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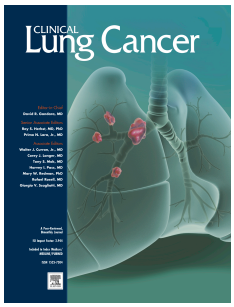

Correlations between the immune-related adverse events spectrum and efficacy of anti-PD1 immunotherapy in NSCLC patients

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Running title: immune-related adverse events in NSCLC

Correlations between the immune-related adverse events spectrum and efficacy of anti-PD1 immunotherapy in NSCLC patients

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Micro-abstract

The aim of this study is to investigate with a large sample size, the predictive and prognostic positive roles of occurrence of immune-related adverse events in NSCLC patients, treated with PD-1 inhibitors. The study confirmed that immune-related adverse events are independent predictors of higher ORR, longer PFS and longer OS.

Clinical Practice Points

What is already known about this subject?

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.

What are the new findings?

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 NSCLC patients. We also demonstrated that not all the irAEs have the same impact on clinical outcome.

How might it impact on clinical practice in the foreseeable future?

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 epatic and penumological irAEs, “multiple-site” irAEs and in general G3/G4 irAEs.

Abstract

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) patients.

Methods: NSCLC patients, 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), ORR, PFS and OS. We further performed a 6-weeks landmark analysis.

Results: 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 ($p < 0.0001$), "single-site" irAEs ($p < 0.0001$), endocrine ($p = 0.0043$) and skin irAEs ($p = 0.0005$). Longer PFS was related to: irAEs of any grade ($p < 0.0001$), "single-site" irAEs ($p < 0.0001$), "multiple-site" irAEs ($p = 0.0374$), endocrine ($p = 0.0084$) and skin irAEs ($p = 0.0001$). Longer OS was related to: irAEs of any grade ($p < 0.0001$), "single-site" irAEs ($p < 0.0001$), endocrine irAEs ($p = 0.0044$), GI irAEs ($p = 0.0437$), skin irAEs ($p = 0.0006$) and others irAEs ($p = 0.0378$). At 6-weeks landmark analysis irAEs of any grade was confirmed an 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 NSCLC patients.

Keywords: pembrolizumab, nivolumab, immunotherapy, efficacy, immune-related adverse events, NSCLC.

Introduction

The advent of immune checkpoint inhibitors (ICIs), particular those acting on the PD-1/PD-L1 axis (Programmed Death-1/ Programmed Death-Ligand 1), 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). Immune-related AEs 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 over than 5.000 NSCLC patients treated with PD-1 inhibitors reported an overall incidence of irAEs of 64%, with 14% of G3/G4 irAEs [3].

The timing of irAEs onset is widely different when compared to the timing of chemotherapy toxicities 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 NSCLC patients 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 ICIs mechanism of action, it has been speculated that patients who experience irAEs might derive a greater clinical benefit from these compounds. Consistently 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 also evidence that patients who discontinue immunotherapy due to irAEs, tend to maintain the benefit from the treatment, an element which further suggest a mechanistic association between irAEs and ICIs 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 to derive definitive results.

Against this background, we conducted a multi-center retrospective study in order to evaluate the impact of different types 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 one cycle of anti-PD-1 agents, regardless of treatment line, at 11 Italian centers, between September 2013 and May 2018 (supplementary file 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 24th July 2018).

Study design

A "real-life" multi-center retrospective observational study of advanced NSCLC patients, 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 one 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: objective 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* $\geq$ 2), age ($<$ 70 *vs* $\geq$ 70 years old) [13 – 16], number of metastatic sites ($\leq$ 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. In order to weigh its role, the incidence of irAEs among subgroups of patients with different PD-L1 expression was compared with the $\chi 2$ test[17]. A further analysis was performed, to evaluate the incidence of irAEs of any grade according to sex, ECOG-PS, age, number of metastatic sites, treatment line (categorized as above 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 [18]. $\chi 2$ test was used to compare ORR and the incidence of irAEs of any

grade among subgroups [17]. In the multivariate analysis, logistic regression was used to evaluate the role of parameters which proved significant at the univariate analyses of ORR and irAEs of any grade [19]. PFS and OS were calculated from the date of the start of immunotherapy (Day1, cycle 1). Median PFS and median OS were evaluated using the Kaplan-Meier method [20]. Patients who had not progressed/not died at data cut-off were censored at the time of the last clinical visit. Median follow-up was calculated according to the reverse Kaplan-Meier method [21]. Cox proportional hazards model [23] 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 to those who did not progress, therefore they had few chances of experience irAEs. In order to overcome the lack of data availability regarding time to develop irAEs among the study population, we performed a further 6-weeks landmark analysis [10], by including only patients with a minimum follow-up for PFS of 6 weeks, regardless of disease progression. The data cut-off 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) was computed on the basis of the percentage of stained tumor cells.

Categorization and definition of single/multiple-sites irAEs

They were graduated according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (CTCAE; version 4.0) and cumulatively reported. Immune-related AEs were categorized on the basis of the organ/system involved as follows: endocrine irAEs (including thyroid disorders), gastro-intestinal (GI) irAEs (excluding pancreatitis), skin irAEs, pneumological irAEs, hepatic irAEs and others irAEs (including rheumatologic, neuro-muscolar, pancreatitis, fever, asthenia and anorexia). Immune-related AEs were defined “single-site” if the patient experienced just one

category of irAEs among the abovementioned; 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 ites were performed only for irAEs of any grade and not for G3/G4 irAEs. Patients were clinically monitored, for safety evaluation, at every pre-administration visit (according to the technical files of the drugs), and as clinically indicated by the investigators subsequently.

Results

Patients' characteristics

Five hundred and fifty-nine, consecutive advanced NSCLC patients were enrolled. The initiation date of the anti-PD-1 treatment ranged from September 2013 to April 2018. The patients' 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%) have ≤ 2 metastatic sites while 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. Thirty-four patients (6.1%) discontinued the treatment due to AEs. Immune-related rAEs were summarized in Table2.

Table 3 summarized 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 to male patients (50% and 37.2% respectively, p = 0.0041), as non-elderly patients compared to elderly patients (45.7% and 36.3% respectively, p = 0.0249) and patients with ECOG-PS 0-1 compared to whom with ECOG-PS ≥ 2 (43.9% and 24.3% respectively, p 0.0014). Treatment line and number of metastatic sites were not significantly related to the incidence of irAEs of any grade. Sex (p = 0.0254) and ECOG-PS (p = 0.0046), whilst not age, 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 abovementioned

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 term of irAEs incidence according to PD-L1 TPS (p = 0.0845).

Activity analysis

Univariate and multivariate analysis 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 experienced irAEs the ORRs were 46.5% and 25.7% respectively (p < 0.0001). 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 = 0.3641). ORR of patients who experienced “single-site” irAEs was significantly higher when compared to ORR of patients who did not developed irAEs (p < 0.0001), while we did not find any association between ORR and “multiple-site” irAEs (p = 0.1773). No difference in ORR was observed between patients who developed single-site and multiple-site irAEs (p = 0.1428). Endocrine and skin irAEs were associated with a significantly higher ORR (p = 0.0007 and p = 0.0004 respectively). After adjusting for ECOG-PS, treatment line and number of metastatic sites irAEs of any grade (p < 0.0001), “single-site” irAEs (< 0.0001), endocrine irAEs (p = 0.0043) and skin irAEs (p = 0.0005) 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), while 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 = 0.1261).

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 moths (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), while 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), while median OS of patients who did not experienced 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), while among patients who did not was 6.2 months (95% CI: 5.1 – 7.1; 315 events), with no statistically significant differences ($p = 0.0666$). Among patients who discontinued the treatment due to irAEs median OS was 24.4 months (95% CI: 5.8 – 48.9; 18 censored patients), while among patients who did not was 12.3 months (95% CI: 10.8 – 15.9; 283 censored patients), with no statistically significant differences between the two groups ($p = 0.3844$).

Six-weeks landmark analysis

Five-hundred and twenty-four patients (93.7%) were included in the 6-weeks 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 overall study population ($p = 0.7618$). 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), while among 271 patients who did not experienced irAEs of any grade ORR was 27.2 % (95% CI: 21.3 – 34.1). The difference was statistically significant at the univariate analysis ($p < 0.0001$).

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), while among patients who did not experienced irAEs of any grade was 4.9 months (95% CI: 3.8 – 6.1; 181 progression events), with a statistically significant difference at univariate analysis ($p < 0.0001$) 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), while among patients who did not experienced irAEs was 10.3 months (95% CI: 7.5 – 14.1; 158 censored patients), with a statistically significant difference at the univariate analysis ($p < 0.0001$) 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. Despite the incidence of irAEs in our cohort is lower than that reported in clinical trials [3], it appears realistic when compared to other “real-life” studies involving NSCLC patients treated with anti-PD-1 mono-therapy [23]. 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 NSCLC patients treated with ICIs. In our study “single-site” irAEs were more frequent than “multiple-site” irAEs (82.6% vs 17.4%), suggesting that the underling 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, where the pathologic mechanisms are based on tissue-specific T-cells and B-cells mediated cross-reactions [24], 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 auto-immunity not only with a T-cell mediated mechanisms but also by modulation of humoral immune response, through B-cells mediated mechanisms [25-27].

Consistently with the available data [6-11, 28], 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-weeks 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), while G3/G4 irAEs were not. These evidences suggest 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 to pneumological and hepatic irAEs. Nevertheless, when looking at the analyses of “multiple-site”, GI and others irAEs, we have the cue to 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 NSCLC patients treated with nivolumab, revealed a statistically significant association between any irAEs, cutaneous irAEs, endocrine irAEs and a longer PFS, while just for any irAEs and cutaneous irAEs with a longer OS [10]. As the mechanisms which 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. Whit this in mind, the latent “tissue-specific” autoimmunity would not only be treatment-related, but also patient-related.

Interestingly, we found a significant greater incidence of irAEs of any grade among females and among patients with ECOG-PS 0-1. Sex could surely affect immune responses [29], even though our knowledge about sexual dimorphism in ICIs response is still scanty [30]. The greater incidence of irAEs is concordant to the trend of a longer

OS among females patients, which at the same time is aligned with both evidences of sex-related difference in survival among cancer patients overall [31], and of a greater benefit from immunotherapy with ICIs in male patients [32, 33].

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 implies a kind of "repressed immune-reactivity", and thus a lower incidence of irAEs with corresponding shorter survivals.

Despite the big 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-weeks landmark analysis confirmed our observations. Amongst 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 NSCLC patients. We can now hypothesize more confidently that the mechanisms that underlie tumor-immune response, are the same that trig immune self-response and autoimmunity. Probably the activation of the "ideal immune system" must to 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.

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Availability of data and materials: the datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

All authors contributed to the publication according to the ICMJE guidelines for the authorship.

All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Consent for publication

Not applicable.

Conflicts of Interest: dr Alessio Cortellini received grants as speaker by MSD; dr Melissa Bersanelli received grants as speker by BMS; dr Marcello Tiseo: advisory boards and speakers' fee for BMS and MSD. All other authors declare no competing interests.

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Table and Figure legend

Table 1: Patients' characteristics.

Table 2: Summary of immune-related adverse events.

Table 3: Univariate and multivariate analyses of incidence of immune related adverse events of any grade.

Table 4: Univariate and multivariate analyses for Overall Response Rate.

Table 5: Cox proportional-hazards regression: univariate and multivariate analyses of Progression Free Survival.

Table 6: Cox proportional-hazards regression: univariate and multivariate analyses of Overall Survival.

Table 7: 6-weeks landmark multivariate analyses. 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). *Adjusted OR, Nagelkerke R2 = 0.1505. In Cox proportional-hazard regression for PFS covariates were: ECOG-PS, treatment line and number of metastatic site. In Cox proportional-hazard regression for OS covariates were: sex and ECOG-PS.

Figure 1: Kaplan-Meier survival curves according to irAEs of any grade. (A) Progression Free Survival; (B) Overall Survival

	N° (%)
	559
AGE, (years)	
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)
Non-squamous	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)
≥ 50%	100 (17.9)

	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)
Haepatic	10 (4.3)	6 (12)
Others	46 (19.9)	6 (12)

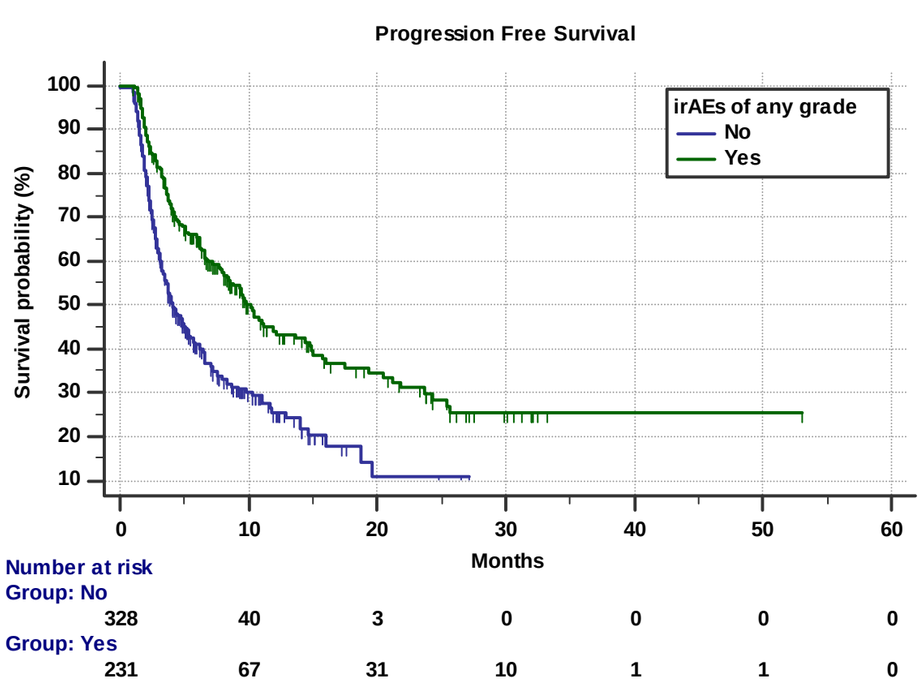
irAEs of any grade - UNIVARIATE ANALYSIS			
Variable (comparator)	**Events Ratio**	**Incidence (95% CI)**	***p - value***
Overall	231/559	41.3 (36.1 – 47.0)	-
Sex			
Female	90/180	50 (40.2 – 61.4)	*0.0041*
Male	141/379	37.2 (31.3 – 43.8)	
Age			
Elderly	94/259	36.3 (29.3 – 44.4)	*0.0249*
Non-elderly	137/300	45.7 (38.3 – 53.9)	
ECOG-PS			
0-1	231/485	43.9 (38.2 – 50.2)	*0.0014*
≥ 2	18/74	24.3 (14.4 – 38.4)	
Treatment line			
First	42/116	36.2 (26.1 – 48.9)	*0.2091*
Further lines	189/443	42.7 (36.8 – 49.2)	
Burden of disease			
≤ 2 site	110/240	45.8 (37.6 – 55.2)	*0.0693*
> 2 site	121/317	38.2 (31.6 – 45.6)	
irAEs of any grade – MULTIVARIATE ANALYSIS			
Variable (comparator)	**Coefficient**	**Std. Error**	***p – value***
Sex	-0.4209	0.1882	*0.0254*
Age	-0.2878	0.1778	*0.1056*
ECOG-PS	-0.8175	0.2886	*0.0046*
			Coefficient of Determination R^2: 0.0482

	UNIVARIATE ANALYSIS			MULTIVARIATE											
				irAEs of any grade			Type of irAEs			Endocrine irAEs			Skin irAEs		
Variable (comparator)	**Response/ Ratio**	**ORR (95% CI)**	***p - value***	**Coeff.**	**St. Err.**	***p - value***	**Coeff.**	**St. Err.**	***p - value***	**Coeff.**	**St. Err.**	***p - value***	**Coeff.**	**St. E.**	***p - value***
Overall	175/507	34.5 (29.5–40.0)	-	-	-	-	-	-	-	-	-	-	-	-	-
irAEs of any grade															
Yes	100/215	46.5 (37.8–56.6)	*< 0.0001*	-0.8769	0.2015	*< 0.0001*	-	-	-	-	-	-	-	-	-
No	75/292	25.7 (20.2–32.2)													
G3/G4 irAEs															
Yes	16/39	41.0 (23.4–66.6)	*0.3641*	-	-	-	-	-	-	-	-	-	-	-	-
No	158/468	33.8 (28.7–39.5)													
Type of irAEs (No)															
Single site	86/176	48.9 (39.1-60.3)	*<0.0001*	-	-	-	-0.9679	0.2123	*<0.0001*	-	-	-	-	-	-
Multiple site	14/39	35.9 (19.6-60.2)	*0.1773*				-0.4601	0.3718	*0.2159*						
Endocrine															
Yes	40/78	51.2 (36.6–69.8)	*0.0007*	-	-	-	-	-	-	-0.7415	0.2597	*0.0043*	-	-	-
No	135/429	31.4 (26.3-37.2)													
GI															
Yes	20/51	39.2 (23.9–60.6)	*0.4572*	-	-	-	-	-	-	-	-	-	-	-	-
No	155/301	51.5 (43.7-60.3)													
Skin															
Yes	32/58	55.1 (37.7–77.9)	*0.0004*	-	-	-	-	-	-	-	-	-	-1.0203	0.2940	*0.0005*
No	143/449	31.8 (26.8-37.5)													
Pneumological															
Yes	9/23	39.1 (17.8–74.2)	*0.6342*	-	-	-	-	-	-	-	-	-	-	-	-
No	166/484	34.3 (29.3-39.9)													
Hepatic															
Yes	4/10	40 (10.9–102.4)	*0.7431*	-	-	-	-	-	-	-	-	-	-	-	-
No	171/497	24.4 (29.4-39.9)													
Others															
Yes	16/46	34.7 (19.8–56.4)	*0.9683*	-	-	-	-	-	-	-	-	-	-	-	-
No	159/461	34.4 (29.3-40.3)													
Sex															
Female	58/166	34.9 (26.5–45.1)	*0.8889*	-	-	-	-	-	-	-	-	-	-	-	-
Male	117/341	34.3 (28.3–41.1)													
Age															
Elderly	78/232	33.6 (26.6–41.9)	*0.6970*	-	-	-	-	-	-	-	-	-	-	-	-
Non-elderly	97/275	35.2 (28.6–43.0)													
ECOG-PS															
0-1	165/439	37.6 (32.1–43.8)	*0.0002*	1.1629	0.3697	*0.0017*	1.1241	0.3700	*0.0015*	1.2058	0.3660	*0.0010*	1.2810	0.3682	*0.0005*
≥ 2	10/68	14.7 (7.1–27.0)													
Treatment line															
First	48/95	50.5 (37.2–66.9)	*0.0003*	1.1059	0.2517	*<0.0001*	1.1031	0.2526	*<0.0001*	1.0344	0.2473	*<0.0001*	1.0868	0.2478	*<0.0001*
Further lines	127/412	30.8 (25.7–36.6)													
N° of metastatic sites															
≤ 2	95/224	42.4 (34.3–51.8)	*0.0009*	0.7203	0.2838	*0.0004*	0.7066	0.2044	*0.0005*	0.7567	0.2014	*0.0231*	0.7609	0.2022	*0.0002*
> 2	80/283	28.3 (22.4–35.2)													
				Nagelkerke R2: 0.1616			**Nagelkerke R2: 0.1661**			**Nagelkerke R2: 0.1343**			**Nagelkerke R2: 0.1441**		

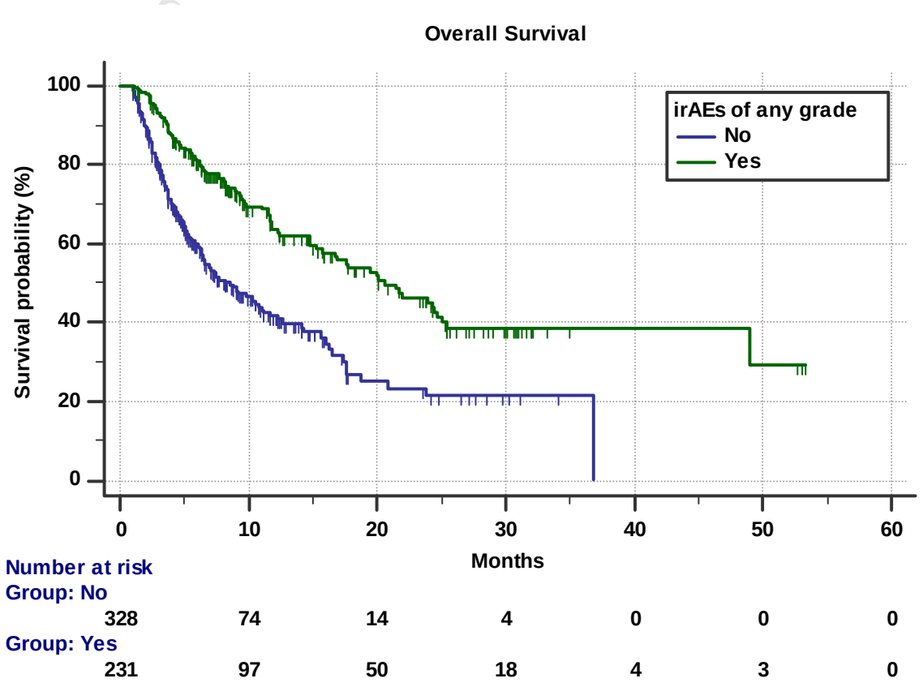
	Progression Free Survival				
	Univariate Analysis	Multivariate Analysis			
		irAEs of any grade	Sites of irAEs	Endocrine irAEs	Skin irAEs
VARIABLE (Comparator)	**HR (95% CI)** ***p - value***	**HR (95% CI)** ***p - value***	**HR (95% CI)** ***p - value***	**HR (95% CI)** ***p - value***	**HR (95% CI)** ***p - value***
irAEs of any grade (Yes vs No)	0.53 (0.42–0.66) *p<0.0001*	0.57 (0.45–0.72) *p<0.0001*	-	-	-
G3/G4 irAEs (Yes vs No)	0.75 (0.51–1.11) *p=0.1556*	-	-	-	-
Sites of irAEs					
Single site vs No	0.51 (0.41-0.65) *p<0.0001*	-	0.55 (0.43-0.71) *p<0.0001*		
Multiple site vs No	0.59 (0.39-0.89) *p=0.0133*		0.64 (0.42-0.97) *p=0.0374*		
Endocrine irAEs (Yes vs No)	0.57 (0.41–0.81) *p=0.0011*	-	-	0.63 (0.45–0.89) *p=0.0084*	-
GI irAEs (Yes vs No)	0.68 (0.47–1.01) *p=0.0531*	-	-	-	-
Skin irAEs (Yes vs No)	0.41 (0.28–0.62) *p<0.0001*	-	-	-	0.46 (0.31–0.69) *p=0.0001*
Pneumological irAEs (Yes vs No)	1.20 (0.76–1.92) *p=0.4203*	-	-	-	-
Hepatic irAEs (Yes vs No)	1.47 (0.72–2.96) *p=0.2817*	-	-	-	-
Others irAEs (Yes vs No)	0.84 (0.57–1.23) *p=0.3723*	-	-	-	-
Sex (Male vs Female)	1.08 (0.86–1.36) *p=0.4914*	-	-	-	-
Age (Elderly vs Non-elderly)	0.88 (0.71–1.09) *p=0.2709*	-	-	-	-
Treatment line (Non-first vs First)	1.62 (1.16–2.25) *p=0.0042*	1.70 (1.22-2.37) *p=0.0017*	1.70 (1.22-2.37) *p=0.0017*	1.67 (1.20-2.33) *p=0.0024*	1.70 (1.22-2.37) *p=0.0016*
N° of metastatic sites (>2 vs ≤ 2)	1.28 (1.02–1.59) *p=0.0277*	1.21 (0.96-1.51) *p=0.0910*	1.21 (0.96-1.51) *p=0.0962*	1.27 (1.02-1.59) *p=0.0307*	1.27 (1.02-1.58) *p=0.0345*
ECOG PS (≥2 vs 0-1)	2.42 (1.82–3.22) *p<0.0001*	2.12 (1.59–2.83) *p<0.0001*	2.12 (1.59-2.83) *p<0.0001*	2.23 (1.67–2.97) *p<0.0001*	2.28 (1.72–3.04) *p<0.0001*

	Overall Survival						
	Univariate Analysis	Multivariate Analysis					
		irAEs of any grade	Sites of irAEs	Endocrine irAEs	GI irAEs	Skin irAEs	Others irAEs
VARIABLE (Comparator)	**HR (95% CI)** ***p - value***	**HR (95% CI)** ***p - value***	**HR (95% CI)** ***p - value***	**HR (95% CI)** ***p - value***	**HR (95% CI)** ***p - value***	**HR (95% CI)** ***p - value***	**HR (95% CI)** ***p - value***
irAEs of any grade (Yes vs No)	0.47 (0.36–0.60) *p<0.0001*	0.53 (0.41–0.69) *p<0.0001*	-	-	-	-	-
G3/G4 irAEs (Yes vs No)	0.76 (0.48–1.21) *p=0.2483*	-	-	-	-	-	-
Sites of irAEs Single site vs No Multiple site vs No	0.45 (0.34-0.59 *p<0.0001* 0.54 (0.33-0.87) *P=0.0111*	-	0.51 (0.38-0.68) *p<0.0001* 0.63 (0.39-1.01) *p=0.0558*				
Endocrine irAEs (Yes vs No)	0.48 (0.32–0.72) *p=0.0004*	-	-	0.55 (0.37–0.83) *p=0.0044*	-	-	-
GI irAEs (Yes vs No)	0.55 (0.34–0.88) *p=0.0131*	-	-	-	0.61 (0.38–0.98) *p=0.0437*	-	-
Skin irAEs (Yes vs No)	0.39 (0.24–0.63) *p=0.0001*	-	-	-	-	0.43 (0.27–0.70) *p=0.0006*	-
Pneumological irAEs (Yes vs No)	1.32 (0.79–2.19) *p=0.2770*	-	-	-	-	-	-
Hepatic irAEs (Yes vs No)	1.09 (0.48–2.45) *p=0.8290*	-	-	-	-	-	-
Others irAEs (Yes vs No)	0.61 (0.38–0.98) *p=0.0432*	-	-	-	-	-	0.61 (0.38–0.97) *p=0.0378*
Sex (Male vs Female)	1.43 (1.09–1.88) *p=0.0099*	1.28 (0.97–1.60) *p=0.0782*	1.28 (0.97-1.69) *p=0.0797*	1.33 (1.01–1.75) *p=0.0407*	1.33 (1.01–1.76) *p=0.0378*	1.34 (1.01–1.76) *p=0.0366*	1.33 (1.01–1.76) *p=0.0384*
Age (Elderly vs Non-elderly)	1.18 (0.92–1.51) *p=0.1823*	-	-	-	-	-	-
Treatment line (Non-first vs First)	1.38 (0.92–2.06) *p=0.1116*	-	-	-	-	-	-
N° of metastatic sites (>2 vs ≤ 2)	1.13 (0.88–1.45) *p=0.3167*	-	-	-	-	-	-
ECOG PS (≥2 vs 0-1)	3.15 (2.34–4.23) *p<0.0001*	2.71 (2.01–3.66) *p<0.0001*	2.72 (2.02-3.67) *p<0.0001*	2.89 (2.15–3.90) *p<0.0001*	2.99 (2.22–4.03) *p<0.0001*	2.92 (2.17–3.92) *p<0.0001*	3.10 (2.31–4.17) *p<0.0001*

	Multivariate Analysis					
	ORR		**PFS**		**OS**	
	OR* (95% CI)	***p - value***	**HR (95% CI)**	***p - value***	***HR (95% CI)***	***p - value***
irAEs of any grade (Yes vs No)	0.43 (0.29–0.65)	*< 0.0001*	0.59 (0.47 – 0.76)	*< 0.0001*	0.55 (0.41 – 0.72)	*< 0.0001*



A



B

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