



Correlations Between the Immune-related Adverse Events Spectrum and Efficacy of Anti-PD1 Immunotherapy in NSCLC Patients

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Abstract

The aim of this study was to investigate with a large sample size, the predictive and prognostic positive roles of occurrence of immune-related adverse events in patients with non–small-cell lung cancer treated with PD-1 inhibitors. The study confirmed that immune-related adverse events are independent predictors of higher overall response rate, longer progression-free survival and longer overall survival.

Background: Immune-related adverse events (irAEs) developed during immunotherapy with anti-PD-1 agents, could be a predictive surrogate marker of clinical benefit in patients with advanced non–small-cell lung cancer (NSCLC).

Methods: Patients with NSCLC, treated with anti-PD-1 agents, were retrospectively evaluated. Univariate and multivariate analyses were performed to evaluate the relationships between types of irAEs (differentiated according to system/organ involved and to single-site/multiple-site), overall response rate (ORR), progression-free survival (PFS) and overall survival (OS). We further performed a 6-week landmark analysis. **Results:** A total of 559 patients were enrolled; 231 patients (41.3%) developed irAEs of any grade and 50 patients (8.9%) G3/G4 events; 191 of them (82.6%) developed “single-site” irAEs and 40 (17.4%) “multiple-site” irAEs. At multivariate analysis, higher ORR was related to irAEs of any grade ($P < .0001$), “single-site” irAEs ($P < .0001$), endocrine ($P = .0043$) and skin irAEs ($P = .0005$). Longer PFS was related to irAEs of any grade ($P < .0001$), “single-site” irAEs ($P < .0001$), “multiple-site” irAEs ($P = .0374$), endocrine irAEs ($P = .0084$) and skin irAEs ($P = .0001$). Longer OS was related to irAEs of any grade ($P < .0001$), “single-site” irAEs ($P < .0001$), endocrine irAEs ($P = .0044$), gastrointestinal irAEs ($P = .0437$), skin irAEs ($P = .0006$), and others irAEs ($P = .0378$). At the 6-week landmark analysis, irAEs of any grade was confirmed an

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independent predictor of higher ORR, longer PFS, and longer OS. **Conclusion:** Our study confirmed that irAEs are concordantly related to higher ORR, longer PFS, and longer OS with anti-PD-1 immunotherapy in patients with NSCLC.

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Introduction

The advent of immune checkpoint inhibitors (ICIs), particularly those acting on the programmed death-1/programmed death-ligand 1 (PD-1/PD-L1) axis, have radically changed the treatment algorithm of patients with non-small-cell lung cancer (NSCLC). To date, this revolution has moved forward to the first-line treatment, leading to an unprecedented improvement in the natural history of these patients.¹ By using anti-PD-1 agents, clinicians have been called to manage new kinds of toxicities, the so-called immune-related adverse events (irAEs). The irAEs result from an aberrant activation of T cells, triggered by ICIs, leading to a "self-response" of the immune system. Overall, the reported incidence of irAEs of any grade with anti-PD-1 treatments in literature is approximately 25%.² A recent systematic review including more than 5000 patients with NSCLC treated with PD-1 inhibitors reported an overall incidence of irAEs of 64%, with 14% of G3/G4 irAEs.³

The timing of irAE onset is widely different when compared with the timing of chemotherapy toxicity onset. Indeed, it is well known that irAEs tend to develop quite late after the commencement of treatment, as if they need a certain time of exposure to the drug, although in some respects are not dose-dependent.⁴ Particularly, the median time to the onset of irAEs in patients with NSCLC treated with PD-1 inhibitors ranged from 4.9 weeks of gastrointestinal (GI) irAEs to 30.3 weeks of pulmonary irAEs.⁵

As the development of irAEs directly depends on the mechanism of action of ICIs, it has been speculated that patients who experience irAEs might derive a greater clinical benefit from these compounds. Consistent with this hypothesis, several studies have reported a significant association between development of irAEs and improved clinical outcomes across different tumor types.⁶⁻¹¹ In addition, there is evidence that patients who discontinue immunotherapy due to irAEs, tend to maintain the benefit from the treatment, an element that further suggests a mechanistic association between irAE and ICI efficacy.¹² However, studies conducted on the topic have been flawed by several limitations, including the small sample size and a short follow-up time, which hindered the possibility of deriving definitive results.

Against this background, we conducted a multicenter retrospective study to evaluate the impact of different types of irAEs on clinical outcomes in a large cohort of patients with advanced NSCLC, treated with PD-1 inhibitors.

Materials and Methods

Patient Eligibility

In this study we enrolled patients with histologically confirmed advanced NSCLC, who had received at least 1 cycle of anti-PD-1 agents, regardless of treatment line, at 11 Italian centers, between September 2013 and May 2018 (Supplemental Table 1). All

patients provided written, informed consent to treatment with immunotherapy. All patients alive at the time of data collection provided an informed consent to participate to the analysis. The procedures followed were in accordance with the precepts of Good Clinical Practice and the declaration of Helsinki. The study was approved by the local responsible committee on human experimentation (University of L'Aquila, Internal Review Board protocol number 32865, approved on July 24, 2018).

Study Design

A "real-life" multicenter retrospective observational study of patients with advanced NSCLC, who had been treated with anti-PD-1 mono-therapy (standard doses and schedules) was performed. The aims of this study were to evaluate the incidence of "single-site" and "multiple-site" irAEs, to compare clinical outcomes of patients who experienced 1 or more irAEs with those of patients who did not experience irAEs, and to evaluate the possible influence of the category of irAEs on clinical outcomes. Measured clinical outcomes were overall response rate (ORR), median progression-free survival (PFS), and median overall survival (OS). The following covariates were analyzed: sex (male vs. female), Eastern Cooperative Oncology Group Performance Status (ECOG-PS) (0-1 vs. ≥ 2), age (< 70 vs. ≥ 70 years old), number of metastatic sites (≤ 2 vs. > 2), and treatment line (first vs. non-first). PD-L1 expression was not used as a covariate because it was not available for all the patients. To weigh its role, the incidence of irAEs among subgroups of patients with different PD-L1 expression was compared with the χ^2 test. A further analysis was performed to evaluate the incidence of irAEs of any grade according to sex, ECOG-PS, age, number of metastatic sites, and treatment line (categorized as previously mentioned), and to compare the incidence of "single-site" and "multiple-site" irAEs among the same subgroups.

Responses were evaluated with RECIST criteria (version 1.1), according to the local clinical practice of the participating centers and to the respective investigators' evaluation.¹³ The χ^2 test was used to compare ORR and the incidence of irAEs of any grade among subgroups. In the multivariate analysis, logistic regression was used to evaluate the role of parameters that proved significant at the univariate analyses of ORR and irAEs of any grade. PFS and OS were calculated from the date of the start of immunotherapy (day 1, cycle 1). Median PFS and median OS were evaluated using the Kaplan-Meier method.¹⁴ Patients who had not progressed/not died at data cutoff were censored at the time of the last clinical visit. Median follow-up was calculated according to the reverse Kaplan-Meier method.¹⁵ The Cox proportional-hazards model¹⁶ was used to evaluate predictor variables in univariate and multivariate analysis for PFS and OS.

As previously mentioned, irAEs are "time-dependent"^{4,5}; thus, we can suppose that early-progressor patients, interrupting the anti-PD-1 treatment, are exposed to the potential "triggering effect" for a shorter time, when compared with those who did not progress, therefore they had few chances of experience irAEs. To overcome the lack of data availability regarding time to develop irAEs among the study population, we performed a further 6-week landmark analysis¹⁰ by including only patients with a minimum follow-up for PFS of 6 weeks, regardless of disease progression. The data cutoff period was August 2018. All statistical analyses were performed using MedCalc Statistical Software version 18.6 (MedCalc Software bvba, Ostend, Belgium; <http://www.medcalc.org>; 2018).

PD-L1 Determination

PD-L1 protein expression was evaluated according to clinical practice on paraffin-embedded tissues with immunohistochemistry techniques (22C3 PharmDx Agilent and SP263 Ventana); tumor proportion scores (TPS) were computed on the basis of the percentage of stained tumor cells.

Categorization and Definition of Single/Multiple-Site irAEs

The irAEs were graduated according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (version 4.0) and cumulatively reported. The irAEs were categorized on the basis of the organ/system involved as follows: endocrine irAEs (including thyroid disorders), GI irAEs (excluding pancreatitis), skin irAEs, pneumological irAEs, hepatic irAEs, and others irAEs (including rheumatologic, neuromuscular, pancreatitis, fever, asthenia, and anorexia). The irAEs were defined as "single-site" if the patient experienced just 1 category of irAEs among those previously mentioned; they were defined "multiple-site" if occurring in patients who experienced irAEs belonging to different categories. The analyses by categories and by number of involved sites were performed only for irAEs of any grade and not for G3/G4 irAEs. Patients were clinically monitored, for safety evaluation, at every preadministration visit (according to the technical files of the drugs), and as clinically indicated by the investigators subsequently.

Results

Patients Characteristics

A total of 559 consecutive patients with advanced NSCLC were enrolled. The initiation date of the anti-PD-1 treatment ranged from September 2013 to April 2018. The patient characteristics are summarized in Table 1. The median age was 69 years (range: 24-88), male/female ratio was 379/180. Primary tumors were non-squamous NSCLC in 324 patients (57.9%) and squamous NSCLC in 235 patients (42.2%). ECOG-PS was 0/1 in 485 patients (86.7%), and ≥ 2 in 74 patients (13.3%); 242 patients (43.2%) had ≤ 2 metastatic sites and 317 (56.8%) had more than 2 metastatic sites. PD-1 inhibitors were administered as first-line of treatment in 116 patients (20.8%).

Immune-related Adverse Events Analysis

Overall, 231 patients (41.3%) developed irAEs of any grade and 50 patients (8.9%) had G3/G4 events. Of them, 191 (82.6%) developed "single-site" irAEs and 40 (17.4%) "multiple-site" irAEs.

Table 1 Patient Characteristics

	n (%)
	559
Age, y	
Median	69
Range	24-88
Elderly (≥ 70)	259 (46.3)
Sex	
Male	379 (67.8)
Female	180 (32.2)
ECOG-PS	
0-1	485 (86.7)
≥ 2	74 (13.3)
Histology	
Squamous	235 (42.1)
Nonsquamous	324 (57.9)
No. of metastatic sites	
≤ 2	242 (43.2)
> 2	317 (56.8)
Type of anti-PD-1	
Pembrolizumab	123 (22)
Nivolumab	436 (78)
Line of immunotherapy	
First	116 (20.8)
Non-first	443 (79.2)
irAEs	231 (41.3)
Single site	191 (82.6)
Multiple site	40 (17.4)
PD-L1 expression (TPS)	
Not available	354 (63.3)
Negative	45 (8.1)
1%-49%	60 (10.7)
$\geq 50\%$	100 (17.9)

Abbreviations: irAE = immune-related adverse event; ECOG-PS = Eastern Cooperative Oncology Group Performance Status; PD-1 = programmed death 1; PD-L1 = programmed death-ligand 1; TPS = tumor proportion score.

Thirty-four patients (6.1%) discontinued treatment due to AEs. The irAEs are summarized in Table 2.

Table 3 summarizes the univariate and multivariate analysis of irAEs of any grade. At univariate analysis, female patients had a significantly higher incidence of irAEs of any grade, compared with

Table 2 Summary of Immune-related Adverse Events (irAEs)

	irAEs of Any Grade	G3/G4 irAEs
Patients	231	50
Endocrine	78 (33.8)	4 (8)
Gastrointestinal	51 (22.1)	15 (30)
Skin	59 (24.2)	7 (14)
Pneumological	23 (9.9)	12 (24)
Hepatic	10 (4.3)	6 (12)
Others	46 (19.9)	6 (12)

Table 3 Univariate and Multivariate Analyses of Incidence of Immune-related Adverse Events of Any Grade

irAEs of Any Grade: Univariate Analysis			
Variable (Comparator)	Events Ratio	Incidence (95% CI)	P
Overall	231/559	41.3 (36.1-47.0)	-
Sex			
Female	90/180	50 (40.2-61.4)	.0041
Male	141/379	37.2 (31.3-43.8)	
Age			
Elderly	94/259	36.3 (29.3-44.4)	.0249
Non-elderly	137/300	45.7 (38.3-53.9)	
ECOG-PS			
0-1	231/485	43.9 (38.2-50.2)	.0014
≥ 2	18/74	24.3 (14.4-38.4)	
Treatment line			
First	42/116	36.2 (26.1-48.9)	.2091
Further lines	189/443	42.7 (36.8-49.2)	
Burden of disease			
≤ 2 sites	110/240	45.8 (37.6-55.2)	.0693
> 2 sites	121/317	38.2 (31.6-45.6)	
irAEs of Any Grade: Multivariate Analysis			
Variable (Comparator)	Coefficient	Standard Error	P
Sex	-0.4209	0.1882	.0254
Age	-0.2878	0.1778	.1056
ECOG-PS	-0.8175	0.2886	.0046

Coefficient of determination R²: 0.0482

Abbreviations: irAE = immune-related adverse event; ECOG-PS = Eastern Cooperative Oncology Group Performance Status.

male patients (50.0% and 37.2%, respectively, $P = .0041$), as non-elderly patients compared with elderly patients (45.7% and 36.3%, respectively, $P = .0249$) and patients with ECOG-PS 0-1 compared with ECOG-PS ≥ 2 (43.9% and 24.3%, respectively, $P = .0014$). Treatment line and number of metastatic sites were not significantly related to the incidence of irAEs of any grade. Sex ($P = .0254$) and ECOG-PS ($P = .0046$), were confirmed as independent predictors for irAEs of any grade at the multivariate analysis. Histological subtype was not related to the incidence of irAEs of any grade (data not shown). None among the previously mentioned factors resulted to be significantly related to the incidence of "single-site" nor "multiple-site" irAEs (data not shown).

PD-L1 expression was available in 205 patients (36.7%): 45 of them (21.9%) had no expression, 60 (29.2%) had a TPS from 1% to 49%, and 100 (48.7%) ≥ 50%. Among these patients, 2 (4.4%), 4 (6.6%), and 15 (15%) experienced irAEs of any grade, respectively. No statistically significant differences were found in terms of irAE incidence according to PD-L1 TPS ($P = .0845$).

Activity Analysis

Univariate and multivariate analyses for ORR are detailed in Table 4. Overall, among 507 patients evaluable for activity, 175

responses of disease were observed: ORR was 34.5%. Among patients who experienced irAEs of any grade and those who did not experience irAEs, the ORRs were 46.5% and 25.7%, respectively ($P < .0001$). Among patients who experienced G3/G4 irAEs and those who did not experienced G3/G4 irAEs, the ORRs were 41.0% and 33.8%, respectively ($P = .3641$). ORR of patients who experienced "single-site" irAEs was significantly higher when compared with the ORR of patients who did not develop irAEs ($P < .0001$), whereas we did not find any association between ORR and "multiple-site" irAEs ($P = .1773$). No difference in ORR was observed between patients who developed single-site and multiple-site irAEs ($P = .1428$). Endocrine and skin irAEs were associated with a significantly higher ORR ($P = .0007$ and $P = .0004$, respectively). After adjusting for ECOG-PS, treatment line and number of metastatic site irAEs of any grade ($P < .0001$), "single-site" irAEs ($P < .0001$), endocrine irAEs ($P = .0043$), and skin irAEs ($P = .0005$) were confirmed as predictors of higher ORR in multivariate analysis.

Among 27 evaluable patients who discontinued the treatment due to irAEs, the ORR was 48.1% (95% CI, 25.6-82.3; 13 responses of disease), whereas among the 480 evaluable patients who did not discontinued the treatment due to irAEs ORR was 33.7% (95% CI, 28.7-39.3; 162 responses of disease). There were no statistically significant differences between patients who discontinued the treatment due to irAEs and those who did not ($P = .1261$).

Efficacy Analysis

At a median follow-up of 11.2 months, median PFS was 6.3 months (95% CI, 5.1-7.5; 333 events) and median OS was 12.7 months (95% CI, 11.0-16.5; 301 censored patients). Median PFS of patients who experienced irAEs of any grade was 10.1 months (95% CI, 8.3-13.6; 128 events), whereas median PFS of patients who did not experienced irAEs of any grade was 4.1 months (95% CI, 3.5-5.2; 205 events) (Figure 1A). As shown in Table 5, irAEs of any grade, "single-site" irAEs, endocrine irAEs, and skin irAEs, were significantly related to a longer PFS at univariate analysis. All of them were confirmed significant predictors of a longer PFS at multivariate analysis together with treatment line, number of metastatic sites, and ECOG-PS.

Median OS of patients who experienced irAEs of any grade was 20.5 months (95% CI, 15.7-25.1; 137 censored patients), whereas median OS of patients who did not experience irAEs of any grade was 8.5 months (95% CI, 6.5-11; 164 censored patients) (Figure 1B). As shown in Table 6, irAEs of any grade, both "single-site" and "multiple-site" irAEs, endocrine irAEs, GI irAEs, skin irAEs, and others irAEs, were significantly related to a longer OS at univariate analysis. All but "multiple-site" irAEs were confirmed significant predictors of a longer OS at multivariate analysis along with female sex and ECOG-PS 0-1.

Among patients who discontinued the treatment due to irAEs, median PFS was 14.3 months (95% CI, 3.7-25.4; 18 events), whereas among patients who did not discontinue treatment due to irAEs, median PFS was 6.2 months (95% CI, 5.1-7.1; 315 events), with no statistically significant differences ($P = .0666$). Among patients who discontinued the treatment due to irAEs, median OS was 24.4 months (95% CI, 5.8-48.9; 18 censored patients), whereas among patients who did not, the median OS was 12.3 months

Table 4 Univariate and Multivariate Analyses for Overall Response Rate

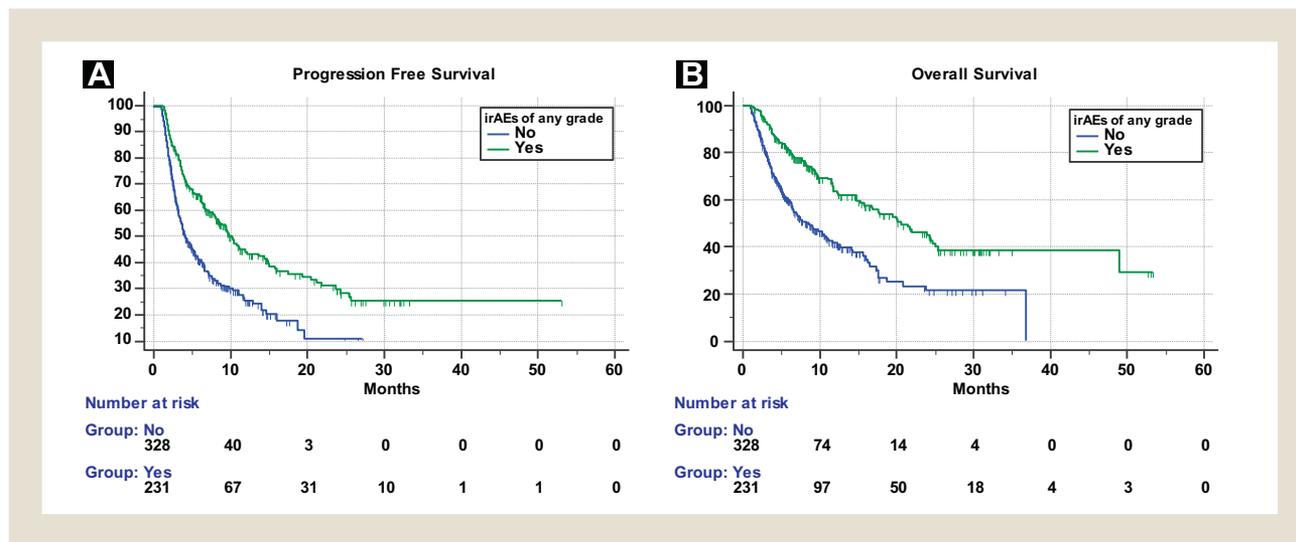
Variable (Comparator)	Univariate Analysis			Multivariate											
				irAEs of Any Grade			Type of irAEs			Endocrine irAEs			Skin irAEs		
	Response/Ratio	ORR (95% CI)	P	Coeff	SE	P	Coeff	SE	P	Coeff	SE	P	Coeff	SE	P
Overall	175/507	34.5 (29.5-40.0)	-	-	-	-	-	-	-	-	-	-	-	-	-
irAEs of any grade															
Yes	100/215	46.5 (37.8-56.6)	< .0001	-0.8769	0.2015	< .0001									
No	75/292	25.7 (20.2-32.2)													
G3/G4 irAEs															
Yes	16/39	41.0 (23.4-66.6)	.3641												
No	158/468	33.8 (28.7-39.5)													
Type of irAEs (No)															
Single site	86/176	48.9 (39.1-60.3)	<.0001				-0.9679	0.2123	<.0001						
Multiple site	14/39	35.9 (19.6-60.2)	.1773				-0.4601	0.3718	.2159						
Endocrine															
Yes	40/78	51.2 (36.6-69.8)	.0007							-0.7415	0.2597	.0043			
No	135/429	31.4 (26.3-37.2)													
GI															
Yes	20/51	39.2 (23.9-60.6)	.4572												
No	155/301	51.5 (43.7-60.3)													
Skin															
Yes	32/58	55.1 (37.7-77.9)	.0004										-1.0203	0.2940	.0005
No	143/449	31.8 (26.8-37.5)													
Pneumological															
Yes	9/23	39.1 (17.8-74.2)	.6342												
No	166/484	34.3 (29.3-39.9)													
Hepatic															
Yes	4/10	40 (10.9-102.4)	.7431												
No	171/497	24.4 (29.4-39.9)													
Others															
Yes	16/46	34.7 (19.8-56.4)	.9683												
No	159/461	34.4 (29.3-40.3)													
Sex															
Female	58/166	34.9 (26.5-45.1)	.8889												
Male	117/341	34.3 (28.3-41.1)													

Table 4 Continued

Variable (Comparator)	Univariate Analysis			Multivariate											
				irAEs of Any Grade			Type of irAEs			Endocrine irAEs			Skin irAEs		
	Response/Ratio	ORR (95% CI)	P	Coeff	SE	P	Coeff	SE	P	Coeff	SE	P	Coeff	SE	P
Age				-	-	-	-	-	-	-	-	-	-	-	-
Elderly	78/232	33.6 (26.6-41.9)	.6970												
Non-elderly	97/275	35.2 (28.6-43.0)													
ECOG-PS															
0-1	165/439	37.6 (32.1-43.8)	.0002	1.1629	0.3697	.0017	1.1241	0.3700	.0015	1.2058	0.3660	.0010	1.2810	0.3682	.0005
≥2	10/68	14.7 (7.1-27.0)													
Treatment line															
First	48/95	50.5 (37.2-66.9)	.0003	1.1059	0.2517	<.0001	1.1031	0.2526	<.0001	1.0344	0.2473	<.0001	1.0868	0.2478	<.0001
Further lines	127/412	30.8 (25.7-36.6)													
No. of metastatic sites															
≤2	95/224	42.4 (34.3-51.8)	.0009	0.7203	0.2838	.0004	0.7066	0.2044	.0005	0.7567	0.2014	.0231	0.7609	0.2022	.0002
>2	80/283	28.3 (22.4-35.2)													
				Nagelkerke R^2 : 0.1616			Nagelkerke R^2 : 0.1661			Nagelkerke R^2 : 0.1343			Nagelkerke R^2 : 0.1441		

Abbreviations: Coeff = coefficient; GI = gastrointestinal; irAE = immune-related adverse event; ECOG-PS = Eastern Cooperative Oncology Group Performance Status; ORR = overall response rate; SE = standard error.

Figure 1 Kaplan-Meier Survival Curves According to irAEs of any Grade. (A) Progression-free Survival; (B) Overall Survival



(95% CI, 10.8-15.9; 283 censored patients), with no statistically significant differences between the 2 groups ($P = .3844$).

Six-Week Landmark Analysis

A total of 524 patients (93.7%) were included in the 6-week landmark analysis; 224 of them experienced irAEs of any grade: 42.7% (95% CI, 37.3-48.7). There were no statistically significant differences with global incidence of irAEs in the overall study population ($P = .7618$). Among 485 patients who were evaluable for activity, ORR was 35.9% (95% CI, 30.7-41.6; 174 responses of disease); among 213 patients who experienced irAEs of any grade, ORR was 46.9% (95% CI, 38.2-57.1; 100 responses of disease), whereas among 271 patients who did not experience irAEs of any grade ORR was 27.2% (95% CI, 21.3-34.1). The difference was statistically significant at the univariate analysis ($P < .0001$).

After a median follow-up of 11.6 months, median PFS was 6.6 months (95% CI, 5.9-8.3; 305 events) and median OS was 14.7 months (95% CI, 11.7-17.6; 292 censored patients). Among patients who experienced irAEs of any grade, median PFS was 10.2 months (95% CI, 8.5-14.4; 124 events), whereas among patients who did not experience irAEs of any grade median PFS was 4.9 months (95% CI, 3.8-6.1; 181 progression events), with a statistically significant difference at univariate analysis ($P < .0001$) and a hazard ratio of 0.56 (95% CI, 0.44-0.71).

Among patients who experienced irAEs of any grade, median OS was 21.4 months (95% CI, 16.8-25.1; 134 censored patients), whereas among patients who did not experience irAEs median OS was 10.3 months (95% CI, 7.5-14.1; 158 censored patients), with a statistically significant difference at the univariate analysis ($P < .0001$) and a hazard ratio of 0.49 (95% CI, 0.37-0.65). As summarized in Table 7, the occurrence of irAEs of any grade was confirmed to be an independent predictor for higher ORR, longer PFS, and longer OS at the multivariate analysis.

Discussion

In this study, irAEs of any grade and G3/G4 irAEs occurred in 41.3% and 8.9% of patients, respectively. Although the incidence

of irAEs in our cohort is lower than that reported in clinical trials,³ it appears realistic when compared with other “real-life” studies involving patients with NSCLC treated with anti-PD-1 monotherapy.¹⁷ To the best of our knowledge, no studies have clearly addressed the question whether “single-site” and “multiple-site” irAEs could have a different impact on the clinical outcome of patients with NSCLC treated with ICIs. In our study “single-site” irAEs were more frequent than “multiple-site” irAEs (82.6% vs. 17.4%), suggesting that the underlying pathological mechanism tends to involve a specific system/organ. As previously stated, irAEs result from an aberrant immune self-response elicited by the ICIs; it is reasonable to assume that, as in autoimmune/immune system disorders, in which the pathologic mechanisms are based on tissue-specific T-cell- and B-cell-mediated cross-reactions,¹⁸ even in the case of irAEs there can be a similar kind of specificity regarding the system/organ involved. Indeed, targeting the PD-1/PD-L1 axis could trig latent autoimmunity not only with T-cell-mediated mechanisms but also by modulation of humoral immune response, through B-cell-mediated mechanisms.¹⁹⁻²¹

Consistent with the available data,^{6-11,22} our results confirmed the correlation between clinical benefit from anti-PD-1 immunotherapy and the development of irAEs considering both our analyses (overall population and 6-week landmark analysis). However, thanks to the wide sample size, our study is the first that revealed a concordant correlation between the occurrence of irAEs, higher ORR, longer PFS, and longer OS at the multivariate analyses.

Interestingly, irAEs of any grade, “single-site” irAEs, endocrine irAEs, and skin irAEs were concordantly associated to better clinical outcomes (ORR, PFS, and OS), whereas G3/G4 irAEs were not. This evidence suggests that irAEs less “clinically impacting,” which usually do not have serious sequelae, could be those with a positive predictive role, as the balance between the advantage or disadvantage of the irAE itself would depend on its severity. Skin irAEs and endocrine irAEs (thyroid dysfunctions prevalently) are surely clinically more manageable and less serious, when compared with pneumological and hepatic irAEs. Nevertheless, when looking at the analyses of “multiple-site,” GI, and other irAEs, we have the cue to

Table 5 Cox Proportional-Hazards Regression: Univariate and Multivariate Analyses of Progression-Free Survival

Variable (Comparator)	Progression-Free Survival					
	Univariate Analysis	Multivariate Analysis				
		irAEs of Any Grade	Sites of irAEs		Endocrine irAEs	Skin irAEs
HR (95% CI) P	HR (95% CI) P	HR (95% CI) P		HR (95% CI) P	HR (95% CI) P	
irAEs of any grade (Yes vs. No)	0.53 (0.42-0.66) P < .0001	0.57 (0.45-0.72) P < .0001	-		-	-
G3/G4 irAEs (Yes vs. No)	0.75 (0.51-1.11) P = .1556	-	-		-	-
Sites of irAEs		-				
Single site versus No	0.51 (0.41-0.65) P < .0001		0.55 (0.43-0.71) P < .0001			
Multiple site versus No	0.59 (0.39-0.89) P = .0133		0.64 (0.42-0.97) P = .0374			
Endocrine irAEs (Yes vs. No)	0.57 (0.41-0.81) P = .0011	-	-		0.63 (0.45-0.89) P = .0084	-
GI irAEs (Yes vs. No)	0.68 (0.47-1.01) P = .0531	-	-		-	-
Skin irAEs (Yes vs. No)	0.41 (0.28-0.62) P < .0001	-	-		-	0.46 (0.31-0.69) P = .0001
Pneumological irAEs (Yes vs. No)	1.20 (0.76-1.92) P = .4203	-	-		-	-
Hepatic irAEs (Yes vs. No)	1.47 (0.72-2.96) P = .2817	-	-		-	-
Others irAEs (Yes vs. No)	0.84 (0.57-1.23) P = .3723	-	-		-	-
Sex (Male vs. Female)	1.08 (0.86-1.36) P = .4914	-	-		-	-
Age (Elderly vs. Non-elderly)	0.88 (0.71-1.09) P = .2709	-	-		-	-
Treatment line (Non-first vs. First)	1.62 (1.16-2.25) P = .0042	1.70 (1.22-2.37) P = .0017	1.70 (1.22-2.37) P = .0017		1.67 (1.20-2.33) P = .0024	1.70 (1.22-2.37) P = .0016
N° of metastatic sites (>2 vs. ≤ 2)	1.28 (1.02-1.59) P = .0277	1.21 (0.96-1.51) P = .0910	1.21 (0.96-1.51) P = .0962		1.27 (1.02-1.59) P = .0307	1.27 (1.02-1.58) P = .0345
ECOG-PS (≥2 vs. 0-1)	2.42 (1.82-3.22) P < .0001	2.12 (1.59-2.83) P < .0001	2.12 (1.59-2.83) P < .0001		2.23 (1.67-2.97) P < .0001	2.28 (1.72-3.04) P < .0001

Abbreviations: CI = confidence interval; GI = gastrointestinal; HR = hazard ratio; irAE = immune-related adverse event; ECOG-PS = Eastern Cooperative Oncology Group Performance Status.

Table 6 Cox Proportional-Hazards Regression: Univariate and Multivariate Analyses of Overall Survival

Variable (Comparator)	Overall Survival						
	Univariate Analysis	Multivariate Analysis					
		irAEs of Any Grade	Sites of irAEs	Endocrine irAEs	GI irAEs	Skin irAEs	Others irAEs
		HR (95% CI) P					
irAEs of any grade (Yes vs. No)	0.47 (0.36-0.60) P < .0001	0.53 (0.41-0.69) P < .0001	-	-	-	-	-
G3/G4 irAEs (Yes vs. No)	0.76 (0.48-1.21) P = .2483	-	-	-	-	-	-
Sites of irAEs							
Single site versus No	0.45 (0.34-0.59) P < .0001		0.51 (0.38-0.68) P < .0001				
Multiple site versus No	0.54 (0.33-0.87) P = .0111		0.63 (0.39-1.01) P = .0558				
Endocrine irAEs (Yes vs. No)	0.48 (0.32-0.72) P = .0004	-	-	0.55 (0.37-0.83) P = .0044	-	-	-
GI irAEs (Yes vs. No)	0.55 (0.34-0.88) P = .0131	-	-	-	0.61 (0.38-0.98) P = .0437	-	-
Skin irAEs (Yes vs. No)	0.39 (0.24-0.63) P = .0001	-	-	-	-	0.43 (0.27-0.70) P = .0006	-
Pneumological irAEs (Yes vs. No)	1.32 (0.79-2.19) P = .2770	-	-	-	-	-	-
Hepatic irAEs (Yes vs. No)	1.09 (0.48-2.45) P = .8290	-	-	-	-	-	-
Others irAEs (Yes vs. No)	0.61 (0.38-0.98) P = .0432	-	-	-	-	-	0.61 (0.38-0.97) P = .0378
Sex (Male vs. Female)	1.43 (1.09-1.88) P = .0099	1.28 (0.97-1.60) P = .0782	1.28 (0.97-1.69) P = .0797	1.33 (1.01-1.75) P = .0407	1.33 (1.01-1.76) P = .0378	1.34 (1.01-1.76) P = .0366	1.33 (1.01-1.76) P = .0384
Age (Elderly vs. Non-elderly)	1.18 (0.92-1.51) P = .1823	-	-	-	-	-	-
Treatment line (Non-first vs. First)	1.38 (0.92-2.06) P = .1116	-	-	-	-	-	-
No. of metastatic sites (>2 vs. ≤ 2)	1.13 (0.88-1.45) P = .3167	-	-	-	-	-	-
ECOG-PS (≥2 vs. 0-1)	3.15 (2.34-4.23) P < .0001	2.71 (2.01-3.66) P < .0001	2.72 (2.02-3.67) P < .0001	2.89 (2.15-3.90) P < .0001	2.99 (2.22-4.03) P < .0001	2.92 (2.17-3.92) P < .0001	3.10 (2.31-4.17) P < .0001

Abbreviations: CI = confidence interval; GI = gastrointestinal; HR = hazard ratio; irAE = immune-related adverse event; ECOG-PS = Eastern Cooperative Oncology Group Performance Status.

Table 7 Six-Week Landmark Multivariate Analyses

	Multivariate Analysis					
	ORR		PFS		OS	
	OR ^a (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
irAEs of any grade (Yes vs. No)	0.43 (0.29-0.65)	<.0001	0.59 (0.47-0.76)	<.0001	0.55 (0.41-0.72)	<.0001

In logistic regression for ORR covariates were: ECOG-PS (0/1 vs. ≥ 2), treatment line (first vs. non-first) and number of metastatic sites (≤ 2 vs. > 2). Abbreviations: CI = confidence interval; HR = hazard ratio; irAE = immune-related adverse event; ECOG-PS = Eastern Cooperative Oncology Group Performance Status; OR = odds ratio; ORR = overall response rate; OS = overall survival; PFS = progression-free survival.
^aAdjusted OR, Nagelkerke R² = 0.1505. In Cox proportional-hazard, regression for PFS covariates were ECOG-PS, treatment line, and number of metastatic sites. In Cox proportional-hazard, regression for OS covariates were sex and ECOG-PS.

hypothesize that maybe with an even bigger sample size, also for these variables a concordant statistical significance for ORR, PFS, and OS would emerge.

Otherwise, we could also speculate that the hypothetical predictive role could depend on the system/organ involved. Cutaneous irAEs of anti-PD-1 treatments have been the first ones to be associated with clinical benefit.⁶⁻⁸ Interestingly, also antibody-mediated thyroid dysfunction developed during immunotherapy with pembrolizumab was found to be significantly related to a longer OS.⁹ A recent retrospective study of 134 patients with NSCLC treated with nivolumab, revealed a statistically significant association between any irAEs, cutaneous irAEs, and endocrine irAEs and a longer PFS, whereas just for any irAEs and cutaneous irAEs with a longer OS.¹⁰ As the mechanisms that underlie tumor response during immunotherapy are the same responsible of irAEs, we could imagine that irAEs are epiphenomena, which depend on the activation of “tissue-specific” immune self-response via T-cell— and B-cell—mediated pathways. With this in mind, the latent “tissue-specific” autoimmunity would not only be treatment-related, but also patient-related.

Interestingly, we found a significantly greater incidence of irAEs of any grade among female individuals and among patients with ECOG-PS 0-1. Sex could surely affect immune responses,²³ even though our knowledge about sexual dimorphism in ICI response is still scanty.²⁴ The greater incidence of irAEs is concordant with the trend of a longer OS among female patients, which at the same time is aligned with both evidence of sex-related difference in survival among cancer patients overall,²⁵ and of a greater benefit from immunotherapy with ICIs in male patients.^{26,27}

Our safety analysis also revealed that ECOG-PS ≥ 2 was significantly related to a lower incidence of irAEs of any grade. If irAEs result from pharmacodynamic activity of ICIs and are surrogates of clinical benefit, a poor PS could imply a kind of “repressed immune-reactivity,” and thus a lower incidence of irAEs with corresponding shorter survival.

Despite the large sample size, we must interpret these findings with caution. The retrospective nature of our study exposes us to the risk of selection biases, even if the 6-week landmark analysis confirmed our observations. Among the limits of our study we must recognize also the lack of centralized data review (imaging and toxicity), and the heterogeneous data availability. Indeed, we do not have the data on treatments used in managing irAEs and we are not able to calculate the time to develop irAEs among subgroups.

Conclusion

Our study confirmed that irAEs and their different spectrum are concordantly related to higher ORR, longer PFS, and longer OS with anti-PD-1 immunotherapy in patients with NSCLC. We can now hypothesize more confidently that the mechanisms that underlie tumor-immune response are the same that trigger immune self-response and autoimmunity. Probably the activation of the “ideal immune system” must walk a tightrope between immune response against the tumor and immune self-response. The balance between the advantage and disadvantage of the irAE itself depends on its severity, on the affected system/organ, and on the number of sites involved. Further prospective studies are required to confirm our findings.

Clinical Practice Points

- Immune-related adverse events (irAEs) developed during immunotherapy with anti-PD-1 agents, could be a predictive surrogate marker of clinical benefit. However, studies conducted on the topic have been flawed by several limitations, including the small sample size.
- Our study, thanks to a wide sample size, confirmed that irAEs seem concordantly related to higher ORR, longer PFS, and longer OS with anti-PD-1 immunotherapy in patients with NSCLC. We also demonstrated that not all the irAEs have the same impact on clinical outcome.
- In case of single-site irAEs of the skin or endocrine system, we can assume a certain clinical benefit with PD-1 inhibitors. On the contrary, we must interpret with caution hepatic and pneumological irAEs, “multiple-site” irAEs, and in general G3/G4 irAEs.

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Disclosure

Dr. Alessio Cortellini received grants as speaker from MSD. Dr. Melissa Bersanelli received grants as speaker from BMS. Dr.

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Supplemental Data

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Supplemental Data

Supplemental Table 1 List of the Participating Oncological Institution	
Institution	Department
St. Salvatore Hospital, University of L'Aquila, L'Aquila	Medical Oncology
SS Annunziata Hospital, Chieti	Medical Oncology
University Hospital of Parma, Parma	Medical Oncology
St. Camillo-Forlanini Hospital, Rome	Pulmonary Oncology
University Hospital of Cagliari, Cagliari	Medical Oncology
S Maria Goretti Hospital, Latina	Medical Oncology
St. Andrea Hospital, Rome	Medical Oncology
Campus Bio-Medico University, Rome	Medical Oncology
“Ospedali Riuniti” Hospital, Ancona	Medical Oncology
St. Maria della Misericordia Hospital, Perugia	Medical Oncology
Hospital of Fabriano, Fabriano	Medical Oncology
SS Spirito Hospital, Pescara	Medical Oncology