Immune Checkpoint Inhibitor Nivolumab and Radiotherapy in Pretreated Lung Cancer Patients

Efficacy and Safety of Combination

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Background: In the last decade, the discovery of immune checkpoint inhibitors such as the *PD-1* inhibitor, nivolumab, has revolutionized the treatment of advanced non–small cell lung cancer (NSCLC). Concurrent radiotherapy (RT) is of particular interest in showing the potential role of the combination.

Objective: The purpose of this study was to retrospectively evaluate the addition of RT to an immune checkpoint inhibitor, nivolumab, with regard to activity and feasibility in pretreated, advanced, or metastatic lung cancer patients at our center.

Patients and Methods: We retrospectively identified 35 consecutive patients (30 men and 5 women), who received nivolumab for pretreated NSCLC, between March 2015 to December 2016. Fifteen received hypofractionated RT as a palliative measure, and, in these patients, nivolumab was administered at an interval of at least 1 week from the end of RT.

Results: The median age was 69 years, and 23 patients (65.7%) had an Eastern Cooperative Oncology Group (ECOG) score of 0 to 1. All patients had previously received at least 1 systemic regimen, and, for only 3 (8.6%), nivolumab was a third-line treatment. The 2 treatment arms, RT-nivolumab and only-nivolumab, were well matched for baseline characteristics. At a median follow-up of 7.4 months, the 1-year overall survival rates were 57.8% for patients treated with RT-nivolumab and 27.4% for patients treated with only-nivolumab ($P = 0.043$). The 1-year progression-free survival in the RT-nivolumab group was 57.8% and 20.6% in the only-nivolumab group ($P = 0.040$). No difference in adverse events was detected.

Conclusions: In conclusion, RT and nivolumab can be combined, obtaining a benefit in overall survival and progression-free survival, without an increase in acute toxicities in pretreated advanced NSCLC patients. Prospective studies are needed to confirm these results.

Key Words: nivolumab and radiotherapy, lung cancer, immunotherapy, radiotherapy, combined therapy

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Non–small cell lung cancer (NSCLC) is historically considered a nonimmunogenic tumor. Tumor cells have several strategies to evade immune surveillance: downregulation of major histocompatibility complex (MHC) class I molecules, secretion of immunosuppressive factors, lack of T-cell costimulation, and expression of death ligands or negative ligands.

In lung cancer cells, it has been demonstrated that programmed-death ligand-1 (*PD-L1*) (ligand of *PD-1*), a negative ligand, is the most important strategy to obtain immune tolerance.[1]

First, the CheckMate 057 trial, using the antibody to *PD-1* nivolumab, clearly showed an improvement in overall survival compared with docetaxel in patients with metastatic non-squamous NSCLC with progression on or after platinum-based chemotherapy.[2]

That being so, activation of *PD-1* with *PD-L1* principally leads to exhaustion (progressive impairment and loss of function) of effector T cells and also to inhibition of the activation of antigen-specific dendritic cells (DC).[3]

Inhibition of the *PD-1-PD-L1* axis of the immune system may influence the clinical outcome of patients. In NSCLC, radiotherapy (RT) remains a mainstay therapy option, including in elderly patients.[4] It acts by debulking tumor cells but also stimulates inflammation. Killing tumor cells can create a pool of dying tumor cells that serves as a source of antigen for cross-presentation of MHC I–restricted peptides, thereby enhancing immunogenicity.[5] However, it was demonstrated that, after a radiation treatment, *PD-L1* expression in the microenvironment is upregulated in cancer cells[6]; thus, globally, the tumor response could be affected by this immune tolerance. Several preclinical experiments have developed the rationale for combining an immune checkpoint inhibitor such as nivolumab with RT. There is an increased interest to translate these findings from bench to bedside. Our objective was to determine whether a hypofractionated RT before administration of Nivolumab was associated with better outcomes in pretreated NSCLC patients in "real life" clinical practice.

PATIENTS AND METHODS

Patient Characteristics

Patients with stage IIIB or IV squamous and nonsquamous NSCLC who had disease recurrence after at least 1 prior platinum-containing regimen were treated with the humanized monoclonal antibody to *PD-1*, nivolumab. As reported in the nivolumab registration study, patients did not receive this treatment if they had an *EGFR*-activating mutation or *ALK* rearrangement, were below 18 years of age, had autoimmune diseases, and/or if they received systemic immunosuppression.

Routinely, all patients were assessed with the Eastern Cooperative Oncology Group (ECOG) performance-status score.

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Data were collected from patients who received oncological treatment in University Hospital of Ferrara between March 2015 and December 2016. Institutional review board approval was required according to the local guidelines for retrospective observational studies. We collected informed consent from patients, whenever it is possible.

Treatment Characteristics

Nivolumab was administered intravenously at 3 mg/kg dose every 2 weeks. All patients received at least 4 cycles of monoclonal antibody to *PD-1*; globally, a mean of 10 cycles/patient were administered, and 50% of patients received > 10 cycles. Between these, 15 patients had received hypofractionated RT before nivolumab administration. In all patients but 2, RT was delivered with a conformal technique using computed tomography (CT)-assisted 3-dimensional treatment planning (pinnacle) and 6 to 15 MV photon beams to treat bone metastases or mediastinum nodes.

During bone irradiation, 8 patients received 8 to 16 Gy in 1 or 2 fractions, and 5 patients received RT in the mediastinum with 36 Gy in 12 fractions instead. Furthermore, 2 patients received stereoablative RT for progression in a lung node. The radiation dose to target volume and constraints of organs at risk were in accordance with the international recommendations. In these radio-treated patients, nivolumab was administered at an interval of at least 1 week from the end of RT.

Evaluation

Following an internal protocol, metastatic lung cancer patients were seen before the start of each treatment cycle during the whole course of anti-*PD-1* therapy and then every 3 months after discontinuation of treatment.

During treatment, patients were monitored for adverse events; visits included a clinical assessment and physical examination, complete blood counts, and blood chemistry examinations. Medical records were reviewed to evaluate and classify side effects and toxicity according to the National Cancer Institute (NCI) expanded Common Toxicity Criteria, version 3.0. Thoracic and upper abdomen CT scan was performed after 3 and 6 nivolumab cycles until disease progression or treatment discontinuation. Additional imaging or laboratory investigations were carried out at the discretion of the treating physician on the basis of findings in the history or physical examination. The response data were rereviewed according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.[7]

In case of pseudoprogression, further CT evaluation was performed after 4 weeks to confirm or exclude progression. After progression, further treatment was at the physician's discretion: in 9 patients, a new chemotherapy schedule was administered.

Statistical Analysis

The primary endpoint of the study was overall survival. The secondary endpoints were (a) progression-free survival and (b) tolerance to treatment. The Kaplan-Meier method[8] was used to estimate survival and progression-free survival. Differences in survival and progression-free survival were assessed by the log-rank test. The observed survival time was the interval between diagnosis and death or the final follow-up. For progression-free survival, the tumor-free time was the interval between no evidence of progression of disease and the progression of the same tumor. In this analysis, patients dying without disease progression were censored at the time of death and were classified as progression-free.

The Cox model[9] was used to identify the risk factors for overall survival and progression-free survival. The following variables at baseline were considered for survival univariate analysis: age, sex, performance status, histology, smoking status, and prior systemic regimens. All analyses were conducted with SPSS version 13.0 (SPSS for Windows, Rel. 13.0 2004. Chicago: SPSS Inc.).

RESULTS

Features of Patients at Baseline

This retrospective study enrolled consecutively 35 consecutive patients, 30 male patients (85%) and 5 female patients (15%). The median age was 69 years. Seventeen subjects of 35 (48.6%) were over 70 years of age. The ECOG score was 0 to 1 in 23 patients (65.7%) and 2 in 12 patients (34.3%). Twelve patients (34.3%) had a local advanced disease stage IIIB, and the remaining 23 patients had a metastasized disease in ≥ 1 site. All patients had previously received at least 1 systemic regimen, and, for only 3 (8.6%), nivolumab was a third-line treatment. In this cohort of treated patients, 15 had received previous RT: as palliative treatment of bone metastases in 8 (53.3%) patients, as palliative treatment of the mediastinum in 5 (33.3%) patients, and as stereoablative RT on a single node in 2 (13.4%) patients. In all, RT was delivered hypofractionated, with a single dose ranging from 3 to 12 Gy and a total dose ranging from 8 to 36 Gy. The interval between the end of RT and nivolumab was at least 1 week. Clinical and demographic data of these 2 patient groups were evaluated at baseline in and are presented in Table 1.

Follow-up

In this retrospective analysis, patients with the complete panel of clinical data were considered. The mean length of follow-up after

TABLE 1. Patients Clinical Characteristics

Characteristics	Nivolumab With RT	Nivolumab Without RT
No. patients	15	20
Age (median) (y)	70	69
Range age	44-81	53-77
Age (> 70 y) (n [%])	7 (47)	10 (50)
Male sex (n [%])	11 (73)	19 (95)
ECOG PS (n [%])		
0-1	9 (60)	14 (70)
2	6 (40)	6 (30)
Histology (n [%])		
Squamous	9 (60)	10 (50)
Nonsquamous	6 (40)	10 (50)
Smoking status (n [%])		
Current or former smoker	14 (93)	18 (90)
Never smoker	0	2 (10)
Unknown	1 (7)	0
No. prior systemic regimens (n [%])		
1	11 (73)	16 (80)
2	2 (13)	3 (15)
3	2 (13)	1 (5)
Stage (n [%])		
IIIB	4 (27)	8 (40)
IV	11 (73)	12 (60)
Site of metastasis (n [%])		
Lung	8 (53)	8 (40)
Bone	5 (33)	3 (15)
Lymph node	4 (27)	8 (40)
Liver	2 (13)	3 (15)

ECOG indicates Eastern Cooperative Oncology Group; PS, performance status; RT, radiotherapy.

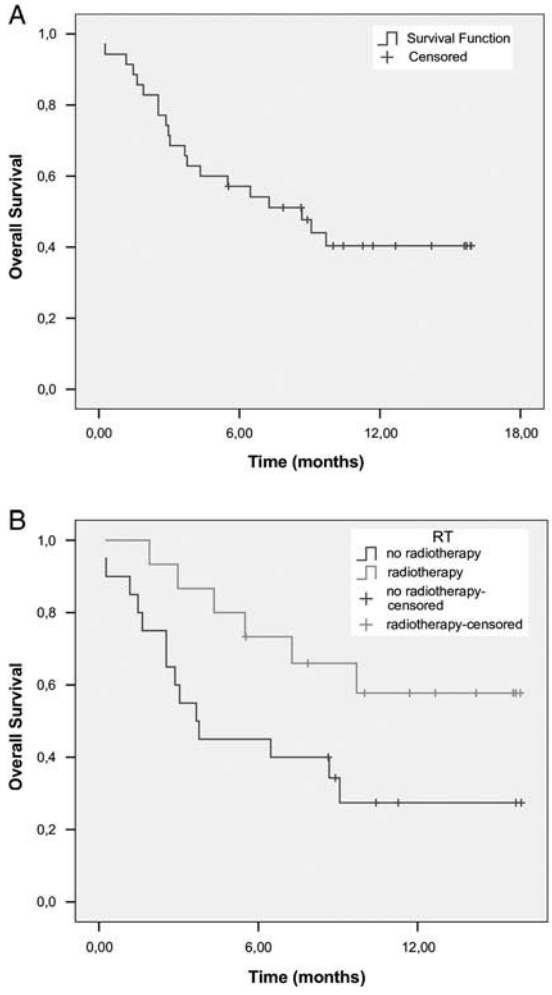

FIGURE 1. A, Overall survival curve for all 35 patients evaluated. B, Overall survival distribution by radiotherapy. RT indicates radiotherapy.

RT was 7.4 ± 5.08 months (median, 7.3 mo) for the entire group. No patients were lost at follow-up visits. During follow-up, a total of 20 deaths occurred, and the median survival time was 8.7 months (95% confidence interval [CI], 4.1-13.2). The actuarial overall survival rates at 6 and 12 months were 54.1% and 40.4%, respectively (Fig. 1A). There were 6 deaths in the RT-nivolumab group, yielding a 1-year overall survival rate of 57.8%. In contrast, there were 14 deaths in the only-nivolumab group at the time of analysis, with a 1-year overall survival rate of 27.4%. As shown in Figure 1B, patients treated with RT before nivolumab had a better survival ($P = 0.043$).

The results of the univariate analysis are displayed in Table 2. The multivariable Cox model included ECOG score index, stage, histology, and RT type and was performed for all models. For overall survival, the final multivariable Cox model maintained an ECOG score of 0 to 1 (hazard ratio [HR], 8.169; 95% CI, 2.592-25.746; $P < 0.001$). In total, 21 patients (60%) experienced tumor progression. The actuarial progression-free survival at 6 and 12 months was 47.8% and 37%, respectively (Fig. 2A). There were 6 patients with disease progression in the RT-nivolumab group (1-year progression-free survival: 57.8%) and 15 events in the only-nivolumab group (1-year progression-free survival: 20.6%) ($P = 0.040$).

TABLE 2. Univariate Analysis of Survival Data According to Various Classifications

Parameters	Groups	β	± SE	*P*	HR (95% CI)
Performance	0: 0-1	—	—	—	—
	1: 2	2.175	0.536	0.001	8.8 (3.075-25.178)
Staging	0: IIIB	—	—	—	—
	1: IV	0.175	0.488	0.717	1.191 (0.457-3.102)
Histology	0: Squamous	—	—	—	—
	1: Nonsquamous	−0.058	0.450	0.898	0.944 (0.390-2.282)
Radiotherapy	0: Yes	—	—	—	—
	1: No	−0.957	0.491	0.041	0.384 (0.147-0.974)

CI indicates confidence interval; HR, hazard ratio.

Treatment Safety

Acute treatment toxicity was recorded by the physician before nivolumab infusion. No treatment-related mortality was

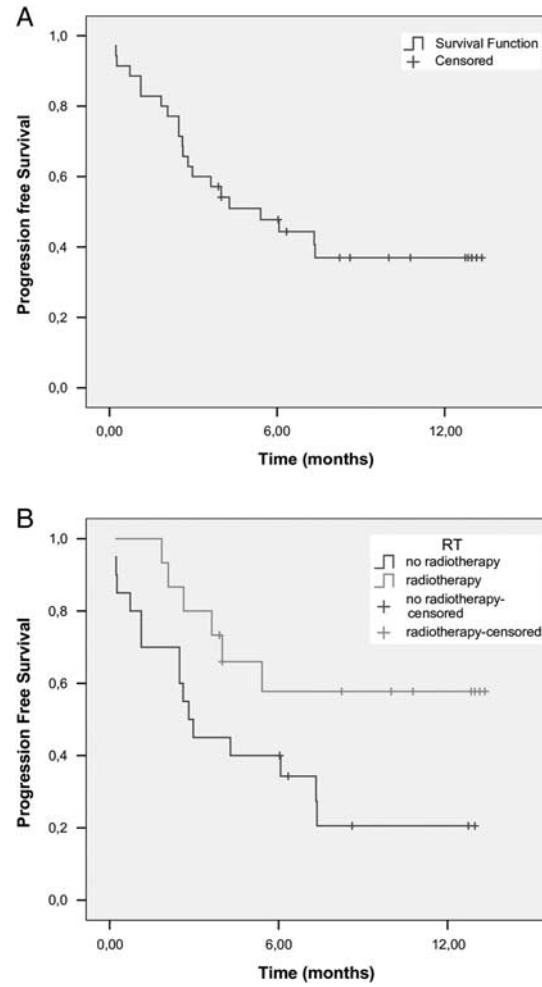

FIGURE 2. Progression-free survival, in overall (A), and in distribution by radiotherapy (B). RT indicates radiotherapy.

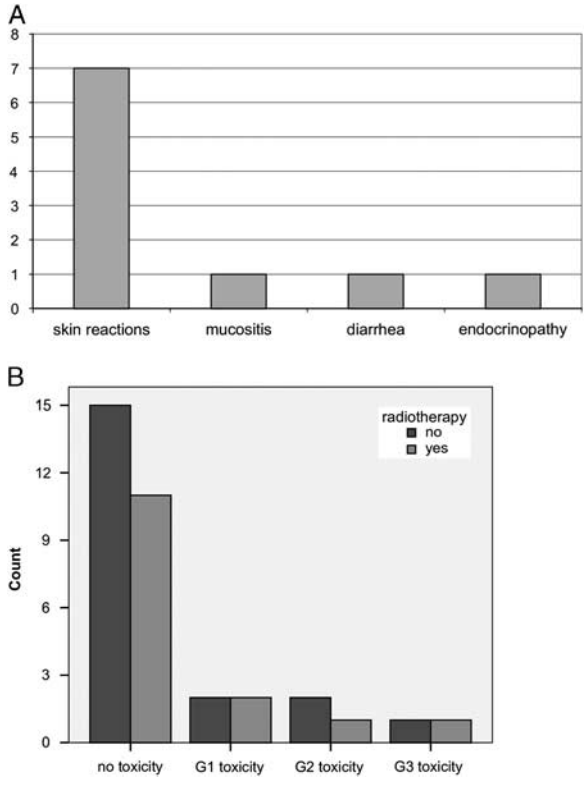

FIGURE 3. Distribution of acute toxicities (A) and distribution by radiotherapy (B).

found. The main treatment-related toxicities were immune-related adverse events (irAEs). In our cohort, these irAEs included colitis, mucositis, endocrinopathy, and cutaneous events. Two patients (5.7%) experienced grade 3 irAEs, 1 in each group ($P = 0.971$): 1 patient, in the RT-nivolumab group, experienced a G3 mucositis, and, another patient, in the only-nivolumab group, had a G3 endocrinopathy of the surrenal gland; in both patients, relief of symptoms was obtained with corticosteroids.

Three patients experienced cutaneous G2 toxicity, 2 patients in the only-nivolumab group and 1 in the RT-nivolumab group. The IrAEs in both groups is shown in Figures 3A and B.

DISCUSSION

Nivolumab has shown an overall survival benefit in advanced NSCLC patients with squamous and nonsquamous histology who have disease progression after first-line chemotherapy. In squamous-cell NSCLC, a 1-year overall survival absolute gain of 18% is observed with nivolumab compared with docetaxel, with an interesting reduction of 41% (HR, 0.59; 95% CI, 0.44-0.79; $P < 0.001$) for risk of death.[10]

In nonsquamous-cell NSCLC, a 1-year overall survival absolute gain of 12% was highlighted, with a reduction of 27% (HR, 0.73; 96% CI, 0.59-0.89; $P = 0.002$) for risk of death.[2] In both histologic lesions, response rates were modest and similar at 19% and 20%. A recent meta-analysis confirms nivolumab as a promising second-line agent for previously treated advanced NSCLC with manageable adverse events, highlighting a similar efficacy for both squamous and nonsquamous NSCLC patients.[11] Increasing the response rate to nivolumab by administering it in combination with chemotherapy and/or target therapy are areas of active research.

Preclinical data showed immunomodulatory activities for several cytotoxic agents[12,13] and antivascular endothelial growth factor agents.[14] The combination between standard NSCLC therapy and immune checkpoint inhibitors could be synergistic.

A single-center, phase I study suggested that combination therapy with nivolumab and standard chemotherapy enhances the antitumor activity, with a response rate between 50% and 100% in first-line therapy and 16.7% in second-line therapy.[15]

Another promising area of development is the combination of immune checkpoint inhibitors with RT. There is a strong rationale to combine these 2 treatment modalities. It is known that radiation can modulate the immune response. RT enhances the expression of MHC I on the surface of tumor cells,[16] so that it could increase immunogenic tumor cell death. The abscopal effect is nothing more than having tumor regression in metastases outside of the radiation treatment field; it is mediated by radiation-induced antitumor T cells, and it can be induced in mice by combining local radiation with growth factors for DCs.[17]

Preclinical studies have also reported that high dose per fraction irradiation has more immunogenic effects than conventionally fractionated treatments. Camphausen et al[18] reported tumor growth inhibition at distant sites following radiation with 10 Gy×5 fractions compared with 2 Gy×12 fractions in mouse models of lung cancer and fibrosarcoma.

A recent meta-analysis evaluated whether the occurrence of abscopal effects may be related to the biologically effective radiation dose. The study shows that the occurrence rate of abscopal effects in preclinical models increases with the biologically effective radiation dose.[19] An explanation for this immune-mediated effect is probably related to an activation of DCs by endogenous signals received from treated cells.[20] Proteins released by radio-treated tumor cells are engulfed by DCs; activated intratumoral DCs have the potential to attract other immune cells and thus obtain a specific antitumor immunity.

Otherwise, a tumor is normally characterized by an immune suppressive microenvironment. Upregulated expression of immune checkpoint ligands in tumor cells is a known immune resistance mechanism.[21] The same radiation treatment can upregulate *PD-L1*, *PD-L2*, and *CTLA4*.[22]

Furthermore, tumors can resist immune elimination by upregulating the expression of *PD-1* on tumor-specific regulator lymphocytes that consequently inhibit antitumor immune responses.[3] Indeed, in lung cancer patients, a high density of regulatory T-cell infiltration in tumor stroma could be recognized as a negative prognostic factor.[23]

There are currently few clinical studies combining immunotherapy and RT, despite preclinical studies highlighting the synergistic effect of interaction and the potential opportunity to improve the therapeutic ratio and to prolong tumor response.

The aim of the present study was to analyze our experience in the use of hypofractionated RT combined with nivolumab in pretreated advanced or metastatic lung cancer patients. Globally, the overall crude survival at 1 year was 40.4. This rate was comparable to those of published randomized clinical trials where the 1-year OS was 42% in squamous NSCLC[10] and 51% in nonsquamous NSCLC.[2] It is important because a "real life" clinical study with consecutive patients proved the efficacy of nivolumab in this patient setting. Otherwise, the difference in 1-year progression-free survival of 37% in our analysis versus 21%[10] and 19%[2] is probably related to the rates of stage IV patients in the randomized clinical trial of 78%[2] and 90%[10] being higher than our population's rate of 65.7%.

The 2 treatment arms of our study, RT-nivolumab and only-nivolumab, were well matched for baseline characteristics, and an impressive absolute gain of 30.4% in 1-year overall survival was demonstrated in patients receiving RT before nivolumab.

This study suggests that the combination of nivolumab and RT can enhance the antitumor activity of the same immune checkpoint inhibitor, as predicted by a preclinical model. Nivolumab can enhance immune responses to RT. Indeed, the association of RT and immunotherapy can modify the tumor microenvironment and render tumors sensitive to the immune system to promote systemic responses.[24] In our study, no differences in acute adverse events were shown, and no autoimmune pneumonitis was highlighted. This is probably related to the irradiation of metastatic sites in 53.3% and to the use of very well-conformed treatments in the mediastinum (33.3%) or lung (13.4%).

In all irradiated patients, we use hypofractionated RT, and preclinical studies indeed suggest a more favorable profile for hypofractionated RT in stimulating immune responses.[24]

In conclusion, our data show that a combined approach with RT added to immunotherapy with immune checkpoint inhibitors such as nivolumab can increase overall survival and progression-free survival in pretreated advanced or metastatic NSCLC patients. Randomized clinical studies are needed to clarify better and define the most appropriate combination of RT with immunotherapy.

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