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ORIGINAL ARTICLE

# Thyroid dysfunction induced by nivolumab: searching for disease patterns and outcomes

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## Abstract

**Purpose** Nivolumab is a monoclonal antibody that blocks the activation of programmed death-1 receptor, promoting T-cell activation against cancer cells. Thyroid dysfunction (TD) is a common immune-related adverse event (irAE) induced by nivolumab. We report the prevalence, patterns and outcomes of nivolumab-induced TD among cancer patients in our center.

**Methods** All patients treated with nivolumab during 2016 were included. We assessed thyroid function tests, thyroid autoimmunity, thyroid imaging, and clinical outcome during nivolumab therapy as well as overall survival (OS).

**Results** Seventy-three patients (55 with non-small-cell lung cancer [NSCLC], 9 with melanoma and 9 with Hodgkin lymphoma) were included. Median of follow up: 390.5 days. Seventeen patients (23.3%) developed TD during treatment. Thyrotoxicosis was reported in seven patients. Serum thyroid-stimulating hormone (TSH) nadir occurred after a median of 51 days (95% CI: 35–71). Thyroid antibodies were positive in three of the seven patients. Five of the seven hyperthyroid patients became hypothyroid later, and four of them required levothyroxine treatment. Primary hypothyroidism occurred in ten patients. Serum TSH peak occurred after a median of 110 days [95% CI: 85.2–197]. Thyroid autoimmunity was positive in one patient. In patients with NSCLC, TD was associated with better OS (HR = 0.4 [95% CI: 0.17–0.94]; $p = 0.035$).

**Conclusions** TD induced by nivolumab is a common and heterogeneous irAE. Thyrotoxicosis develops earlier than hypothyroidism. A pattern consistent with a transient thyroiditis followed by hypothyroidism was observed in one-third of patients. Our results suggest that patients with NSCLC and nivolumab-induced TD might have better survival.

**Keywords** Thyroiditis · Thyrotoxicosis · Hypothyroidism · Cancer · Nivolumab · Immune-checkpoint inhibitors.

## Introduction

Nivolumab is a human monoclonal antibody that binds to the programmed death 1 (PD-1) receptor and blocks its interaction with the PD-1 ligands, PD-L1 and PD-L2. The PD-1 receptor is expressed on the surface of T lymphocytes, while PD-L1 and PD-L2 are expressed on the surface of

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both tumor cells and antigen-presenting cells in the tumor microenvironment. The coupling of PD-1 with PD-L1 or PD-L2 inhibits the proliferation of T lymphocytes and cytokine production [1]. Therapies targeting PD-1, such as nivolumab or pembrolizumab have shown antitumor activity by enhancing adaptive immune response against cancer cells, resulting in significant long-lasting responses [2–6]. Nivolumab has been approved by the US Food and Drug Administration (FDA) and by the European Medicines Agency (EMA) for the treatment of advanced melanoma, non-small-cell lung cancer (NSCLC), renal cell carcinoma, classical Hodgkin lymphoma, recurrent or metastatic head and neck squamous cell carcinoma, locally advanced or metastatic urothelial carcinoma, and is currently in clinical trials for many other malignancies.

Although the toxicity profile of therapies targeting PD-1 is more favorable than conventional chemotherapy, immune-related adverse events (irAEs) can occur as a result of enhancing immune response in normal tissues. IrAEs induced by PD-1 inhibitors can potentially affect every organ in the body, but gastrointestinal tract, skin, liver, and endocrine system are the most commonly involved sites [7, 8].

The thyroid gland is a common site for irAEs, with an overall incidence of thyroid dysfunction (TD) ranging from 4 to 21% with anti-PD-1 agents [9–12]. The TD, includes hypothyroidism and thyrotoxicosis, which are generally mild to moderate. The cause of TD induced by targeting PD-1, as well as the timing and pattern of the TD and prognosis of patients who develop TD remain unclear.

A recent prospective analysis of 51 patients with NSCLC treated with pembrolizumab as part of KEYNOTE-001 study found a positive association between TD and thyroid autoantibodies, as well as a possible improvement in survival in patients who developed pembrolizumab-induced TD [11, 13]. The aim of this study was to assess the prevalence and characteristics of TD induced by nivolumab, and survival in a cohort of patients with solid tumors treated with nivolumab in our center.

## Material and methods

### Study population

We prospectively identified patients who developed TD among all patients who had been treated consecutively with nivolumab at the Institut Català d'Oncologia (L'Hospitalet de Llobregat, Barcelona) during 2016, either as part of a clinical trial or routine care, as monotherapy or combined with other immunotherapies and/or chemotherapies. Nivolumab (Opdivo®; Bristol-Myers Squibb Company) was given according to the US full prescribing information (3 mg/kg intravenously every 2 weeks) until disease progression or lack of clinical benefit.

Patients' characteristics (age, gender, history of TD, and previous cancer treatment), thyroid function tests, thyroid autoantibodies, thyroid imaging, and thyroid treatment were collected from medical records. Patients with previous TD were excluded from the analysis. All patients were followed up for survival until death. Living patients were censored at the last review date on 14 March 2018. This study was approved by the Ethical Committee of the Hospital Universitari de Bellvitge (EPA025/18); written informed consent was obtained from all patients.

### Thyroid function evaluation, thyroid-related adverse events and survival

Thyroid function (thyroid-stimulating hormone [TSH] and serum free T4 [fT4]) was measured at baseline in all patients. Serum TSH and serum fT4 were prospectively measured during treatment with nivolumab usually before each cycle of treatment or as it was indicated by clinical trial protocols. Serum free triiodothyronine (fT3) was determined only in the presence of low-serum TSH. All the patients with TD induced by nivolumab were evaluated and treated by an endocrinologist. Primary hypothyroidism was defined as high serum TSH value with low serum fT4 value. Thyrotoxicosis was defined as low serum TSH value in conjunction with high serum fT4 and/or high serum fT3 concentrations. Subclinical hypothyroidism was defined as high serum TSH value with normal serum fT4 value and subclinical thyrotoxicosis was defined as low serum TSH value with normal serum fT4 and/or serum fT3 value. Serum TSH, fT4, fT3, thyroid peroxidase antibodies (TPOAbs), and TSH receptor antibodies (TRAbs) were analyzed using the Elecsys electro-chemiluminescence immunoassays (Roche Diagnostics). Thyroglobulin antibodies (TGAbs) were analyzed using the chemiluminescent enzyme immunoassay in solid phase (Siemens Healthcare). Reference laboratory values were: serum TSH (0.48–4.36 mUI/L); fT4 (9.7–30.9 pmol/L); fT3 (3.1–6.8 pmol/L); TPOAbs (< 35 kint.U/L); TRAbs (<1.75 int.U/L); TGAbs (<40 kint.U/L). When TD was reported, thyroid autoantibodies (TRAbs, TPOAbs, and TGAbs) were measured and thyroid imaging (thyroid ultrasonography [US] and/or a radioiodine/Tc 99m scintigraphy) were performed. Radioiodine/Tc 99m scintigraphy was performed 2 months after the last computed tomography scan, whenever possible, in order to avoid the potential interference caused by iodinated contrast.

### Statistical analysis

The demographic and clinical variables were described by mean and standard deviation (SD) for continuous variables and frequencies and percentages for categorical variables. Nonparametric quantitative variables were described

through the median and the interquartile range (IQR). A $p$ value < 0.05 was considered statistically significant. All statistical analyses were performed using the statistical program R (version 3.4.4 for Windows).

The prevalence of TD was estimated as the proportion of patients with TD in the whole cohort of patients treated with nivolumab. The number of patients and the 95% confidence interval (CI) of the estimate were provided. The impact of predictors of TD was calculated through odds ratio (OR) and logistic regression with the presence of TD as a response variable.

The survival time was measured as the number of days elapsed between the first day of treatment with nivolumab and the date of exitus or the censoring date. The differences between the survival curves were evaluated through Cox proportional hazard models, stratified by (1) tumor type, (2) TD, or (3) clinical or subclinical TD. The hazard ratio (HR) and 95% CI were calculated, and $p$ values were derived from the models. Time to TD was stratified according to the tumor type. A potential immortal time bias in patients with TD was detected due to the design of the study. In order to correct for this bias, the time until TD was diagnosed, took into account the moment when the situation of each patient changed. These patients had therefore two roles in the study. First, during the period prior to the diagnosis, they were part of the group without TD. From the diagnosis of TD, the survival time of the patient was calculated for the group with TD. For all Cox proportional hazards models, the assumption of proportionality of the risks has been verified.

## Results

### Patients' characteristics

A total of 75 cancer patients consecutively treated with nivolumab from January to December 2016 at the Institut Català d'Oncologia (L'Hospitalet de Llobregat, Barcelona) were identified. Seventy-three patients were included in the study, two patients were excluded due to previous TD (Table 1). Median age (range) at diagnosis was 60.5 (49.8–67). Most patients were male ($n = 50$; 68.5%) and 55/73 patients included in the study had advanced NSCLC (75.4%), 9/73 had melanoma (12.3%), and 9/73 had Hodgkin lymphoma (12.3%). Patients with NSCLC were older than those diagnosed with melanoma or Hodgkin lymphoma. Only three patients diagnosed with melanoma had received previous treatment with nivolumab at a dose of 1 mg/kg plus ipilimumab at a dose of 3 mg/kg every 3 weeks for 4 doses, followed by nivolumab at a dose of 3 mg/kg every 2 weeks. None of the other patients had received prior immunotherapy. Most patients in our study,

**Table 1** Baseline characteristics of patients

| | All ($n = 73$) | | | | NSCLC ($n = 55$) | | Melanoma ($n = 9$) | | Hodgkin lymphoma ($n = 9$) | |
|---|---|---|---|---|---|---|---|---|---|---|
| $n$ (%) except where noted | All ($n = 73$) | No TD ($n = 56$) | TD ($n = 17$) | $p$ Value | No TD ($n = 47$) | TD ($n = 8$) | No TD ($n = 6$) | TD ($n = 3$) | No TD ($n = 3$) | TD ($n = 6$) |
| *Age* | | | | | | | | | | |
| Median (range) | 60.5 (49.8–67) | 62.0 (54.0–67) | 55.0 (41.2–65) | | | | | | | |
| Mean ± SD (years) | 57.4 ± 14.3 | 59.4 ± 12.2 | 50.4 ± 18.7 | 0.09 | 61.3 ± 9.61 | 65.1 ± 8.1 | 59.3 ± 14.5 | 48.5 ± 9.2 | 29.7 ± 6.35 | 31.3 ± 12.6 |
| *Gender* | | | | | | | | | | |
| Female | 23(31.5%) | 15 (26.8%) | 8 (47.1%) | 0.201 | 10 (21.3%) | 4 (50%) | 3 (50%) | 2 (66.7%) | 2 (66.7%) | 2 (33.3%) |
| Male | 50 (68.5%) | 41 (73.2%) | 9 (52.9%) | | 37 (78.7%) | 4 (50%) | 3 (50%) | 1 (33.3%) | 1 (33.3%) | 4 (66.7%) |
| *Nivolumab-based treatment* | | | | | | | | | | |
| Nivolumab (3 mg/kg) | 58 (79.5%) | 49 (87.5%) | 9 (52.9%) | 0.004 | 47 (100%) | 8 (100%) | 2 (33.3%) | 1 (33.3%) | 0 | 0 |
| Nivolumab/ipilimumab | 6 (8.2%) | 4 (7.1%) | 2 (11.8%) | | 0 | 0 | 4 (66.7%) | 2 (66.7%) | 0 | 0 |
| Nivolumab + AVD | 9 (12.3%) | 3 (5.4%) | 6 (35.3%) | | 0 | 0 | 0 | 0 | 3 (100%) | 6 (100%) |
| *Previous immunotherapy* | | | | | | | | | | |
| No | 70 (95.9%) | 54 (96.4%) | 16 (94.1%) | 0.56 | 47 (100%) | 8 (100%) | 4 (66.7%) | 2 (66.7%) | 3 (100%) | 6 (100%) |
| Yes | 3 (4.1%) | 2 (3.6%) | 1(5.9%) | | 0 | 0 | 2 (33.3%) | 1 (33.3%) | 0 | 0 |
| *Baseline TSH* | | | | | | | | | | |
| Mean ± SD (mUI/L) | 2.42 (1.38) | 2.27 ± 1.38 | 2.85 ± 1.30 | 0.125 | 2.25 ± 1.39 | 2.98 ± 1.65 | 2.21 ± 1.76 | 3.08 ± 0.35 | 2.52 ± 0.62 | 2.57 ± 1.17 |

*AVD* doxorubicin, vinblastine and dacarbazine, *NSCLC* non-small-cell lung cancer, *TD* thyroid dysfunction, *TSH* thyroid-stimulating hormone

**Table 2** Clinical and demographical features of patients according to whether they have or not thyroid dysfunction

| | All ($N = 73$) | No TD ($n = 56$) | TD ($n = 17$) | OR (95% CI) |
|---|---|---|---|---|
| *Age, years* | | | | |
| Median (range) | 60.5 (49.8–67) | 62.0 (54.0–67) | 55.0 (41.2–65) | 0.96 (0.92–1.00) |
| *Gender* | | | | |
| Female | 23 (31.5%) | 15 (26.8%) | 8 (47.1%) | – |
| Male | 50 (68.5%) | 41 (73.2%) | 9 (52.9%) | 0.42 (0.13–1.32) |
| *Type of tumor* | | | | |
| Melanoma | 9 (12.3%) | 6 (10.7%) | 3 (17.6%) | – |
| Hodgkin lymphoma | 9 (12.3%) | 3 (5.4%) | 6 (35.3%) | 3.61 (0.52–31.7) |
| NSCLC | 55 (75.4%) | 47 (83.9%) | 8 (47.1%) | 0.34 (0.07–2.00) |
| *Nivolumab-based treatment* | | | | |
| Nivolumab (3 mg/kg) | 58 (79.5%) | 49 (87.5%) | 9 (52.9%) | – |
| Nivolumab/ipilimumab | 6 (8.2%) | 4 (7.1%) | 2 (11.8%) | 2.74 (0.3–17.5) |
| Nivolumab + AVD | 9 (12.3%) | 3 (5.4%) | 6 (35.3%) | 10.1 (2.18–58.9) |
| *Previous immunotherapy* | | | | |
| No | 70 (95.9%) | 54 (96.4%) | 16 (94.1%) | – |
| Yes | 3 (4.1%) | 2 (3.6%) | 1 (5.9%) | 1.77 (0.05–23.2) |
| *Baseline TSH* | | | | |
| Mean ± SD (mUI/L) | 2.43 (1.38) | 2.27 (1.38) | 2.85 (1.30) | 1.36 (0.91–2.05) |

Odds ratio (OR) was calculated by logistic regression to predict TD based on distinct clinical covariates. For categorical variables, the first feature was considered as reference

*AVD* doxorubicin, vinblastine and dacarbazine, *NSCLC* non-small-cell lung cancer, *SD* standard deviation, *TD* thyroid dysfunction, *TSH* thyrotropin stimulating hormone

77.3% (all 55 patients with NSCLC and 2 patients with melanoma), were treated with nivolumab as part of routine clinical practice (outside of a clinical trial).

## TD induced by nivolumab

During nivolumab treatment, 17 patients developed TD (23.3% [95% CI: 13.6–33]). Prevalence of TD was highest in patients with Hodgkin lymphoma (66.7% [95% CI: 35.9–97.5]), followed by melanoma (33.3% [95% CI: 2.5–64.1]), and NSCLC (14.6% [95% CI: 5.2–23.9]). Among the 17 patients who developed TD, two patients had a previous history of nontoxic multinodular goiter but with a normal baseline thyroid function. Table 2 shows clinical and demographical features of patients according to the presence of TD. Patients with TD were slightly younger than non-TD patients (55 (range: 41.2–65) vs. 62 (range: 54–67) years; OR = 0.96 [95% CI: 0.92; 1]).

Regarding the oncological treatment, an OR = 10.1 [95% CI: 2.1–58.9] was observed. There were no differences between patient groups according to the other variables that were analyzed (gender, tumor location, previous immunotherapy treatments, number of cycles of immunotherapy, and baseline serum TSH).

## Thyrotoxicosis induced by nivolumab

Seven patients (9.6%; five women, median age 51 [IQR: 45.5–56.5]) developed thyrotoxicosis during treatment with nivolumab. All the patients were followed up at least 6 months after the development of TD. We found two patterns of TD in those patients (Table 3): a pattern consistent with a transient thyrotoxicosis followed by hypothyroidism (thyroiditis) (pattern I) and subclinical thyrotoxicosis (pattern II). Three patients developed positive thyroid autoimmunity: case 2 (TGAbs positive), case 3 (TGAbs and TPOABs positive), and case 6 (TRAbs positive). TSH nadir occurred after a median of 51 days (95% CI: 35–71). Most of the patients had mild clinical and biochemical thyrotoxicosis. Two patients (case 5 and 6) with thyrotoxicosis were treated successfully with high dose of steroids (prednisone 0.8 mg/kg/day) and nivolumab was temporarily withdrawn. Thyroiditis was seen in five patients. Four of them required replacement treatment with levothyroxine, mean (SD) dose of levothyroxine was 1.72 ± 0.13 μg/kg/day. Thyroid imaging was pathological in five cases (four with thyroiditis and one with multinodular goiter) (Fig. 1). The time frame between imaging and TD was less than 30 days.

**Table 3** Patients with nivolumab-induced thyrotoxicosis during follow up

| Baseline | | | | | Thyroid function and management at TSH nadir | | | | | | | Course of the disease: TSH peak | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pattern | Case | Age | Gender | Tumor/drug | TSH (mUI/L) | Free T4 (pmol/L) | Time (days) | TSH nadir (mUI/L) | Free T4 (pmol/L) | Thyroid Abs (type) | Imaging | Treatment | Time (days) | TSH peak (mUI/L) | Free T4 (pmol/L) | T4 replacement/µg per kg per day |
| I | 1 | 43 | F | Hodgkin Nivo + ADV | 1.26 | 13.7 | 45 | <0.01 | 33 | Negative | 18F-FDG PET (thyroiditis) | B-blockers | 87 | 57.1 | <3 | yes 1.85 |
| | 2 | 42 | F | Melanoma Nivo/ Ipi | 2.67 | 14.9 | 63 | 0.09 | 14.4 | Positive (TGAbs) | SCINT (multinodular goiter) | none | 84 | 98.79 | 4.2 | yes 1.67 |
| | 3 | 73 | F | NSCLC Nivo | 3.76 | 14.5 | 25 | 0.33 | 20.31 | Positive (TGAbs; TPOAbs) | US (thyroiditis) | none | 87 | 82.2 | 4.6 | yes 1.54 |
| | 4 | 26 | M | Hodgkin Nivo | 2.23 | 17 | 295 | 0.27 | 19.5 | Negative | Normal | none | 372 | 13.32 | 9.5 | no |
| | 5 | 48 | M | Melanoma Nivo/ Ipi | 3.24 | 16 | 15 | <0.01 | >100 | n.a | SCINT/18 FDG-PET (thyroiditis) | steroid | 58 | 96 | <3 | yes 1.83 |
| II | 6 | 58 | F | NSCLC Nivo | 0.9 | 17.1 | 51 | 0.01 | 23 | Positive (TRAbs) | SCINT (thyroiditis) | steroid | 83 | 3.55 | 16.3 | no |
| | 7 | 55 | F | NSCLC Nivo | 1.12 | 12.4 | 79 | 0.03 | 14.9 | Negative | Normal | none | 126 | 0.81 | 10.2 | no |

*AVD* doxorubicin, vinblastine and dacarbazine, *Pattern thyroid pattern*. *18F-FDG PET* fluorine-18-fluorodeoxyglucose positron emission tomography, *Ipi* Ipilimumab, *LT4* levothyroxine, *n.a* not available data, *Nivo* Nivolumab, *NSCLC* non-small-cell lung cancer, *SCINT* thyroid scintigraphy, *TGAbs* thyroglobulin antibodies, *Thyroid Abs* thyroid autoimmunity, *TPOAbs* thyroid peroxidase antibodies, *TRAbs* TSH receptor antibodies, *TSH* thyroid-stimulating hormone, *T4* thyroxine, *US* thyroid ultrasonography. TSH value in mUI/L. Normal serum TSH value: 0.48–4.36 mUI/L; normal serum free T4 value: 9.7–30.9 pmol/L

I: Pattern 1: transient thyrotoxicosis evolved to hypothyroidism II: Pattern 2: subclinical thyroiditis

## Hypothyroidism induced by nivolumab

Ten patients (13.7%; 1 woman, median age = 57 [IQR: 40.8–67]) developed nivolumab-induced hypothyroidism (Table 4). Autoimmunity was measured in half of the patients, being positive only in one case: case 1 (TGAbs and TPOAbs positive). TSH peak occurred after a median of 110 days (95% CI: 85.2–197). All cases of hypothyroidism were mild and only two patients (20%) (cases 1 and 2) developed overt hypothyroidism requiring replacement treatment with levothyroxine. The mean dose of levothyroxine was 0.63 ± 0.06 µg/kg/day in these two patients. No patients required discontinuation of nivolumab because of hypothyroidism.

## Survival analysis

Median of follow up of the patients was 364.5 days (IQR: 120–597.3) and during this period, 45 deaths occurred. In an unadjusted model, TSH was a protective factor (HR = 0.28 [95% CI: 0.13–0.64]), whereas age was a risk factor for death (HR = 1.04 [95% CI: 1.01–1.07]; Table 5). There were no differences in overall survival between patients with or without TD induced by nivolumab, or in patients with subclinical or clinical TD. When we performed survival analysis by tumor location, TD was associated with better overall survival among NSCLC (HR = 0.4 [95% CI: 0.17–0.94]; $p = 0.035$; Fig. 2). The median survival for the non-TD group was 244 days. Due to the lower mortality data among NSCLC patients with TD, median survival could not be calculated.

# Discussion

Our results show real-life data on the prevalence, clinical features, patterns, evolution, and prognosis of TD induced by nivolumab in cancer patients treated at a single cancer center, regardless of whether patients had been evaluated in routine clinical practice or in the setting of a clinical trial.

We observed a higher prevalence of TD than that reported in previous clinical trials of patients receiving nivolumab at similar doses. Barroso-Sousa et al. [9] performed a systematic review and meta-analysis with 7551 patients from 38 randomized clinical trials evaluating immune-checkpoint inhibitors for treatment of advanced solid tumors [12]. In patients treated with nivolumab in monotherapy, they reported a frequency of hypothyroidism and hyperthyroidism of 6.5% and 2.5%, respectively. Incidence of TD was higher in patients receiving treatment with a combination of immune-checkpoint inhibitors; hypothyroidism was reported in 13.2% of patients and hyperthyroidism in 8%. In our cohort, one out of four patients

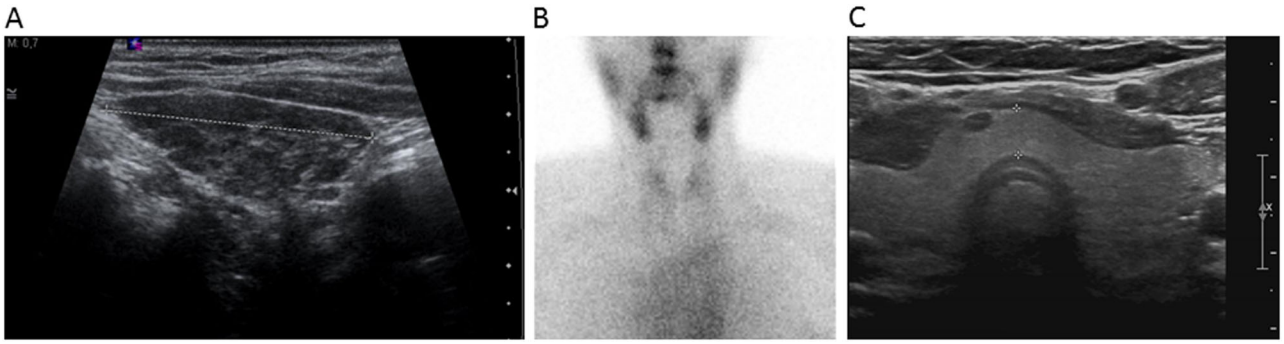


**Fig. 1** Imaging for each pattern of TD. **a** Thyroid US of a NSCLC patient treated with nivolumab with transient thyrotoxicosis followed by hypothyroidism, pattern I (thyroiditis). **b** Scintigraphy of a melanoma patient treated with nivolumab with subclinical thyrotoxicosis, pattern II (thyroiditis). **c** Thyroid US of a patient with Hodgkin Lymphoma treated with nivolumab plus *AVD* with hypothyroidism, pattern III (goiter). All three patients had positive thyroid autoimmunity. *AVD* doxorubicin, vinblastine, and dacarbazine, *NSCLC* non-small-cell lung cancer, *TD* thyroid dysfunction, *US* ultrasonography

**Table 4** Patients with nivolumab-induced hypothyroidism during follow up

| Baseline | | | | | | Course of the disease | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Case | Age | Gender | Tumor | TSH (mUI/L) | Free T4 (pmol/L) | Time (days) | TSH peak (mUI/L) | Free T4 (pmol/L) | Thyroid Abs (type) | T4 replacement/μg per kg per day |
| 1 | 77 | F | NSCLC | 3.54 | 19.1 | 89 | 13.90 | 15.0 | Positive (TGAbs; TPOAbs) | Yes/0.68 |
| 2 | 56 | M | Melanoma | 3.32 | n.a | 84 | 13.76 | 10.2 | Negative | Yes/0.57 |
| 3 | 19 | M | Hodgkin | 3.97 | 19 | 96 | 10.19 | 15.1 | n.a | No |
| 4 | 46 | M | Hodgkin | 1.65 | 18.2 | 32 | 4.70 | 15.9 | Negative | No |
| 5 | 17 | M | Hodgkin | 2.26 | 18.7 | 142 | 5.29 | 17.8 | Negative | No |
| 6 | 39 | M | Hodgkin | 4.03 | 14.1 | 245 | 4.81 | 16.0 | Negative | No |
| 7 | 64 | M | NSCLC | 5.60 | 18.3 | 70 | 8.02 | 15.0 | n.a | No |
| 8 | 58 | M | NSCLC | 2.70 | n.a | 125 | 6.37 | 18.3 | n.a | No |
| 9 | 71 | M | NSCLC | 3.88 | n.a | 215 | 9.07 | 14.6 | n.a | No |
| 10 | 68 | M | NSCLC | 1.75 | n.a | 245 | 6.22 | 16.9 | n.a | No |

*F* female, *M* male, *NSCLC* non-small-cell lung cancer, *TGAbs* thyroglobulin antibodies, *Thyroid Abs* thyroid autoimmunity, *TPOAbs* thyroid peroxidase antibodies, *TSH* thyroid-stimulating hormone, *T4* thyroxine, *n.a* not available data; TSH value in mUI/L; T4: Levothyroxine

Normal serum TSH value: 0.48–4.36 mUI/L; normal serum free T4 value: 9.7–30.9 pmol/L

developed TD during treatment with nivolumab. Our data are comparable with what has been reported in other cohort studies. Morganstein et al found a TD incidence of 39% in patients with melanoma treated with anti-PD-1 therapy and of 50% in patients treated with anti-PD-1/anti CTLA-4 combination therapy [10]. Osorio et al. [11] reported a TD incidence of 21% in a cohort of NSCLC patients treated with pembrolizumab monotherapy within a clinical trial at a single center.

In our study, the higher prevalence of TD might be due to two reasons: first, all patients had a prospective and systematic surveillance including thyroid function tests. When TD occurred, patients were quickly referred to an endocrinologist for an active follow up. And secondly, an unexpectedly high rate of nivolumab-induced TD in patients with Hodgkin lymphoma (six out of nine patients) was observed. In a phase 2 study of nivolumab in newly diagnosed advanced stage Hodgkin lymphoma, hypothyroidism was reported in 12% of patients [14]. This value is higher than that reported in recent phase 2 studies with nivolumab in solid malignancies [15, 16]. Mancuso et al. [17] highlighted a possible relationship between lymphomas and TD, reporting an involvement of thyroid disorders in 11–27% of patients with this malignancy. Preexisting chronic autoimmune thyroiditis is also a well-recognized risk factor of thyroid lymphoma. Nevertheless, none of our patients with Hodgkin lymphoma and TD had positive autoimmunity or a baseline thyroid uptake on fluorine-18-fluorodeoxyglucose positron emission tomography (18F-FDG PET). On the other hand, some treatments of lymphoma, such as radiotherapy or rituximab may induce TD [17], but all patients with Hodgkin lymphoma in our cohort were treatment naïve. Our data suggest that those patients treated with Nivolumab plus ADV (doxorubicin, vinblastine and dacarbazine) could have a higher risk of TD. In fact, all of our patients with Hodgkin lymphoma were

**Table 5** Univariate survival analysis

| *n* (%), except where noted | No event | Event (death) | HR (95% CI) | *p* Value |
|---|---|---|---|---|
| *Age, years* | | | | |
| Mean (SD) | 49.2 (16.7) | 61.6 (11.2) | 1.04 (1.01–1.07) | 0.004 |
| *Gender* | | | | |
| Female | 12 (48.0%) | 10 (22.2%) | Ref. | Ref. |
| Male | 13 (52.0%) | 35 (77.8%) | 1.58 (0.77–3.28) | 0.216 |
| *Type of tumor* | | | | |
| Melanoma | 4 (16%) | 5 (11.1%) | Ref. | Ref. |
| Hodgkin lymphoma | 9 (36%) | 0 | 0 | 0 |
| NSCLC | 12 (48%) | 40 (88.9%) | 1.61 (0.62–4.24) | 0.327 |
| *Treatment* | | | | |
| Nivolumab (3 mg/kg) | 14 (56%) | 41 (91.1%) | Ref. | Ref. |
| Nivolumab/Ipilimumab | 2 (8.00%) | 4 (8.9%) | 1.06 (0.37–3.04) | 0.911 |
| Nivolumab + AVD | 9 (36%) | 0 | 0 | 0 |
| Previous immunotherapy: Yes | 2 (8.00%) | 1 (2.22%) | 0.28 (0.05–1.66) | 0.159 |
| TD induced by nivolumab | 12 (48.0%) | 5 (11.1%) | 0.93 (0.73–1.19) | 0.55 |
| *Baseline TSH* | | | | |
| Mean ± SD (mUI/L) | 2.56 (1.30) | 2.37 (1.44) | 0.28 (0.13–0.64) | 0.002 |

*AVD* doxorubicin, vinblastine and dacarbazine, *CI* confidence interval, *HR* hazard ratio, *NSCLC* non-small-cell lung cancer, *TD* thyroid dysfunction, TSH thyroid-stimulating hormone

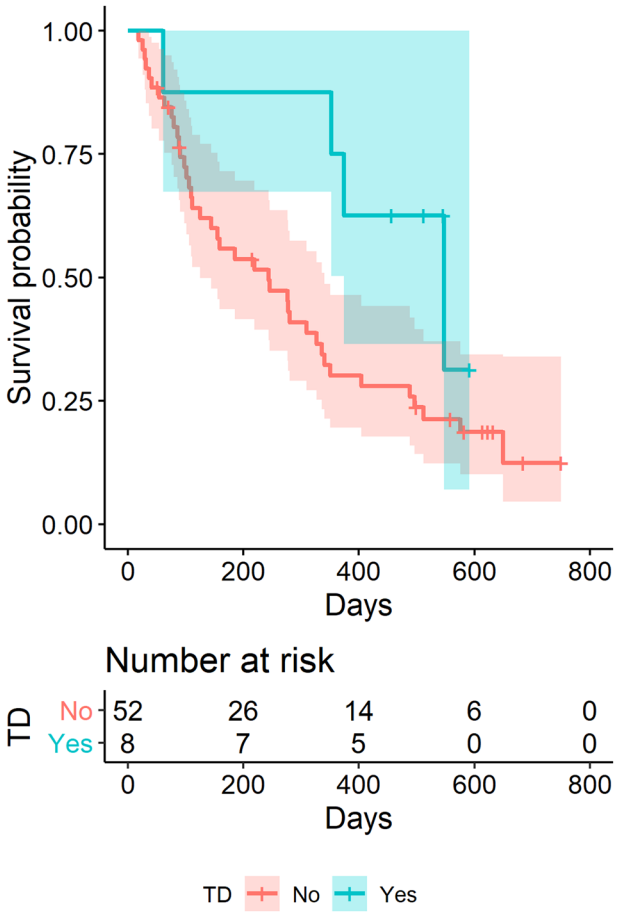


**Fig. 2** Survival analysis according to thyroid dysfunction in patients with non-small cell lung cancer. HR = 0.4 (95% CI: 0.17–0.94); $p = 0.035$. *CI* confidence interval, *HR* hazard ratio

treated with this combined therapy. However, due to the small number of patients with Hodgkin lymphoma in our study and limited data available about TD in these patients, all these findings found in lymphoma patients should be interpreted with care and must be confirmed in future studies.

In our study, most cases of TD were diagnosed on routine screening before the appearance of TD symptoms. We identified three patterns of TD induced by nivolumab: the most common was subclinical primary hypothyroidism. The other patterns were thyroiditis with transient thyrotoxicosis that evolved to hypothyroidism and subclinical thyrotoxicosis. Data from thyroid imaging showed a destructive thyroiditis as reported in previous studies [10, 18–22]. Angell et al. [23] reported a melanoma case treated with nivolumab plus ipilimumab who developed thyrotoxicosis, thyroid biopsy showed abundant clusters of necrotic cells along with lymphocytes and CD163-positive histiocytes indicating a thyroiditis associated with the immunotherapy. On the other hand, Yamauchi et al. [22] found than PD-L1 and PD-L2 were expressed in normal thyroid tissue, suggesting that nivolumab may reduce immune tolerance leading to the development of thyroiditis.

One-third of our patients had positive autoimmunity when TD occurred. Osorio et al. [11] reported development of thyroid autoantibodies shortly after initiation of pembrolizumab, suggesting that PD-1 inhibitors may be modulating autoimmune balance and unmasking latent autoimmunity. Kobayashi et al. [19] in a prospective small study of patients treated with nivolumab showed a positive association of baseline autoimmunity with the development of thyroiditis. Unfortunately, data about baseline autoimmunity were not collected in our study.

As reported in previous clinical trials, TD was generally mild in our study. This suggests that treatment should be used only in symptomatic patients with TD, as recommended by the Society for Immunotherapy of Cancer Toxicity Management Working Group [24–26].

We observed a better overall survival in lung cancer patients with TD induced by nivolumab compared with patients that did not develop TD. Although the underlying mechanism of TD is still unknown, a correlation between endocrine irAEs and response to treatment has been suggested. Faje et al. [27] reported that patients with melanoma who developed hypophysitis induced by ipilimumab achieved better survival outcomes. A correlation between irAEs and survival has been previously described in lung cancer patients [11, 13, 28]. In the study of Osorio et al. [11], median overall survival with pembrolizumab was significantly longer in patients who developed TD as compared with euthyroid patients (HR = 0.29 [95% CI: 0.09–0.94]; $p = 0.04$). Those results can potentially be overestimated by an immortal time bias [29, 30]. As described in the Section Methods, we proposed an alternate statistical analysis in order to avoid this time bias in our analyses.

Limitations of our study include the small sample size and the relatively short follow up, and the lack of assessment of baseline autoimmunity as well as US. Indeed, our study was made in a real-life setting. Strengths of our study are the prospective data collection and follow up of patients with TD, the quick referral to an endocrinologist when TD occurred, and the specific statistical analysis used to correct a potential immortal time bias.

In summary, nivolumab in real-life is associated with higher prevalence of TD than previously described in clinical trials. Thyrotoxicosis occurs earlier than hypothyroidism and in most of cases is related to destructive thyroiditis. Our data suggest an association among TD induced by nivolumab and favorable clinical outcome in patients with advanced NSCLC. Large prospective studies are required in order to assess the prevalence of TD in lymphoma, the impact of autoimmunity on the development of TD induced by nivolumab, and its potential effect on overall survival and specific cancer survival data.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all individual participants included in the study.



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