 (15.8)	158 (32.4)	
BMI (kg/m^2) [b]					
Median [range]	24.2 [14.0–44.9]	23.8 [14.0–44.9]	24.5 [16.6–38.1]	24.3 [16.2–43.5]	P = 0.4328
Underweight (≤18.5)	27 (4.4)	16 (59.3)	1 (3.7)	10 (37.0)	
Normal weight (18.5–25)	348 (56.4)	203 (58.3)	47 (13.5)	98 (28.2)	
Overweight (25–30)	177 (28.7)	93 (52.5)	25 (14.1)	59 (33.3)	
Obese (≥30)	65 (10.5)	37 (56.9)	12 (18.5)	16 (24.6)	

[a] Available for 488 patients.
[b] Available for 617 patients.

perfectly paired. Matched patients receiving pembrolizumab plus LATs achieved a median ppOS of 13.9 months (95%CI: 7.9–14.3; 17 events), while matched patients receiving pembrolizumab ByPD alone reported a median ppOS of 7.8 months (95%CI: 3.3–17.6; 22 events) (log-rank: p = 0.0179) (Fig. 2B).

3.3. Second line PFS and OS analysis

At the data cut off, 241 (35.5%) among the 678 patients who had experienced disease progression received a second-line systemic treatment; 191 patients (79.3%) received platinum-based doublet chemotherapy, 44 (18.3%) single-agent chemotherapy and 6 (2.5%) other regimens (Supplementary Fig. 2). Forty-six patients (19.1%) had received previous pembrolizumab ByPD.

Patients' characteristics at second-line commencement are summarised in Table 2. As compared to the baseline (at the first-line treatment commencement), there was a significantly higher proportion of patients aged under 70 years old (p = 0.0244), and having CNS (p = 0.0001), bone (p = 0.0266) and liver metastases (p = 0.0148). Noteworthy, at the second-line treatment commencement, there was also a significantly higher proportion of patients with a poorer ECOG-PS (p < 0.0001).

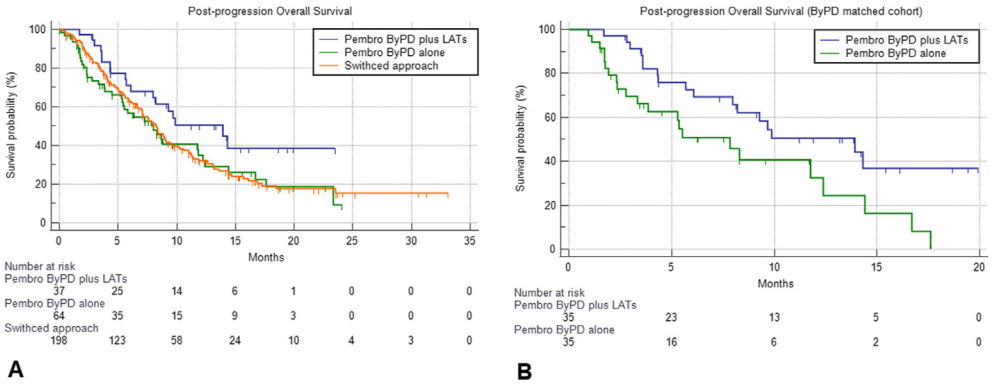

Fig. 2. Kaplan–Meier survival estimate of post-progression overall survival according to the therapeutic strategies chosen by clinicians at the moment of progressive disease (PD): patients who received pembrolizumab beyond PD (ByPD), (with or without local ablative treatments – LATs) and patients who received other post-progression systemic treatments (switched approach).

Table 2
Patients' characteristics at second-line treatment commencement.

	Baseline	II line setting	χ2 test
	678 N° (%)	241 N° (%)	
AGE, (years)			
Median	70.2	67.9	P = 0.0244
Range	28–92	29–86	
Elderly (≥70)	347 (51.2)	103 (42.7)	
ECOG PS			
0	180 (26.5)	28 (11.6)	P < 0.0001
1	353 (52.1)	156 (64.7)	
≥2	145 (21.4)	57 (23.7)	
CNS metastases			
Yes	133 (19.6)	78 (32.4)	P = 0.0001
No	545 (80.4)	163 (67.6)	
Bone metastases			
Yes	257 (37.9)	111 (46.1)	P = 0.0266
No	421 (62.1)	130 (53.9)	
Liver metastases			
Yes	127 (18.7)	63 (26.1)	P = 0.0148
No	551 (81.3)	178 (73.9)	
Smoking status			
Never smokers	85 (12.5)	33 (13.7)	P = 0.6048
Former smokers	370 (56.6)	137 (56.8)	
Current smokers	223 (32.9)	71 (29.5)	
Sex			
Male	449 (66.2)	165 (68.5)	P = 0.5260
Female	229 (33.8)	76 (31.5)	
Histology			
Squamous	156 (23.0)	47 (19.5)	P = 0.2599
Non-squamous	522 (77.0)	194 (80.5)	
PD-L1 expression[a]		[b]	
<90%	408 (83.6)	133 (86.4)	P = 0.4130
≥90%	80 (16.4)	21 (13.6)	

[a] Available for 488 patients.
[b] Available for 154 patients.

With a second-line median follow-up of 12.1 months (95%CI: 10.5–32.5), II line PFS and II line OS overall were 3.9 months (95%CI: 3.1–4.8; 206 events) and 6.7 months (95%CI: 5.7–7.9; 158 events), respectively. Patients who received platinum-based doublet chemotherapy had a median II line PFS of 4.1 months (95%CI: 3.2–5.3; 162 events), while those who received single-agent chemotherapy and other regimens had a median II line PFS of 2.8 months (95%CI: 1.8–4.0; 39 events) and 4.0 months (95%CI: 4.3–5.3; 5 events), respectively (log-rank test: p = 0.5628) (Fig. 3A). II line OS was 7.5 months (95%CI: 5.9–8.9; 119 events) for patients treated with platinum-based doublet chemotherapy, 5.3 months (95%CI: 2.7–6.9; 34 events) for those receiving single-agent chemotherapy and 3.4 months (95%CI: 1.3–7.9; 5 events) for patients receiving other regimens (log-rank test: 0.0289) (Fig. 3B).

Table 3 summarized univariable and multivariable analyses for II line PFS and II line OS. In the multivariable analysis, only ECOG-PS ≥2 was confirmed to be significantly associated with an increased risk of progressive disease as compared to ECOG-PS 0 (HR = 3.09 [95%CI: 1.84–5.19], p < 0.001). Patients receiving other regimens had an increased risk of death as compared to platinum-based doublet chemotherapy (HR = 2.53 [95%CI: 1.02–6.27]; p = 0.0447), as well as patients with an ECOG-PS ≥ 2 compared to ECOG-PS 0 (HR = 3.61 [95%CI: 1.90–6.83], p = 0.0001). Among the evaluable patients, PD-L1 expression (cut off 90%) was neither associated with II line PFS (HR = 0.81 [95%CI:0.49–1.35]; p = 0.4305) nor with II line OS (HR = 0.81 [95%CI: 0.47–1.38]; p = 0.4328).

4. Discussion

Clinical decision-making in advanced disease has always been a contentious topic in NSCLC, and while the advent of ICIs has been a game-changer, it does not simplify treatment algorithms. Recently, a review of real-world observational studies reported a median OS ranging from 4.6 to 12.8 months in the second-line setting [22]. We report ppOS ranging from 8.0 months to 13.9 months, findings that somehow mirror the incremental benefit already reported in the post-immunotherapy setting [23–26].

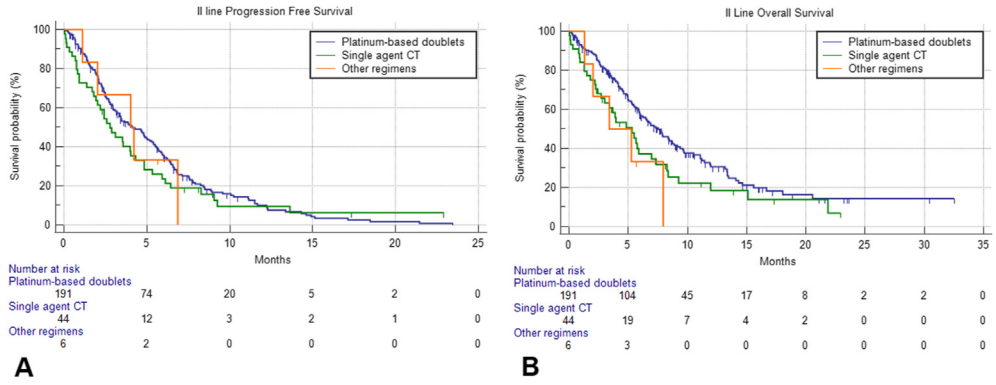

Fig. 3. Kaplan–Meier survival estimate of II line progression-free survival (PFS) (A) and II line overall survival (OS) (B) according to the received second-line regimen: platinum-based doublet chemotherapy, single-agent chemotherapy and other regimens.

Our study conveys a credible portrait of contemporary routine clinical practice in advanced NSCLC. The median OS for the entire population was 15.8 months, a significantly worse estimate compared to the 26.3 months reported in Keynote-024 [8]. These results are not unsurprising, considering the higher proportion of patients with adverse prognostic factors present in our cohort (i.e. those with ECOG-PS $\geq$ 2, receiving corticosteroids, aged more than 70 years old). Whilst accounting for the OS discrepancy, data on real-world populations are highly important to confirm RCT findings, where participants are highly selected for lower co-morbidity burden and feature portending to indolent disease. In this respect, it has been already demonstrated that NSCLC patients with PD-L1 expression $\geq$50% and poor baseline PS, particularly if related to disease burden [27], experience inferior outcomes with first-line single-agent pembrolizumab [28].

Considering that with a shorter follow-up, the OS of our cohort was 17.2 months [12], it can be assumed that post-progression outcomes played their specific detrimental role, reflecting the downside of having included frail patients. The impressively high proportions of patients who did not receive any further treatment at the data cut off (55.9%), and who died without receiving any subsequent treatments (52.9%), which are worse than reported in clinical trials [8,9], mirror these findings. Accordingly, the correlation analysis revealed that baseline (at the first-line treatment) characteristics significantly associated with not having received any further treatment are typical features of patients' frailty, including older age ($p = 0.0011$), higher ECOG-PS (p 0.0001) and baseline corticosteroids administration ($p = 0.0024$). These results suggest that NSCLC patients with PD-L1 expression $\geq$50% aged $\geq$70 years old, with an ECOG-PS $\geq$ 2, and receiving systemic corticosteroids before starting the first-line pembrolizumab, are at higher risk of life-threatening progressive disease; therefore, the treatment sequencing approach (first-line immunotherapy followed by second-line chemotherapy) is unlikely to be completely pursued. However, a tailored decision-making process at the first-line treatment commencement should always take into account that frail/older patients are unlikely to be treated with thefirst-line chemoimmunotherapy combinations without experiencing limiting side effects.

Our results regarding the ppOS are partially aligned with similar studies reported in this setting [29]. The case-control matching analysis confirmed that patients receiving pembrolizumab ByPD in combination with LATs achieved the best post-progression outcome; therefore, a combinational approach should always be considered at the moment of disease progression (when feasible), as confirmed in a recent prospective study [30]. However, although the random-matching included key baseline characteristics (CNS and bone metastases, ECOG-PS and previous best response to pembrolizumab) that might have affected clinicians' choice regarding post-progression treatments, we have not been able to entirely mitigate the positive selection bias associated with the oligo-progressive disease, which is known to be related with a better prognosis [16,31]. In fact, in our population LATs were significantly associated with oligo-progressive disease ($p < 0.0001$), and considering the retrospective nature of the study we could not evaluate the criteria associated with post-progression choices.

The II line PFS and II line OS analyses revealed that patients who had reached the second-line setting tended to be younger compared to the first-line setting. They also had poorer PS and a higher prevalence of CNS, bone and liver metastases. This is probably related to the natural history of the disease, which tends to worsen throughout treatment lines. These negative baseline characteristics could explain the low median II line PFS and II line OS in absolute terms and when compared to other studies in the post-immunotherapy setting [25,26,32]. Nevertheless, we found an incremental benefit for patients who received platinum-based doublet chemotherapy, while ECOG-PS still remains the major determinant of II line PFS and II line OS.

Table 3
Univariable and multivariable analyses for II line PFS and II line OS.

Variable (Comparator)	II line progression-free survival		II line overall survival	
	UVA HR (95% CI); *p-value*	MVA HR (95% CI); *p-value*	UVA HR (95% CI); *p-value*	MVA HR (95% CI); *p-value*
Treatment regime (Platinum doublets)				
Single agent CT	1.20 (0.84–1.71); *p = 0.3038*	–	1.51 (1.03–2.21); *p = 0.0337*	1.01 (0.66–1.51); *p = 0.9802*
Others	1.17 (0.48–2.87); *p = 0.7172*		2.21 (0.90–5.46); *p = 0.0829*	2.53 (1.02–6.27); *p = 0.0447*
ECOG-PS (0)				
1	1.35 (0.85–2.15); *p = 0.1958*	1.37 (0.86–2.20); *p = 0.1796*	1.49 (0.83–2.66); *p = 0.1766*	1.56 (0.87–2.80); *p = 0.1339*
≥ 2	3.12 (1.87–5.20); *p < 0.0001*	3.09 (1.84–5.19); *p < 0.0001*	3.63 (1.96–6.72); *p < 0.0001*	3.61 (1.90–6.83); *p = 0.0001*
Age				
Elderly vs non-Elderly	1.05 (0.79–1.39); *p = 0.7065*	–	1.12 (0.82–1.53); *p = 0.4661*	–
CNS metastases				
Yes vs No	1.34 (1.01–1.79); *p = 0.0489*	1.33 (0.98–1.79); *p = 0.0629*	1.22 (0.87–1.71); *p = 0.2369*	–
Bone metastases				
Yes vs No	1.34 (1.01–1.78); *p = 0.0378*	1.15 (0.86–1.55); *p = 0.3198*	1.31 (0.95–1.80); *p = 0.0885*	–
Liver metastases				
Yes vs No	1.14 (0.84–1.55); *p = 0.3824*	1.11 (0.81–1.52); *p = 0.5025*	1.17 (0.83–1.65); *p = 0.3472*	–
Previous ByPD				
Yes vs No	0.83 (0.61–1.26); *p = 0.4999*	–	0.91 (0.61–1.36); *p = 0.6573*	–
Smoking status (Never smoker)				
Former smoker	0.99 (0.66–1.49); *p = 0.9970*	–	1.24 (0.77–2.02); *p = 0.3661*	–
Current smoker	0.77 (0.49–1.21); *p = 0.2694*		0.95 (0.56–1.61); *p = 0.8523*	
Histology				
Non-sq. vs Squamous	0.99 (0.70–1.41); *p = 0.9951*	1.15 (0.86–1.55); *p = 0.3198*	0.96 (0.64–1.42); *p = 0.8449*	–
PD-L1 expression[a]				
≥90% vs <90%	0.81 (0.49–1.35); *p = 0.4305*	–	0.81 (0.47–1.38); *p = 0.4328*	–
Sex				
Male vs Female	1.26 (0.93–1.70); *p = 0.1282*	–	1.50 (1.05–2.13); *p = 0.0246*	1.36 (0.94–1.97); *p = 0.0954*

[a] Available for 154 patients. UVA: univariable analysis; MVA: multivariable analysis.

Several limitations of the present study must be acknowledged. The retrospective design and the lack of centralised imaging review expose to selection biases. Moreover, patients' outcomes assessment performed according to the respective clinical practice of the participating centres might have affected the analysis, including the definition of oligo-progressive disease. Additionally, also the lack of a centralised review of PD-L1 expression, as well as missing data about its discrete/absolute value for some patients, might have affected the reliability of our analysis. More than one-third of the patients have been tested using the SPS263 and other antibodies. Despite the harmonisation evidence [33,34], we have to consider that only the 22C3 has been clinically validated in relation to pembrolizumab as a companion diagnostic assay, and some evidence has underlined possible discrepancies at clinically relevant cutoffs (TPS 1% and 50%) [35].

5. Conclusion

Our study portrays the significant heterogeneity in the outcome of NSCLC patients with PD-L1 expression ≥50% treated with first-line single-agent pembrolizumab in routine practice as compared to RCTs. In comparison with the Keynote-024 [1,2], patients achieve worse outcomes in the real-world scenario. These findings provide an important benchmark that is characteristic of patients usually not enrolled in RCTs: older age, with poorer PS and who were receiving corticosteroids prior to immunotherapy. Attrition between the first and

second line is common, and the post-progression outcome is a major determinant of the global outcome. Among patients who are able to receive further treatments, pembrolizumab ByPD ± LATs represents a viable option. Among patients who reach a second-line treatment, ECOG-PS still remains the major determinant of clinical outcomes.

Ethics approval and consent to participate

All patients provided 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 centre (Comitato Etico per le provice di L'Aquila e Teramo, verbale N.15 del 28 Novembre 2019).

Authors' contributions

All authors contributed to the publication according to the ICMJE guidelines for authorship (study conception and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision). All authors read and approved the submitted version of the manuscript (and any substantially modified version that involves the author's contribution to the study). Each author has agreed to be both personally accountable for the own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

Funding

No funding was received.

Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Consent for publication

Not applicable.

Conflict of interest statement

Dr Alessio Cortellini received speaker fees and grant consultancies by Astrazeneca, MSD, BMS, Roche, Novartis, Istituto Gentili and Astellas. Dr Raffaele Giusti received speaker fees and grant consultancies by Astrazeneca and Roche. Dr Joachim GJV Aerts reports receiving commercial research grants from Amphera and Roche, holds ownership interest (including patents) in Amphera BV, and is a consultant/advisory board member for Amphera, Boehringer Ingelheim, Bristol-Myers Squibb, Eli-Lilly, MSD and Roche. Dr Alex Friedlaender received grant consultancies by Roche, Pfizer, Astellas and BMS. Dr Alessandro Morabito received speaker fees by Astra, Roche, BMS, MSD, Boehringer, Pfizer, Takeda. Dr Francesca Mazzoni received grant consultancies by MSD and Takeda. 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. Dr Marco Russano received honoraria for scientific events by Roche, Astrazeneca, BMS, MSD and Boehringer Ingelheim. Dr Marcello Tiseo received speakers' and consultants' fee from Astra-Zeneca, Pfizer, Eli-Lilly, BMS, Novartis, Roche, MSD, Boehringer Ingelheim, Otsuka, Takeda, Pierre Fabre. 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. Dr David J Pinato received lecture fees from ViiV Healthcare, Bayer Healthcare and travel expenses from BMS and Bayer Healthcare; consulting fees for Mina Therapeutics, EISAI, Roche, Astra Zeneca; received research funding (to institution) from MSD, BMS. All other authors declare no competing interests.

Acknowledgements

A special thanks to the 'Consorzio Interuniversitario Nazionale per la Bio-Oncologia' for their support in this study. M.T. is currently supported by the Associazione Italiana per la Ricerca sul Cancro (AIRC) under Investigator Grant (IG) No. IG2017-20074.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2021.02.005.

References

[1] Reck M, Rodríguez-Abreu D, Robinson AG, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. N Engl J Med 2016;375(19):1823-1833. https://doi.org/10.1056/NEJMoa1606774.

[2] Reck M, Rodríguez-Abreu D, Robinson AG, et al. Updated analysis of KEYNOTE-024: pembrolizumab versus platinum-based chemotherapy for advanced non-small-cell lung cancer

with PD-L1 tumor proportion score of 50% or greater. J Clin Oncol 2019;37(7):537-546. https://doi.org/10.1200/JCO.18.00149.

[3] Gandhi L, Rodríguez-Abreu D, Gadgeel S, et al. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. N Engl J Med 2018;378(22):2078–92. https://doi.org/10.1056/NEJMoa1801005. Epub 2018Apr16.

[4] Paz-Ares L, Luft A, Vicente D, et al. Pembrolizumab plus chemotherapy for squamous non-small-cell lung cancer. N Engl J Med 2018;379(21):2040–51. https://doi.org/10.1056/NEJMoa1810865. Epub 2018 Sep. 25.

[5] Zhou Y, Lin Z, Zhang X, et al. First-line treatment for patients with advanced non-small cell lung carcinoma and high PD-L1 expression: pembrolizumab or pembrolizumab plus chemotherapy. J Immunother Canc 2019;7:120.

[6] Addeo A, Banna GL, Metro G, et al. Chemotherapy in combination with immune checkpoint inhibitors for the first-line treatment of patients with advanced non-small cell lung cancer: a systematic review and literature-based meta-analysis. Front Oncol 2019;9:264.

[7] Pathak R, De Lima Lopes G, Yu H, et al. Comparative efficacy of chemoimmunotherapy versus immunotherapy for advanced non-small cell lung cancer: a network meta-analysis of randomized trials. Cancer 2020. https://doi.org/10.1002/cncr.33269.

[8] Brahmer JR, Rodriguez-Abreu D, Robinson AG, et al. KEYNOTE-024 5-year OS update: first-line (1L) pembrolizumab (pembro) vs platinum-based chemotherapy (chemo) in patients (pts) with metastatic NSCLC and PD-L1 tumour proportion score (TPS) ≥50%. Ann Oncol 2020;31(suppl_4):S1142–215. https://doi.org/10.1016/annonc/annonc325.

[9] Mok TSK, Wu YL, Kudaba I, et al. Pembrolizumab versus chemotherapy for previously untreated, PD-L1-expressing, locally advanced or metastatic non-small-cell lung cancer (KEYNOTE-042): a randomised, open-label, controlled, phase 3 trial. Lancet 2019;393(10183):1819–30. https://doi.org/10.1016/S0140-6736(18)32409-7.

[10] Cortellini A, Ficorella C, Crisci R, Divisi D. A reflection on the actual place of osimertinib in the treatment algorithm of EGFR-positive non-small cell lung cancer patients. J Thorac Dis 2020;12(2). https://doi.org/10.2103/jtd-20-1733.

[11] Freeman AT, Lesperance M, Wai ES, et al. Treatment of non-small-cell lung cancer after progression on nivolumab or pembrolizumab. Curr Oncol 2020;27(2):76–82. https://doi.org/10.3747/co.27.5495.

[12] Cortellini A, Tiseo M, Banna GL, et al. Clinicopathologic correlates of first-line pembrolizumab effectiveness in patients with advanced NSCLC and a PD-L1 expression of ≥ 50% Cancer Immunol Immunother 2020;69(11):2209–21. https://doi.org/10.1007/s00262-020-02613-9. Epub 2020 May 30.

[13] Cortellini A, Friedlaender A, Banna GL, et al. Immune-related adverse events of pembrolizumab in a large real-world cohort of patients with NSCLC with a PD-L1 expression ≥ 50% and their relationship with clinical outcomes. Clin Lung Canc 2020;S1525–7304(20):30204–7. https://doi.org/10.1016/j.cllc.2020.06.010. Epub ahead of print.

[14] Cortellini A, Ricciuti B, Tiseo M, et al. Baseline BMI and BMI variation during first line pembrolizumab in NSCLC patients with a PD-L1 expression ≥50%: a multicenter study with external validation. J Immunother Canc 2020;8(2):e001403. https://doi.org/10.1136/jitc-2020-001403.

[15] 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. Thorac Canc 2021. https://doi.org/10.1111/1759-7714.13852. Epub ahead of print.

[16] Kim C, Hoang CD, Kesarwala AH, Schrump DS, Guha U, Rajan A. Role of local ablative therapy in patients with oligometastatic and oligoprogressive non-small cell lung cancer. J Thorac Oncol 2017;12(2):179–93. https://doi.org/10.1016/j.jtho.2016.10.012. Epub 2016Oct22.

[17] Gridelli C, Balducci L, Ciardiello F, 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 Canc 2015;16(5):325–33.

[18] Cortellini A, Bersanelli M, Buti S, et al. A multicenter study of body mass index in cancer patients treated with anti-PD-1/PD-L1 immune checkpoint inhibitors: when overweight becomes favorable. J Immunother Canc 2019;7(1):57. https://doi.org/10.1186/s40425-019-0527-y.

[19] Cortellini A, Bersanelli M, Santini D, et al. Another side of the association between body mass index (BMI) and clinical outcomes of cancer patients receiving programmed cell death protein-1 (PD-1)/Programmed cell death-ligand 1 (PD-L1) checkpoint inhibitors: a multicentre analysis of immune-related adverse events. Eur J Canc 2020;128:17–26. https://doi.org/10.1016/j.ejca.2019.12.031. Epub 2020 Mar 5.

[20] PD-L1 IHC 22C3 pharmDx interpretation manual – NSCLC. Available at: https://www.agilent.com/cs/library/usermanuals/public/29158_pd-l1-ihc-22C3-pharmdx-nsclc-interpretation-manual.pdf. [Last access 13 January 2021].

[21] Aguilar EJ, Ricciuti B, Gainor JF, et al. Outcomes to first-line pembrolizumab in patients with non-small-cell lung cancer and very high PD-L1 expression. Ann Oncol 2019;30(10):1653–9. https://doi.org/10.1093/annonc/mdz288.

[22] Davies J, Patel M, Gridelli C, et al. Real-world treatment patterns for patients receiving second-line and third-line treatment for advanced non-small cell lung cancer: a systematic review of recently published studies. PloS One 2017;12(4):e0175679. https://doi.org/10.1371/journal.pone.0175679. Published 2017 Apr 14.

[23] Park SE, Lee SH, Ahn JS, et al. Increased response rates to salvage chemotherapy administered after PD-1/PD-L1 inhibitors in patients with non-small cell lung cancer. J Thorac Oncol 2018;13:106–11. https://doi.org/10.1016/j.jtho.2017.10.011.

[24] Schvartsman G, Peng SA, Bis G, et al. Response rates to single-agent chemotherapy after exposure to immune checkpoint inhibitors in advanced non-small cell lung cancer. Lung Canc 2017;112:90–5. https://doi.org/10.1016/j.lungcan.2017.07.034.

[25] Kato R, Hayashi H, Chiba Y, et al. Propensity score-weighted analysis of chemotherapy after PD-1 inhibitors versus chemotherapy alone in patients with non-small cell lung cancer (WJOG10217L). J Immunother Canc 2020;8(1):e000350. https://doi.org/10.1136/jitc-2019-000350.

[26] Bersanelli M, Buti S, Giannarelli D, et al. Chemotherapy in non-small cell lung cancer patients after prior immunotherapy: the multicenter retrospective CLARITY study. Lung Canc 2020;150:123–31. https://doi.org/10.1016/j.lungcan.2020.10.008. Epub ahead of print.

[27] Facchinetti F, Mazzaschi G, Barbieri F, et al. First-line pembrolizumab in advanced non-small cell lung cancer patients with poor performance status. Eur J Canc 2020;130:155–67. https://doi.org/10.1016/j.ejca.2020.02.023. Epub 2020 Mar 25.

[28] Friedlaender A, Metro G, Signorelli D, et al. Impact of performance status on non-small-cell lung cancer patients with a PD-L1 tumour proportion score ≥50% treated with front-line pembrolizumab. Acta Oncol 2020;59(9):1058–63. https://doi.org/10.1080/0284186X.2020.1781249. Epub 2020 Jun 17.

[29] Metro G, Addeo A, Signorelli D, et al. Outcomes from salvage chemotherapy or pembrolizumab beyond progression with or without local ablative therapies for advanced non-small cell lung cancers with PD-L1 ≥50% who progress on first-line immunotherapy: real-world data from a European cohort. J Thorac Dis 2019;11(12):4972–81. https://doi.org/10.21037/jtd.2019.12.23.

[30] Campbell AM, Cai WL, Burkhardt D, et al. Final results of a phase II prospective trial evaluating the combination of stereotactic body radiotherapy (SBRT) with concurrent pembrolizumab

in patients with metastatic non-small cell lung cancer (NSCLC). Int J Radiat Oncol Biol Phys 2019;105(1):S36–7.

[31] Bersanelli M, Lattanzi E, D'Abbiero N, et al. Palliative radiotherapy in advanced cancer patients treated with immune-checkpoint inhibitors: the PRACTICE study. Biomed Rep 2020;12(2):59–67. https://doi.org/10.3892/br.2019.1265. Epub 2019 Dec 13.

[32] Talbot D, Fish SM, Jin Ong T, et al. 1497P – second-line (2L) real-world treatment (tx) patterns and outcomes in patients (pts) with advanced/metastatic non-small cell lung cancer (NSCLC) treated with first-line (1L) immuno-oncology (IO) monotherapy (mono tx). Ann Oncol 2019;30(suppl_5):v602–60. https://doi.org/10.1093/annonc/mdz260.

[33] Marchetti A, Barberis M, Franco R, et al. Multicenter comparison of 22C3 PharmDx (agilent) and SP263 (Ventana) assays to test PD-L1 expression for NSCLC patients to Be treated with immune checkpoint inhibitors. J Thorac Oncol 2017;12(11):1654–63. https://doi.org/10.1016/j.jtho.2017.07.031. Epub 2017 Aug 14.

[34] Büttner R, Gosney JR, Skov BG, et al. Programmed death-ligand 1 immunohistochemistry testing: a review of analytical assays and clinical implementation in non-small-cell lung cancer. J Clin Oncol 2017;35(34):3867–76. https://doi.org/10.1200/JCO.2017.74.7642. Epub 2017 Oct 20.

[35] Munari E, Rossi G, Zamboni G, et al. PD-L1 assays 22C3 and SP263 are not interchangeable in non-small cell lung cancer when considering clinically relevant cutoffs: an interclone evaluation by differently trained pathologists. Am J Surg Pathol 2018;42(10):1384–9. https://doi.org/10.1097/PAS.0000000000001105.