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Association of antithyroglobulin antibodies with the development of thyroid dysfunction induced by nivolumab

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Thyroid dysfunction (TD) induced by immune checkpoint inhibitors is not sufficiently understood. The purpose of this retrospective observational study was to identify risk factors and the clinical course of TD induced by nivolumab. Patients with advanced solid tumors who were treated with nivolumab from March 2009 through to March 2016 at the National Cancer Center Hospital (Tokyo, Japan) were included. Thyroid function and antithyroid Abs from serum samples among all patients were evaluated at baseline and during nivolumab treatment. Overt hypothyroidism was defined as low serum-free T4 together with elevated thyroid-stimulating hormone (TSH) >10 μIU/mL. Thyrotoxicosis was defined as low TSH with elevated free T4 and/or free T3. We defined thyroid autoimmunity as the presence of antithyroid Abs at baseline, including antithyroid peroxidase Abs and antithyroglobulin Abs (TgAb). Twenty-three (14%) of a total of 168 patients developed TD, including 17 cases of hypothyroidism and 20 of thyrotoxicosis. Thyrotoxicosis followed by hypothyroidism occurred in 14 cases. Fourteen of 35 patients (40%) with thyroid autoimmunity developed TD vs 9 of 133 (7%) without (odds ratio 9.19; 95% confidence interval [CI], 3.53-23.9). In multivariate analysis, elevated TSH and TgAb at baseline were significantly associated with the development of TD, with odds ratio of 7.36 (95% CI, 1.66-32.7) and 26.5 (95% CI, 8.18-85.8), respectively. Association between TD and elevated antithyroid peroxidase Abs at baseline was not significant. These results suggest that patients with pre-existing TgAb and elevated TSH at baseline are at high risk of TD.

KEYWORDS
antithyroglobulin antibody, antithyroid peroxidase antibody, immune-related adverse event, nivolumab, thyroid dysfunction

1 | INTRODUCTION

The clinical success of immune checkpoint blockade has brought about dramatic "breakthroughs" in oncology. Immune checkpoint inhibitors (ICIs) have emerged as the new standard treatment in many different types of malignant tumor, including malignant melanoma,[1-6] nonsmall cell lung cancer (NSCLC),[7-10] head and neck cancer,[11] renal cell carcinoma,[12] urothelial cancer,[13,14] and Hodgkin's lymphoma.[15,16] Immune checkpoint inhibitors have often achieved a durable response and have unique kinetics of response, such

as pseudoprogression. However, ICIs generate unique immune-mediated side-effects, called immune-related adverse events (irAEs), in nearly all organs.

Nivolumab is a fully human IgG4 programmed cell death-1 (PD-1) checkpoint inhibitor Ab that selectively blocks the interaction of the PD-1 receptor with its known ligands, programmed cell death ligand 1 (PD-L1) and PD-L2, disrupting signals that downregulate T cell activation and proliferation. Immune-related adverse events with anti-PD-1 Ab are typically mild to moderate in intensity, but are rarely severe (<4% of patients). They most commonly occur in

the skin, gastrointestinal tract, and endocrine organs. The majority (~80%) of these irAEs resolve within a median of 5 weeks from the onset by the use of immune-modulating medication.[17]

Endocrine irAEs, including thyroid dysfunction (TD), adrenal insufficiency, hypophysitis, and type 1 diabetes, have been reported.[18] Of these endocrine irAEs, TD, including hypothyroidism, thyrotoxicosis, and thyroiditis, is one of the most frequent irAEs, observed in more than 10% of patients treated with ICIs. Although most cases of TD are mild, high-grade thyrotoxicosis and hypothyroidism are also reported at low frequency (<1%) in clinical trials.[4,6] Even though TD is a common irAE, the etiology and precise clinical course of TD have not been sufficiently identified. Here, to identify the risk factors and clinical course of ICI-induced TD, we retrospectively examined thyroid function and antithyroid Abs among patients treated with nivolumab.

2 | MATERIALS AND METHODS

2.1 | Patients and study design

Patients with advanced solid tumors who were treated with nivolumab from March 2009 through to March 2016 at the National Cancer Center Hospital (Tokyo, Japan) were eligible for this study. Subjects included patients who were treated in clinical practice as well as those who participated in sponsor-initiated investigational trials (ONO-4538-01, ONO-4538-02, ONO-4538-04, ONO-4538-05, ONO-4538-06, and ONO-4538-08).[19-23] Patients were excluded from our study for the following reasons: previous treatment with any ICI, pre-existing hypothyroidism such as thyroid-stimulating hormone (TSH) >10 μIU/mL or concomitant treatment with levothyroxine replacement therapy before nivolumab, insufficient assessment for thyroid function and thyroid Abs, and a short follow-up time of less than 90 days from the initial administration of nivolumab to exclude early progression and death.

This observational study was carried out at the National Cancer Center Hospital. We retrospectively reviewed the medical records and evaluated patient characteristics including age, sex, ECOG performance status (PS), cancer type, clinical outcome, safety of irAEs, and biochemistry tests such as thyroid function and antithyroid Abs.

This study was approved by the Ethics Committee of the National Cancer Hospital (2015-366) and carried out in accordance with the Declaration of Helsinki.

2.2 | Assessment of thyroid function and definition of TD

We assessed thyroid function, namely TSH, free T3 (fT3), and free T4 (fT4) levels and antithyroid Abs, namely antithyroid peroxidase Abs (TPOAb) and antithyroglobulin Abs (TgAb) before and during nivolumab treatment. Thyroid function was assessed every 1 or 2 months. Most patients were evaluated for thyroid function and the titer of antithyroid Abs in clinical practice and sponsor-initiated investigational trials. For missing values, we measured them in

preserved serum samples collected at the National Cancer Center Biobank (Tokyo, Japan). In other words, we prospectively collected serial serum samples from all subjects and retrospectively reviewed them for thyroid function and antithyroid Ab titer. Normal ranges in our laboratory are 0.35-4.94 μIU/mL for TSH, 1.71-3.71 ng/mL for fT3, 0.70-1.48 ng/mL for fT4, <16 IU/mL for TPOAb, and <28 IU/mL for TgAb. Antithyroid Abs were defined as the presence if the titer was elevated above the normal limit and as the absence if the titer was within the normal limit.

The biochemical diagnosis of TD was determined in accordance with the guidelines of the American Thyroid Association, the American Association of Clinical Endocrinologists, and the Endocrine Society. Hypothyroidism was defined as low serum fT4 together with elevated TSH (>10 μIU/mL). Thyrotoxicosis was defined as low TSH with elevated fT4 and/or fT3. The decision to initiate treatment for thyrotoxicosis or hypothyroidism was made by the attending physician based on clinical presentation. An extramural endocrinologist (SI) reviewed all values and confirmed the final clinical diagnosis. In this study, we defined thyroid autoimmunity at baseline as the presence of elevated antithyroid Abs before nivolumab treatment. The interval between thyrotoxicosis and hypothyroidism was defined from the date of onset of thyrotoxicosis to that of hypothyroidism.

2.3 | Statistical analysis

All patients were classified into two cohorts, based on the presence of thyroid autoimmunity at baseline. Differences between covariates in the two cohorts were compared using the Mann-Whitney *U* test or Fisher's exact test. Possible explanatory factors for the development of TD were analyzed using a multivariate logistic regression model. A multivariate model initially included age (<65 years, ≥65 years), sex (male, female), ECOG PS (0-1, 2-3), THS (<5μIU/mL, ≥5μIU/mL), TPOAb (presence, absence), TgAb (presence, absence), and thyroid autoimmunity (presence, absence). A stepwise model selection was carried out with *P* value thresholds of .05 for inclusion and .10 for exclusion. In this model, candidate variables were tested with stepwise forward selection method. Cumulative incidence of TD was estimated using the Kaplan-Meier method. A difference in cumulative incidence between patients with and without thyroid autoimmunity before nivolumab treatment was assessed with the log-rank test, and hazard ratio (HR) and 95% confidence interval (CI) were estimated with a Cox proportion hazard model. In patients who developed TD, association between time-to-onset of TD and titer of TgAb at baseline was examined using the Mann-Whitney test. All statistical analyses were undertaken using SPSS 23.0 (IBM, Armonk, NY, USA).

3 | RESULTS

3.1 | Patient characteristics

A total of 256 patients with malignant solid tumors were treated with nivolumab. Sixty-seven patients were excluded from this study
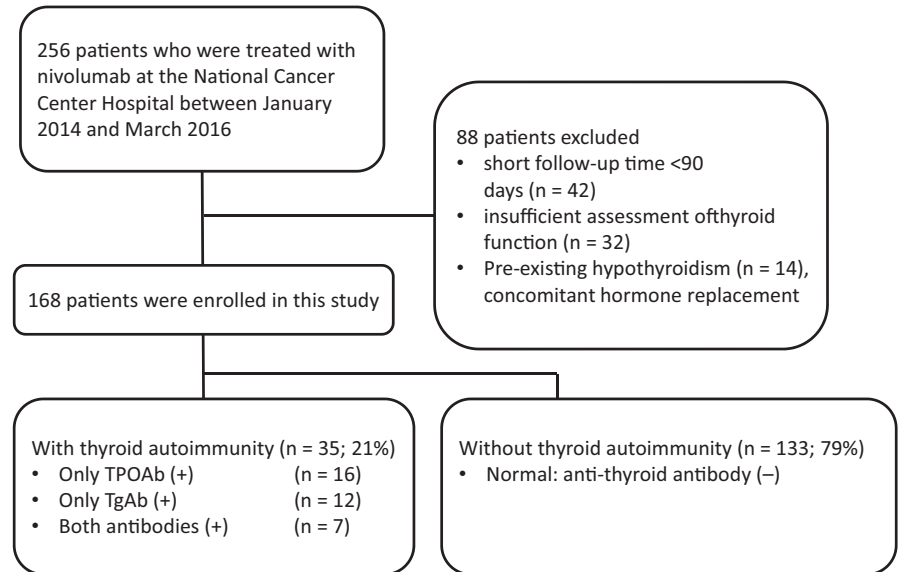
FIGURE 1 Consort diagram of this study of patients with advanced solid tumors treated with nivolumab at the National Cancer Center Hospital (Tokyo, Japan). TgAb, antithyroglobulin Ab; TPOAb, antithyroid peroxidase Ab

TABLE 1 Patient characteristics and thyroid autoimmunity

		Total (N = 168)	Patients with thyroid autoimmunity at baseline (N = 35)	Patients without thyroid autoimmunity at baseline (N = 133)
Age, years	Median (range)	63.5 (17-92)	63 (22-81)	64 (17-92)
Sex, N (%)	Male/female	98 (58)/70 (42)	15 (43)/20 (57)	83 (62%)/50 (38%)
ECOG PS (%)	0-1/2-3	158 (94)/10 (6)	31 (89)/4 (11)	127 (95%)/6 (5%)
Cancer type, N (%)				
Malignant melanoma		92 (54%)	19 (54)	73 (54)
NSCLC		70 (42%)	16 (46)	54 (41)
Others		6 (4%)	0 (0)	6 (5)
Dose of nivolumab	Median (range)	9 (1-76)	9 (1-59)	8 (1-76)
Duration of response, days	Median (range)	201 (14-1665)	237 (18-1063)	185 (14-1665)
Thyroid autoimmunity at baseline, N (%)				
Presence of TPOAb alone (≥16 IU/mL)		16 (10)	16 (46)	0 (0)
Presence of TgAb alone (≥28 IU/mL)		12 (7)	12 (34)	0 (0)
Presence of both TPOAb and TgAb		7 (4)	7 (20)	0 (0)
TSH at baseline, N (%)				
TSH ≥ ULN, <10 µIU/mL		11 (7)	2 (6)	9 (7)

NSCLC, nonsmall cell lung cancer; PS, performance status; TgAb, antithyroglobulin Ab; TPOAb, antithyroid peroxidase Ab; TSH, thyroid-stimulating hormone.

due to a short follow-up time (N = 42), insufficient preserved serum samples for the assessment of thyroid function and thyroid Abs (N = 32), or pre-existing overt hypothyroidism (N = 14). Of the 14 patients with pre-existing hypothyroidism, 9 already received levothyroxine replacement therapy before nivolumab, whereas those with elevated TSH ≥10 µIU/mL did not receive replacement therapy. Accordingly, a total of 168 patients were enrolled in this study (Figure 1). Patient characteristics and nivolumab treatment are shown in Table 1. Seventy patients (42%) were female and 98 (58%) were male, with a median age of 63.5 years (range, 17-92 years). The predominant cancer types were malignant melanoma (N = 92; 54%) and NSCLC (N = 70; 42%). Among 35 patients (21%) with thyroid

autoimmunity at baseline, 16 were positive for TPOAb alone, 12 for TgAb alone, and 7 for both TPOAb and TgAb (Figure 1).

3.2 | Onset and timing of TD

At the time of data cut-off (31 August 2016), median follow-up time was 272 days (range, 100-2529 days). Twenty-three of 168 patients (14%) developed TD, including thyrotoxicosis and hypothyroidism (Table S1). There were no significant differences between patients who developed TD (N = 23) and those who did not (N = 145) in terms of patient characteristics such as age, sex, and ECOG PS, except for the evidence of thyroid autoimmunity at baseline (P < .001, Fisher's exact test; Table 2).

		With thyroid dysfunction (N = 23)	Without thyroid dysfunction (N = 145)
Age, years	Median (range)	66 (46-87)	63 (17-92)
Sex, N (%)	Male/female	10 (43)/13 (57)	88 (61)/57 (39)
ECOG PS, N (%)	0-1/2-3	22 (96)/1 (4)	136 (94)/9 (6)
Dose of nivolumab	Median (range)	11 (2-59)	8 (1-76)
Duration of response, days	Median (range)	261 (69-834)	189 (14-1665)
Thyroid autoimmunity at baseline, N (%)			
Presence/absence		14 (61)/9 (39)	21 (14)/124 (86)
Presence of TPOAb alone (≥16 IU/mL)		2 (9)	14 (9)
Presence of TgAb alone (≥28 IU/mL)		8 (35)	4 (3)
Presence of both TPOAb and TgAb		4 (17)	3 (2)
TSH at baseline, N (%)			
TSH ≥ ULN, <10 μIU/mL		4 (17)	7 (5)

TABLE 2 Characteristics of patients with advanced solid tumors treated with nivolumab who did or did not develop thyroid dysfunction

PS, performance status; TgAb, antithyroglobulin Ab; TPOAb, antithyroid peroxidase Ab; TSH, thyroid-stimulating hormone; ULN, upper limit of normal.

Thyrotoxicosis and hypothyroidism occurred in 20 (12%) and 17 patients (10%), respectively (Table S1). Hypothyroidism following thyrotoxicosis occurred in 14 patients (8.3%). Median onset time of thyrotoxicosis was 43 days (range, 20-149 days) and that of hypothyroidism was 90 days (range, 29-240 days). Median interval between thyrotoxicosis and hypothyroidism in the 14 patients was 45 days (range, 21-91 days). All thyrotoxicosis was transient and recovered with a median duration of 32.5 days (range, 2-91 days), suggesting the development of destructive thyroiditis. Regarding the signs and symptoms of thyrotoxicosis, 16 (80%) patients developed symptomatic thyrotoxicosis, including pyrexia (N = 6), fatigue (N = 5), pruritus (N = 5), tachycardia (N = 5), palpitation (N = 2), anorexia (N = 2), sore throat (N = 2), thirst (N = 1), vomiting (N = 1), and body weight loss (N = 1). Most symptoms were mild, and only 2 patients received beta blockers to manage their symptoms. Eight (47%) developed symptomatic hypothyroidism including fatigue (N = 7), systemic edema (N = 2), increased sensitivity to cold (N = 2), and showed body weight gain (N = 1). Among 17 patients with hypothyroidism, 15 (88%) received levothyroxine replacement at the discretion of the attending physician and 16 (94%) did not experience resolution throughout the observation period.

Most patients (70%) who developed thyrotoxicosis eventually turned out to have hypothyroidism. Interestingly, however, thyrotoxicosis did not progress to hypothyroidism in 5 of 6 patients who received systemic steroid therapy at the onset of thyrotoxicosis for any reason, such as other irAEs of skin rash or symptom relief for brain metastasis.

3.3 | Association between development of TD and thyroid autoimmunity before nivolumab

The incidence of TD in patients with thyroid autoimmunity at baseline was 40% (N = 14/35), whereas it was 7% (N = 9/133) in patients without thyroid autoimmunity (odds ratio [OR] 9.19; 95% CI, 3.53-23.9; P < .01). The incidence of TD in patients with TgAb

was 63% (N = 12/19); it was 7.3% (N = 11/149) in patients without TgAb (OR 21.5; 95% CI, 7.04-65.7; P < .01). Among 16 patients with TPOAb alone at baseline, 2 (12.5%) developed TD. Moreover, these 2 patients with TPOAb alone became positive for TgAb at the onset of TD (titers of TgAb in the 2 patients was from 26 to 274 IU/mL and from 18 to 341 IU/mL). Eventually, the maximum levels of TgAb during nivolumab treatment were more than the upper limit of normal in 17 patients (74%) among 23 who developed TD. On univariate analysis, the level of TSH, the presence of TgAb, and the presence of thyroid autoimmunity before nivolumab were significantly associated with TD (Table 3). When multivariate analysis was carried out with a model including age, sex, ECOG PS, the level of TSH, the presence of TPOAb, and the presence of TgAb, TD was significantly associated with the level of TSH (OR 7.36; 95% CI, 1.66-32.7; P = .01), and the presence of TgAb (OR 26.5; 95% CI, 8.18-85.8; P < .001).

The cumulative incidence of thyrotoxicosis and hypothyroidism using the Kaplan-Meier method was overwhelmingly higher in patients with thyroid autoimmunity than in patients without (Figure 2). The HR in thyrotoxicosis was 11.3 (95% CI, 4.35-29.6; P < .01), and the HR in hypothyroidism was 11.0 (95% CI, 3.89-31.4; P < 0.01). In all patients who developed thyrotoxicosis (N = 20), incidence rates within 3 months and 6 months were 86% and 100% in patients with thyroid autoimmunity and 67% and 100% in those without. In all patients who developed hypothyroidism (N = 17), incidence rates within 3 and 6 months were 58% and 100% in patients with thyroid autoimmunity and 40% and 80% in those without. Additionally, Figure 3 shows the association of baseline titer of TgAb and time-to-onset of TD among patients who developed TD. During the follow-up period, the patients with a higher titer of TgAb than median (>63 IU/mL) tended to develop thyrotoxicosis (median 4.7 weeks [range, 2.9-18.9] vs median 6.9 weeks [range, 2.9-21.3]) and hypothyroidism (median 11 weeks [range, 5.9-18.1] vs median 14.6 weeks

TABLE 3 Logistic regression analysis to identify risk factors for the development of thyroid dysfunction among patients with advanced solid tumors treated with nivolumab

		Univariate analysis			Multivariate analysis		
Covariate		Odds ratio	(95% CI)	P value	Odds ratio	(95% CI)	P value
Age	<65 vs ≥65 years	1.24	(0.51-2.98)	.640			
Sex	Male vs female	2.01	(0.83-4.88)	.130			
ECOG PS	0-1 vs 2-3	0.69	(0.08-5.69)	.730			
TSH, μIU/mL	<5 vs ≥5 μIU/mL	4.15	(1.11-15.5)	.030	7.36	(1.66-32.7)	.010
TPOAb	Presence vs absence	2.66	(0.92-7.67)	.070			
TgAb	Presence vs absence	21.5	(7.04-65.7)	.001	26.5	(8.18-85.8)	<.001
Thyroid autoimmunity at baseline	Presence vs absence	9.19	(3.53-23.9)	<.001	NA		
Thyroid autoimmunity and/ or increased level of TSH at baseline	Presence vs absence	12.4	(4.46-34.4)	<.001	NA		

Univariate analysis included age, sex, ECOG performance status (PS), levels of thyroid-stimulating hormone (TSH), antithyroid peroxidase Ab (TPOAb), and antithyroglobulin Ab (TgAb), thyroid autoimmunity, and thyroid autoimmunity and/or increased level of TSH.
Multivariate analysis included age, sex, ECOG PS, and levels of TSH, TPOAb, and TgAb.
NA, not applicable.
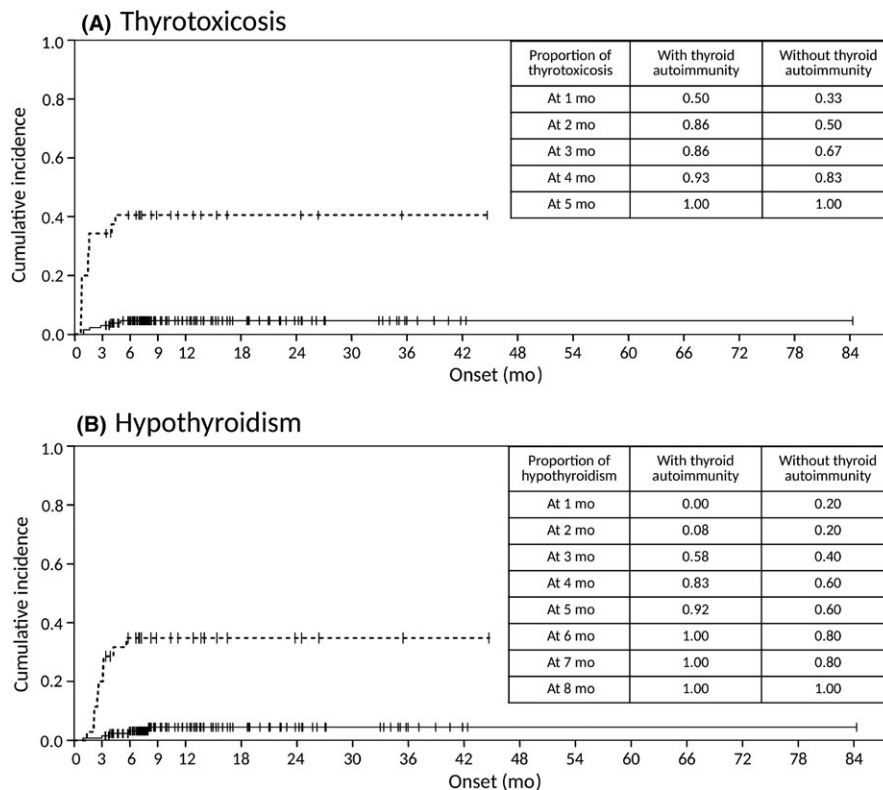
FIGURE 2 Cumulative incidence of thyrotoxicosis and hypothyroidism among patients with advanced solid tumors treated with nivolumab. Broken line, patients with thyroid autoimmunity; solid line, those without. Patients who were lost to follow-up and those still alive at the cut-off date were censored. Termination of nivolumab treatment was not a censored event. Incidence rate indicates the rate of cumulative event at each time point among all patients who developed thyrotoxicosis and hypothyroidism

[range, 4.1-34.3]) at an earlier time than those with a lower titer than median (≤63 IU/mL) (P = 0.190 and 0.321 by Mann-Whitney test, retrospectively).

All TD events except 1 developed within 6 months from the initiation of nivolumab. The cumulative incidence reached a plateau within 9 months, and no events occurred thereafter (Figure 2).

3.4 | Association between development of TD and survival

Median overall survival of all patients was 1.41 years (95% CI, 0.71-2.10; Figure S1A). Overall survival in patients with TD was numerically longer than that without TD, albeit without statistical significance (HR 0.52; 95% CI, 0.25-1.11; *P* = .09) (Figure S1B).

4 | DISCUSSION

Our study showed that TD induced by ICIs was common, occurred early, and had unique kinetics with transient thyrotoxicosis followed by hypothyroidism. The signs and symptoms of TD were mild, various, and non-specific. As patients with hypothyroidism often require hormone replacement therapy for a long period and rarely develop thyroid crisis, the early and precise diagnosis and appropriate management of TD are important for oncologists in practice.

This study can be considered as one utilizing real-world data, given that our study included a large number of patients with NSCLC, malignant melanoma, and other types of cancer who were treated with nivolumab in investigational trials and clinical practice. In fact, the frequency of hypothyroidism induced by nivolumab in our study was comparable to results from clinical trials (9%-10.8%)[24] and post-marketing surveillance (11.3%).[25]

Recently, an irAE management guide has been published, the American Society of Clinical Oncology Clinical Practice Guideline.[26] However, baseline risk of development of TD is not included in this guidance. We identified the increased level of TSH and the presence of TgAb at baseline as risk factors for TD induced by nivolumab

treatment. In addition, the Kaplan-Meier curve indicated that almost all patients developed TD within 6 months from the initiation of nivolumab treatment and that the cumulative incidence achieved a plateau within 9 months. These findings can help to predict who and when TD is induced by nivolumab.

To date, several investigational trials and case series presented TD induced by ICIs as a part of irAEs, but only a few reports have focused on the clinical and immunologic features of TD. Osorio et al showed the clinical course of TD associated with thyroid Abs in patients with stage IV NSCLC treated with pembrolizumab as part of the KEYNOTE-001 study. Among a total of 48 patients, 10 (21%) developed TD, including thyrotoxicosis and hypothyroidism. Six of these 10 patients with hypothyroidism experienced preceding transient thyrotoxicosis.[27] Furthermore, antithyroid Abs were present in 8 of 10 patients who developed hypothyroidism, compared with 3 of 38 who did not (80% vs 8%, *P* < .0001), although only 4 of 11 patients who had positive antithyroid Abs were present at baseline and the remaining 7 developed during pembrolizumab treatment. Another report showed that destructive thyroiditis was developed in 4 out of 66 patients treated with nivolumab and was significantly associated with positive TgAb and/or TPOAb before nivolumab treatment.[28] In the current study, the incidence of TD was significantly higher in patients with thyroid autoimmunity than in those without. These findings consistently suggest that thyroid Abs play essential roles in the development of TD induced by ICIs.

However, whether TgAb and TPOAb have the same impact on the development of TD is not well known. Our study showed that the presence of TgAb before nivolumab was associated with a large and significant HR of TD compared to that of TPOAb (Table 3). Different results between ours and other studies might be based on the number of
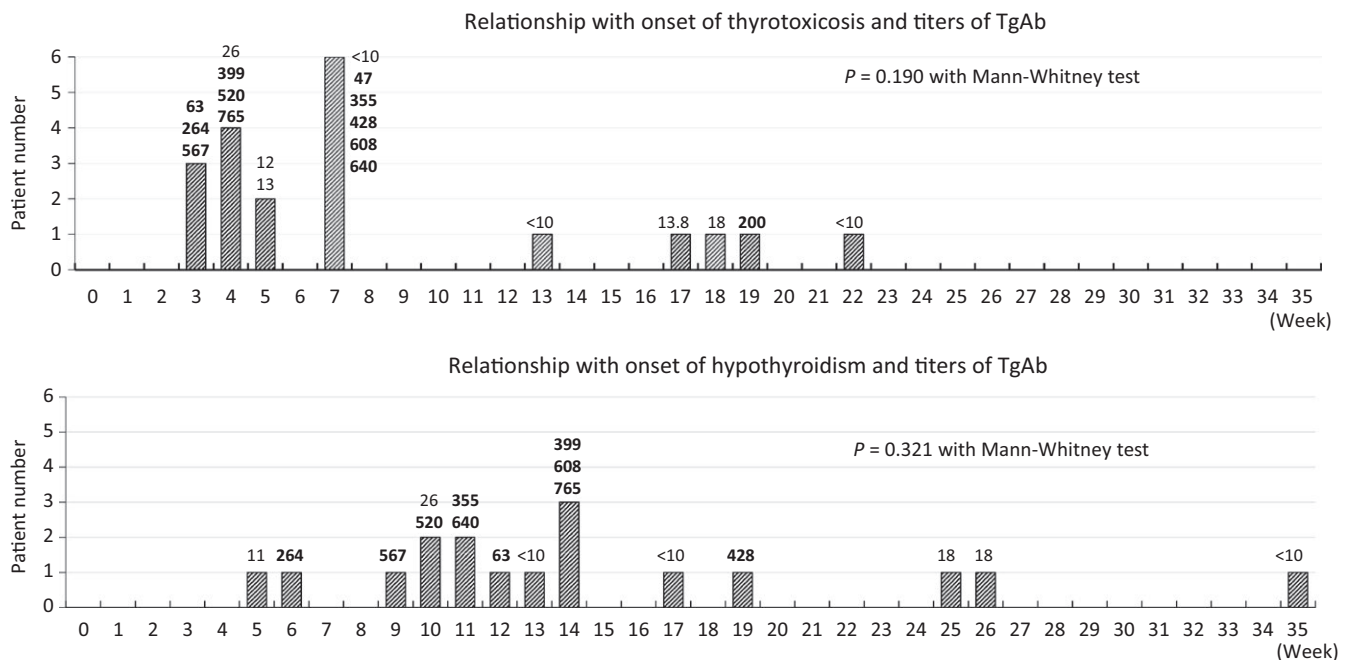
FIGURE 3 Association of thyroid dysfunction with the titers of antithyroglobulin Ab (TgAb) at baseline in patients with advanced solid tumors treated with nivolumab. Numbers beside bars indicate titer. Bold indicates increased titers of anti-Tg Ab

cases and distinction of thyroid Abs. Additionally, among all 23 patients who developed TD, 74% experienced increased levels of TgAb during nivolumab treatment. Furthermore, the 2 patients with TPOAb alone at baseline changed to become positive for TgAb at the onset of TD. We consider that Tg-specific immunity plays a predominant role in destructive thyroiditis induced by nivolumab compared with TPO-specific immunity, whereas both Tg- and TPO-specific CD8-positive T cells are involved in the thyroid destruction of Hashimoto's thyroiditis.[29]

Transient thyrotoxicosis followed by permanent hypothyroidism, as observed in our study, was previously reported.[30] This unique pattern is considered to be caused by acute inflammation and subsequent destruction of the thyroid gland by thyroid autoimmunity. Indeed, all patients recovered from transient thyrotoxicosis, with a median duration of 32.5 days, suggesting the development of destructive thyroiditis. Moreover, patients who developed thyrotoxicosis only and did not develop hypothyroidism were also observed in our study. Interestingly, 5 of 6 patients received systemic steroid therapy during the thyrotoxicosis period for various reasons. Although the dose and duration of systemic steroid therapy varied, use of an immune suppressive agent in the acute inflammation period might avoid permanent destruction of the thyroid gland. Steroid therapy at the onset of thyrotoxicosis could prevent permanent hypothyroidism, but further investigation is warranted.

Several studies have suggested that development of irAEs is associated with better clinical response and outcome.[31-35] Our study also showed that the survival of those who experienced TD tended to be better than that of those who did not, especially patients with malignant melanoma (Figure S1C). These findings imply the same underlying mechanism enhancing the immune response against tumor cells and self-organs.

Our study has several limitations. First, although we evaluated thyroid function at baseline and during nivolumab treatment almost every 2 months, potential measurement bias was present in the different intervals for onset time and duration between events. Second, most patients were evaluated prospectively for thyroid function and antithyroid Abs titer in clinical practice and sponsor-initiated investigational trials. Moreover, missing cases were measured retrospectively from preserved serum samples. The decision to initiate treatment for thyrotoxicosis and hypothyroidism was made by the attending physicians depending on the clinical presentation.

In conclusion, our large cohort study revealed that TgAb was associated with the development of nivolumab-induced TD. This large cohort study in which all subjects could be assessed for thyroid function and antithyroid Abs before and during nivolumab treatment has clearly depicted the clinical course of TD using real-world data. These findings suggest that the optimal management of TD based on risk factors can be clinically useful.

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CONFLICT OF INTEREST

Yutaka Fujiwara reports grants and lecture fees from Bristol-Myers Squibb and Ono Pharmaceutical Co. Shintaro Iwama reports lecture fees from Bristol-Myers Squibb and Ono Pharmaceutical Co. Hiroshi Arima reports grants from Ono Pharmaceutical Co. Naoya Yamazaki reports grants and lecture fees from Bristol-Myers Squibb and Ono Pharmaceutical Co. Shigehisa Kitano reports lecture fees from AstraZeneca, Bristol-Myers Squibb, and Chugai, and grants and lecture fees from Ono Pharmaceutical Co. Noboru Yamamoto reports grants and lecture fees from AstraZeneca, Bristol-Myers Squibb, Chugai, and Ono Pharmaceutical Co. Yuichiro Ohe reports grants from Bristol-Myers Squibb and grants and lecture fees from Ono Pharmaceutical Co. All remaining authors have declared no conflict of interests.

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REFERENCES

1. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010;363:711-723.
2. Robert C, Thomas L, Bondarenko I, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med*. 2011;364:2517-2526.
3. Robert C, Long GV, Brady B, et al. Nivolumab in previously untreated melanoma without BRAF mutation. *N Engl J Med*. 2015;372:320-330.
4. Robert C, Schachter J, Long GV, et al. Pembrolizumab versus ipilimumab in advanced melanoma. *N Engl J Med*. 2015;372:2521-2532.
5. Weber JS, D'Angelo SP, Minor D, et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. *Lancet Oncol*. 2015;16:375-384.
6. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N Engl J Med*. 2015;373:23-34.
7. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. *N Engl J Med*. 2015;373:1627-1639.
8. Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. *N Engl J Med*. 2015;373:123-135.

9. Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med*. 2015;372:2018-2028.

10. Reck M, Rodriguez-Abreu D, Robinson AG, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. *N Engl J Med*. 2016;375:1823-1833.

11. Ferris RL, Blumenschein G Jr, Fayette J, et al. Nivolumab for recurrent squamous-cell carcinoma of the head and neck. *N Engl J Med*. 2016;375:1856-1867.

12. Motzer RJ, Escudier B, McDermott DF, et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. *N Engl J Med*. 2015;373:1803-1813.

13. Rosenberg JE, Hoffman-Censits J, Powles T, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. *Lancet*. 2016;387:1909-1920.

14. Sharma P, Callahan MK, Bono P, et al. Nivolumab monotherapy in recurrent metastatic urothelial carcinoma (CheckMate 032): a multicentre, open-label, two-stage, multi-arm, phase 1/2 trial. *Lancet Oncol*. 2016;17:1590-1598.

15. Younes A, Santoro A, Shipp M, et al. Nivolumab for classical Hodgkin's lymphoma after failure of both autologous stem-cell transplantation and brentuximab vedotin: a multicentre, multicohort, single-arm phase 2 trial. *Lancet Oncol*. 2016;17:1283-1294.

16. Chen R, Zinzani PL, Fanale MA, et al. Phase II study of the efficacy and safety of pembrolizumab for relapsed/refractory classic hodgkin lymphoma. *J Clin Oncol*. 2017;35:2125-2132.

17. Hodi FS, Postow MA, Chesney JA, et al. Overall survival in patients with advanced melanoma (MEL) who discontinued treatment with nivolumab (NIVO) plus ipilimumab (IPI) due to toxicity in a phase II trial (CheckMate 069). *J Clin Oncol*. 2016;34:9518-9518.

18. Corsello SM, Barnabei A, Marchetti P, De Vecchis L, Salvatori R, Torino F. Endocrine side effects induced by immune checkpoint inhibitors. *J Clin Endocrinol Metab*. 2013;98:1361-1375.

19. Yamamoto N, Nokihara H, Yamada Y, et al. Phase I study of Nivolumab, an anti-PD-1 antibody, in patients with malignant solid tumors. *Invest New Drugs*. 2017;35:207-216.

20. Yamazaki N, Kiyohara Y, Uhara H, et al. Efficacy and safety of nivolumab in Japanese patients with previously untreated advanced melanoma: a phase II study. *Cancer Sci*. 2017;108:1223-1230.

21. Kanda S, Goto K, Shiraishi H, et al. Safety and efficacy of nivolumab and standard chemotherapy drug combination in patients with advanced non-small-cell lung cancer: a four arms phase Ib study. *Ann Oncol*. 2016;27:2242-2250.

22. Nishio M, Hida T, Atagi S, et al. Multicentre phase II study of nivolumab in Japanese patients with advanced or recurrent non-squamous non-small cell lung cancer. *ESMO Open*. 2016;1:e000108.

23. Hida T, Nishio M, Nogami N, et al. Efficacy and safety of nivolumab in Japanese patients with advanced or recurrent squamous non-small cell lung cancer. *Cancer Sci*. 2017;108:1000-1006.

24. Cukier P, Santini FC, Scaranti M, Hoff AO. Endocrine side effects of cancer immunotherapy. *Endocr Relat Cancer*. 2017;24:T331-T347.

25. Kiyohara Y, Uhara H, Ito Y, Matsumoto N, Tsuchida T, Yamazaki N. Safety and efficacy of nivolumab in Japanese patients with malignant melanoma: an interim analysis of a postmarketing surveillance. *J Dermatol*. 2018;45:408-415.

26. Brahmer JR, Lacchetti C, Schneider BJ, et al. Management of immune-related adverse events in patients treated with immune checkpoint inhibitor therapy: American society of clinical oncology clinical practice guideline. *J Clin Oncol*. 2018;14:247-249.

27. Osorio JC, Ni A, Chaft JE, et al. Antibody-mediated thyroid dysfunction during T-cell checkpoint blockade in patients with non-small-cell lung cancer. *Ann Oncol*. 2017;28:583-589.

28. Kobayashi T, Iwama S, Yasuda Y, et al. Patients with antithyroid antibodies are prone to develop destructive thyroiditis by nivolumab: a prospective study. *J Endocr Soc*. 2018;2:241-251.

29. Ehlers M, Thiel A, Bernecker C, et al. Evidence of a combined cytotoxic thyroglobulin and thyroperoxidase epitope-specific cellular immunity in Hashimoto's thyroiditis. *J Clin Endocrinol Metab*. 2012;97:1347-1354.

30. Orlov S, Salari F, Kashat L, Walfish PG. Induction of painless thyroiditis in patients receiving programmed death 1 receptor immunotherapy for metastatic malignancies. *J Clin Endocrinol Metab*. 2015;100:1738-1741.

31. Downey SG, Klapper JA, Smith FO, et al. Prognostic factors related to clinical response in patients with metastatic melanoma treated by CTL-associated antigen-4 blockade. *Clin Cancer Res*. 2007;13:6681-6688.

32. Freeman-Keller M, Kim Y, Cronin H, Richards A, Gibney G, Weber JS. Nivolumab in resected and unresectable metastatic melanoma: characteristics of immune-related adverse events and association with outcomes. *Clin Cancer Res*. 2016;22:886-894.

33. Hasan Ali O, Diem S, Markert E, et al. Characterization of nivolumab-associated skin reactions in patients with metastatic non-small cell lung cancer. *Oncoimmunology*. 2016;5:e1231292.

34. Haratani K, Hayashi H, Chiba Y, et al. Association of immune-related adverse events with nivolumab efficacy in non-small-cell lung cancer. *JAMA Oncol*. 2017;4:374-378.

35. Nakamura Y, Kitano S, Takahashi A, et al. Nivolumab for advanced melanoma: pretreatment prognostic factors and early outcome markers during therapy. *Oncotarget*. 2016;7:77404-77415.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.