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Thyroid Dysfunction after Atezolizumab and Bevacizumab Is Associated with Favorable Outcomes in Hepatocellular Carcinoma

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Keywords
Hepatocellular carcinoma · Thyroid adverse events · Atezolizumab · Bevacizumab

Abstract

Introduction: Atezolizumab and bevacizumab (Ate/Bev) combination has become the new first-line systemic therapy for unresectable hepatocellular carcinoma (HCC). Although several studies reported thyroid dysfunction after treatment with immune checkpoint inhibitors, the clinical and immunological significance of thyroid dysfunction in patients treated with Ate/Bev has not been comprehensively addressed. We aimed to comprehensively evaluate the clinical and immunological implications of thyroid dysfunction in unresectable HCC patients treated with Ate/Bev. ***Methods:*** We enrolled 208 patients with unresectable HCC treated with Ate/Bev from three Korean cancer centers. Thyroid adverse events (AEs) were reviewed, and cytokines and T cells in the blood samples were analyzed at baseline. For external validation, we analyzed clinical outcomes according to thyroid AEs in patients treated with Ate/Bev in the IMbrave150 study. ***Results:*** Forty-one (19.7%) out of 208 patients experienced thyroid dysfunction (hypothyroidism [17.3%] and thyrotoxicosis [5.8%]) after Ate/Bev treatment. Median time to onset of hypothyroidism and thyrotoxicosis after Ate/Bev treatment was 3.5 and 1.3 months, respectively. Patients with thyroid AEs demonstrated significantly better progression-free survival, overall survival, and objective response rate than those without thyroid AEs. These findings were still consistent even

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after adjusting for confounding factors. Furthermore, favorable survival outcomes in patients with thyroid AEs were also validated in a cohort of IMbrave150 patients. While patients with thyrotoxicosis showed a significantly lower level of baseline IL-6, those with hypothyroidism did not show significant differences in circulating cytokine levels and $CD8^+$ T-cell fractions. ***Conclusions:*** A fraction of patients with HCC treated with Ate/Bev experienced thyroid dysfunction, and the development of thyroid AEs was associated with favorable clinical outcomes.

Introduction

In the IMbrave150 phase III trial, atezolizumab, an anti-programmed death-1 (PD-1) ligand 1 (PD-L1) antibody, and bevacizumab, an anti-vascular endothelial growth factor antibody, demonstrated statistically significant survival benefit over sorafenib, thus becoming the new first-line systemic therapy for unresectable hepatocellular carcinoma (HCC) [1–3]. Although immune checkpoint inhibitor (ICI) therapy is generally well tolerated, it can induce immune-related adverse events (irAEs) [4]. The exact mechanisms of irAEs have not yet been fully elucidated; however, they are assumed to be caused by the bystander effects of ICI-induced T-cell activation [5, 6]. In addition, the association between irAEs and various circulating cytokines has also been suggested in recent studies [7, 8]. In some reports, the occurrence of irAEs after ICI therapy was associated with improved survival outcomes in various types of solid tumors [9, 10]. Thyroid dysfunction is one of the most common adverse events (AEs) after ICI treatment and can be easily monitored using a thyroid function test in clinical practice [11]. In the IMbrave150 trial, 15.5% of patients have been reported to develop thyroid dysfunction after atezolizumab and bevacizumab (Ate/Bev) treatment. However, the clinical and immunological significance of thyroid AEs in patients treated with Ate/Bev has not yet been comprehensively addressed. Therefore, this study aimed to comprehensively evaluate the clinical and immunological implications of thyroid AEs in unresectable HCC patients treated with Ate/Bev.

Materials and Methods

Study Design and Patients

This study prospectively enrolled patients with unresectable HCC who were treated with Ate/Bev between June 2020 and December 2021 at three tertiary cancer centers in Korea for the analysis of cytokines and T cells in the peripheral blood (n = 276; CONSORT diagram shown in online suppl. Fig. 1 left; for all online suppl. material, see https://doi.org/10.1159/000531182). We retrospectively reviewed treatment-related AEs, including thyroid AEs. The exclusion criteria were as follows: Eastern Cooperative Group (ECOG) performance status of 2 (n = 8), Child-Pugh score of C (n = 1), patients who received Ate/Bev treatment as second-line therapy (n = 11), patients who had no follow-up thyroid function test data after initiation of treatment (n = 21), and those who had thyroid dysfunction before initiation of treatment (n = 27). Finally, 208 HCC patients were eligible for analysis in this study. This study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and was approved by the participating hospitals' Institutional Review Boards (CHA Bundang Medical Center, CHA-2017-11-052, CHA-2017-11-054; Ulsan University Hospital, 2020-12-006; Haeundae Paik Hospital, 2020-12-019-001). Written informed consent was obtained from all the patients. As an external validation cohort, we analyzed clinical outcomes according to thyroid AEs in patients treated with Ate/Bev in the IMbrave150 study (ClinicalTrials.gov, NCT03434379), a prospective, randomized, phase III trial (CONSORT diagram shown in online suppl. Fig. 1 right).

Treatment and Outcome Evaluation

All patients were treated with atezolizumab (1,200 mg fixed dose) and bevacizumab (15 mg/kg) every 3 weeks following the IMbrave150 protocol [12]. Treatment was continued until disease progression, intolerable toxicities, or withdrawal of consent. Tumor response was evaluated every 6 or 9 weeks using computed tomography or magnetic resonance imaging, following the Response Evaluation Criteria in Solid Tumors version 1.1. AEs were assessed using the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

Definitions

Thyroid AE was defined as a newly developed thyroid dysfunction after the initiation of Ate/Bev treatment. Overt hypothyroidism was defined as a thyroid-stimulating hormone (TSH) level above the upper reference limit with a free thyroxine (T4) level below the lower reference limit or by TSH ≥10.0 μIU/mL regardless of the free T4 level. Overt thyrotoxicosis was defined as a TSH level below the lower reference limit with a free T4 level above the upper reference limit. Subclinical hypothyroidism was defined as normal free T4 levels with elevated TSH levels above the upper reference limit and <10 μIU/mL, and subclinical thyrotoxicosis was defined as normal free T4 levels with reduced TSH levels below the lower reference limit. Isolated hypo- and hyperthyroxinemia were defined as normal TSH levels with free T4 levels below or above the reference interval, respectively [13, 14]. Normal TSH and free T4 levels were determined based on the reference ranges of each hospital.

Blood Sample Collection and Measurement of Serum Cytokines

Blood samples were obtained immediately before the first administration of Ate/Bev. The blood was centrifuged at 1,000 g for 5 min to isolate the serum, which was stored at −80°C. Serum cytokine levels were analyzed using a cytometric bead array (560484, BD Biosciences), according to the manufacturer's instructions.

Table 1. Baseline clinical characteristics

	All patients (n = 208)	Thyroid AE (n = 41)	No thyroid AE (n = 167)	p value
Age, median (IQR), years	61.0 (54.3–68.0)	62.0 (54.0–67.5)	61.0 (54.0–68.0)	0.822
Male sex, n (%)	179 (86.1)	33 (80.5)	146 (87.4)	0.251
ECOG performance status, n (%)				
0	97 (46.6)	19 (46.3)	78 (46.7)	0.967
1	111 (53.4)	22 (53.7)	89 (53.3)	
Child-Pugh classification, n (%)				
A	169 (81.3)	35 (85.4)	134 (80.2)	0.451
B	39 (18.8)	6 (14.6)	33 (19.8)	
Barcelona clinical liver cancer stage, n (%)				
B	38 (18.3)	9 (22.0)	29 (17.4)	0.496
C	170 (81.7)	32 (78.0)	138 (82.6)	
AFP ≥400 ng/mL, n (%)	72 (34.6)	9 (22.0)	63 (37.7)	0.057
NLR, median (IQR)	2.6 (1.7–4.2)	2.1 (1.6–3.5)	2.8 (1.8–4.2)	**0.040**
Presence of MVI, n (%)	84 (40.4)	17 (41.5)	67 (40.1)	0.875
Presence of extrahepatic spread, n (%)	127 (61.1)	23 (56.1)	104 (62.3)	0.467
Etiology of HCC, n (%)				
Hepatitis B	143 (68.8)	28 (68.3)	115 (68.9)	0.663
Hepatitis C	13 (6.3)	1 (2.4)	12 (7.2)	
Alcohol	25 (12.0)	6 (14.6)	19 (11.4)	
Other or unknown	27 (13.0)	6 (14.6)	21 (12.6)	
Prior local therapy for HCC, n (%)	145 (69.7)	32 (78.0)	113 (67.7)	0.195

Significant p values in bold. AE, adverse event; IQR, interquartile range; ECOG, Eastern Cooperative Oncology Group; HCC, hepatocellular carcinoma; AFP, alpha-fetoprotein; MVI, macrovascular invasion.

T-Cell Proliferation and Cytokine Production Assay

Peripheral blood mononuclear cells were isolated using the density gradient centrifugation with Ficoll-Paque PLUS (GE Healthcare). T-cell proliferation and cytokine production were analyzed as previously described [15, 16].

Flow Cytometry Analysis

Flow cytometry analysis was performed as previously described [16, 17]. Briefly, single-cell suspensions were stained with the following fluorochrome-conjugated antibodies: anti-human CD3 (clone SK7, eBioscience), anti-human CD8 (clone RPA-T8, eBioscience), anti-human PD-1 (clone EH12.2H7, BioLegend), anti-human TIM-3 (clone F38-2E2, BioLegend), and anti-human TIGIT (clone A15153G,. BioLegend). For intracellular staining, cells were fixed and permeabilized using a FoxP3 staining buffer kit (Thermo Fisher Scientific) and stained with the following antibodies: anti-human Ki67 (clone Ki67, BioLegend), anti-human interferon-γ antibody (clone B27, BD Bioscience), and anti-human tumor necrosis factor (TNF)-α antibody (clone Mab11, BD Bioscience). All flow data were acquired using a CytoFLEX flow cytometer (Beckman Coulter) and analyzed using FlowJo software (Tree Star Inc.).

Statistical Analysis

Categorical variables were compared using either Pearson's χ^2 test or Fisher's exact test. For continuous variables, the Kolmogorov-Smirnov test was used to test the normality of data, and either the independent t test or Wilcoxon-Mann-Whitney test was used for comparisons. Survival analysis was performed using the Kaplan-Meier method, and the subgroups were compared using log-rank statistics. Cox proportional hazards regression was used to perform univariate and multivariate analyses of the survival outcomes. In time-dependent Cox regression model, the development of thyroid AEs was used as a time-dependent exposure variable. All statistical analyses were performed using SPSS Statistics software version 26 (IBM, Armonk, NY, USA). Statistical significance was defined as two-sided p values of <0.05.

Results

Patient Characteristics and Treatment Outcomes

The baseline patient characteristics are presented in Table 1. The median patient age was 61.0 (interquartile range [IQR]: 54.3–68.0) years, and 86.1% of the patients were male. Most patients had Child-Pugh class A (81.3%) and Barcelona clinical liver cancer stage C (81.7%). Hepatitis B (68.8%) was the most common cause of HCC. Most patients (69.7%) had received at least one prior local therapy for HCC. The median follow-up duration was 11.0 (IQR, 8.2–15.9) months. The objective response rate (ORR) was 31.3% (95% confidence interval

Table 2. Characteristics of thyroid AEs

Type of thyroid dysfunction, *n* (%)	
Hypothyroidism	36 (17.3)
Overt hypothyroidism	29 (13.9)
Subclinical hypothyroidism	7 (3.4)
Thyrotoxicosis	12 (5.8)
Overt thyrotoxicosis	6 (2.9)
Subclinical thyrotoxicosis	6 (2.9)
Isolated hypothyroxinemia	3 (1.4)
Isolated hyperthyroxinemia	0 (0.0)
Time to onset of thyroid dysfunction, median (IQR), months	
Hypothyroidism	3.5 (2.6–6.0)
Thyrotoxicosis	1.3 (1.2–2.7)
Isolated hypothyroxinemia	3.1 (2.2–5.0)
Duration of thyroid dysfunction, median (IQR), months	
Hypothyroidism	8.4 (4.0–14.4)
Thyrotoxicosis	2.4 (1.8–4.2)
Isolated hypothyroxinemia	10.7 (10.1–12.7)
Resolution of thyroid dysfunction, *n* (%)	
Hypothyroidism	4/36 (11.1)
Thyrotoxicosis	12/12 (100.0)
Isolated hypothyroxinemia	1/3 (33.3)
CTCAE grade, *n* (%)	
Grade 1	25 (49.0)
Grade 2	26 (51.0)

IQR, interquartile range; CTCAE, Common Terminology Criteria for Adverse Events.

[CI], 25.0–37.6%), and the median progression-free survival (PFS) and overall survival (OS) were 7.6 (95% CI, 6.0–9.3) and 17.2 (95% CI, 14.9–19.5) months, respectively.

Characteristics of Thyroid AEs after Ate/Bev in HCC

The Ate/Bev-associated thyroid AEs occurred in 41 (19.7%) patients during the study period, and the median time to onset of thyroid dysfunction was 2.7 (IQR, 1.3–4.5) months. Primary hypothyroidism was the most common thyroid AE, affecting 36 (17.3%) patients (Table 2). Overt and subclinical hypothyroidism occurred in 29 (13.9%) and seven (3.4%) patients, respectively. Primary thyrotoxicosis occurred in 12 (5.8%) patients, six of whom were overt, and six were subclinical. There were three (1.4%) patients with isolated hypothyroxinemia and no patients with isolated hyperthyroxinemia. The median time to onset of hypothyroidism and thyrotoxicosis after Ate/Bev treatment was 3.5 (IQR, 2.6–6.0) and 1.3 (IQR, 1.2–2.7) months, respectively (Table 2). In addition, the median duration of hypothyroidism and thyrotoxicosis after Ate/Bev treatment was 8.4 (IQR, 4.0–14.4) and 2.4 (IQR, 1.8–4.2) months, respectively. Of the 36 patients who experienced hypothyroidism, four (11.1%) recovered to normal thyroid function, while the remaining patients had permanent hypothyroidism. In accordance with clinical practice guidelines [18, 19], thyroid hormone supplementation was prescribed for patients with permanent hypothyroidism who had symptoms or a sustained TSH level over 10 μIU/mL. None of the patients who experienced thyrotoxicosis continued to have thyrotoxicosis or required antithyroid drug treatment during the follow-up period. While 3/12 (25.0%) patients recovered to normal thyroid function, 9/12 (75.0%) patients progressed to hypofunction of the thyroid (seven overt hypothyroidism, one subclinical hypothyroidism, and one isolated hypothyroxinemia). All cases were classified as grade 1 or 2 by CTCAE 4.0 criteria. Table 1 shows the baseline characteristics according to the thyroid AEs. Notably, the baseline neutrophil-lymphocyte ratio (NLR) was significantly lower in patients with thyroid AEs than in those without thyroid AEs. In addition, among the Ate/Bev treatment-related AEs, dermatological AEs and adrenal insufficiency were significantly more common in patients with thyroid AEs than in those without thyroid AEs (online suppl. Table 1).

Association of Thyroid AEs with Favorable Clinical Outcomes of Ate/Bev in HCC

We examined whether the development of thyroid AEs was associated with the clinical outcomes of the Ate/Bev treatment. Patients with thyroid AEs had higher ORR compared to those without thyroid AEs (58.5 vs. 24.6%, respectively, $p < 0.001$; Fig. 1a). Moreover, PFS and OS were significantly better in patients with thyroid AEs than in those without thyroid AEs (median PFS, 21.0 vs. 6.3 months, respectively, $p < 0.001$; median OS, not reached vs. 15.3 months, respectively, $p = 0.001$; Fig. 1b, c). Furthermore, among irAEs, thyroid AEs had the greatest impact on both DFS and OS (online suppl. Table 2). Because the incidence of thyroid dysfunction increased with longer duration of Ate/Bev treatment and survival of patients, we further performed a time-dependent analysis using a time-dependent Cox regression model in which the development of thyroid AEs was used as a time-dependent variable. Even in this analysis, PFS and OS were consistently longer in patients with thyroid AEs compared with those without thyroid AEs ($p = 0.024$ and $p = 0.027$, respectively; online suppl. Fig. 2a, b).

To minimize false-negatives from patients with short-term Ate/Bev exposure, we excluded patients with progressive disease at the first response evaluation and performed a subgroup analysis in the remaining patients. Consistently, patients with thyroid AEs showed better PFS and OS than those without thyroid AEs ($p < 0.001$ and $p = 0.035$, respectively; online suppl. Fig. 2c, d).

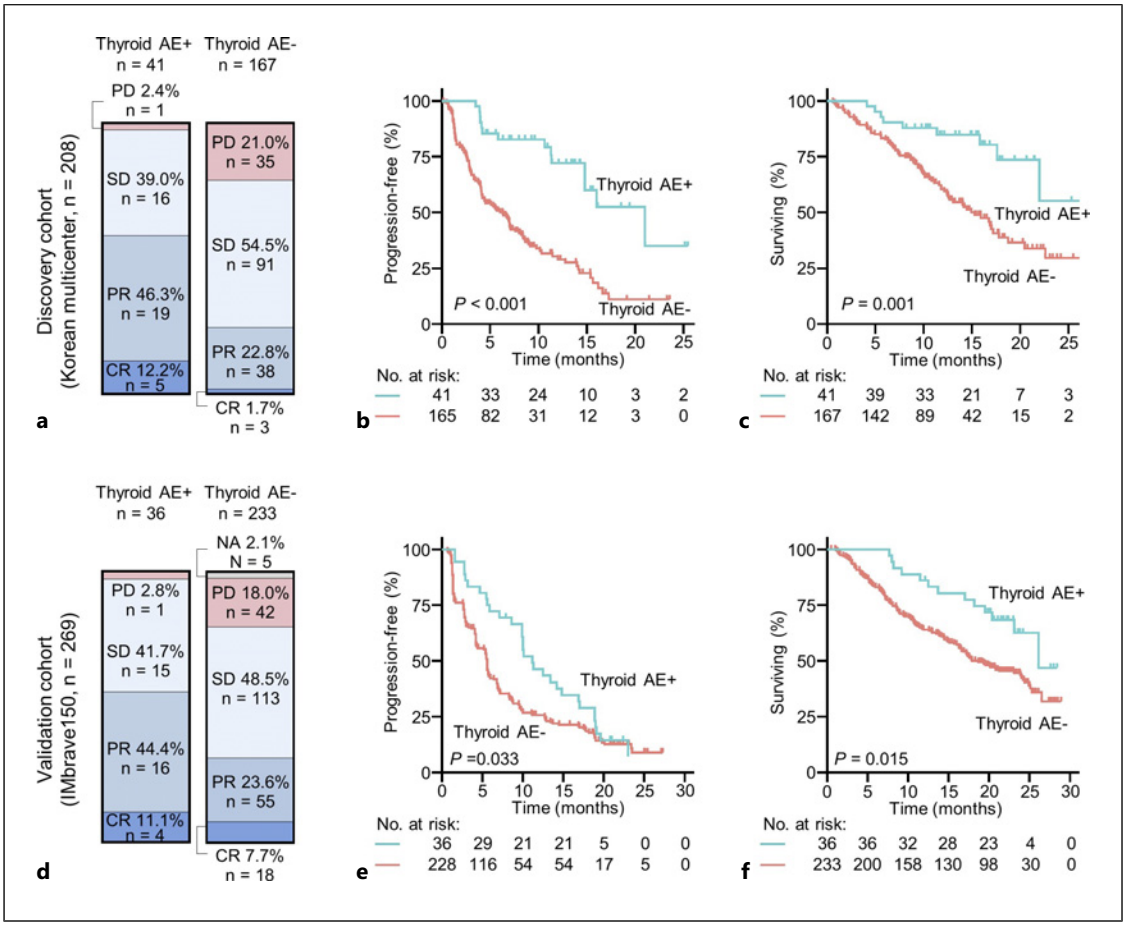


Fig. 1. Best response to therapy, PFS, and OS according to thyroid AEs. **a–c** Discovery cohort. **d–f** Validation cohort. AE, adverse event; PD, progressive disease; SD, stable disease; PR, partial response; CR, complete response.

On categorizing the patients into overt and subclinical thyroid AE groups, patients in both the overt and subclinical thyroid dysfunction groups were strongly associated with better PFS than those without thyroid AEs (online suppl. Fig. 2e). However, OS was significantly better in patients in the overt thyroid dysfunction group than in those without thyroid AEs, but not in patients in the subclinical thyroid dysfunction group (online suppl. Fig. 2f). In particular, the median OS in the overt thyroid dysfunction group was significantly longer than that in the subclinical thyroid dysfunction group ($p = 0.005$). In addition, when patients with thyroid AEs were categorized into early- and late-onset groups according to the median time to onset of thyroid dysfunction, they were strongly associated with better PFS and OS than patients without thyroid AEs (online suppl. Fig. 2g, h). There was no difference in PFS and OS between the early- and late-onset groups.

Because various factors could confound the association of thyroid AEs with a favorable prognosis of HCC, we further validated the clinical implications using the multivariate Cox proportional hazard model. Even after adjusting for age, sex, ECOG performance status, Child-Pugh score, alpha-fetoprotein level, macroscopic vascular invasion, extrahepatic spread, etiology of HCC, and NLR, the development of thyroid AEs during Ate/Bev treatment remained the most significant factor associated with better PFS and OS (hazard ratios, 0.3 [95% CI, 0.1–0.4; $p < 0.001$] and 0.3 [95% CI, 0.1–0.5; $p < 0.001$], respectively; Figure 2a, b).

Validation of Thyroid AEs with Favorable Clinical Outcomes of Ate/Bev in HCC

To externally validate our finding, we analyzed clinical outcomes according to thyroid AEs in patients treated with Ate/Bev in the IMbrave150 study. Of a total of 336

patients, 269 were included in the analysis, excluding 64 with data sharing prohibited due to consent, legal, regulatory, or contractual constraints and three with thyroid dysfunction before initiation of treatment or related to other medical conditions. The baseline patient characteristics of the IMbrave150 study cohort are presented in online supplementary Table 3. In the IMbrave150 study cohort, 13.4% (36/269) of patients had thyroid AEs following Ate/Bev treatment, of whom 26 were hypothyroidism and 10 were hyperthyroidism. Patients with thyroid AEs had higher ORR than those without thyroid AEs (55.6 vs. 31.3%, $p = 0.004$, Fig. 1d). Moreover, patients with thyroid AEs exhibited better PFS and OS than those without thyroid AEs ($p = 0.033$ and 0.015, respectively, Fig. 1e, f). Therefore, we confirmed the association between thyroid AEs and favorable clinical outcomes of Ate/Bev in our multicenter cohort and the IMbrave150 study cohort.

Association of the Development of Thyrotoxicosis with Baseline IL-6 Levels

As ICI-induced immune responses may mediate thyroid AEs, we compared circulating cytokine levels and early T-cell responses in patients with and without thyroid AEs. When peripheral blood cytokine levels at baseline were compared, baseline IL-6 levels were significantly lower in patients with thyrotoxicosis. However, there were no significant differences in the levels of IL-2, IL-4, IL-10, IL-17A, TNF-α, and IFN-γ (Fig. 3a, b). Next, we compared the proportion of $CD8^+$ T cells and their effector cytokine production. At baseline, the fractions of $PD\text{-}1^+CD8^+$ T cells, $TIM3^+PD1^+CD8^+$ T cells, and $TIGIT^+PD1^+CD8^+$ T cells did not differ between the two groups (Fig. 3c). Moreover, the production of effector cytokines (TNF-α and IFN-γ) in $CD8^+$ T cells was comparable between the two groups (Fig. 3d). However, patients with hypothyroidism did not show significant differences in circulating cytokine levels and T-cell subsets (online suppl. Fig. 3a–c). Overall, the development of thyrotoxicosis was significantly associated with peripheral IL-6 levels at baseline, but not with other cytokines and T-cell subsets.

Discussion

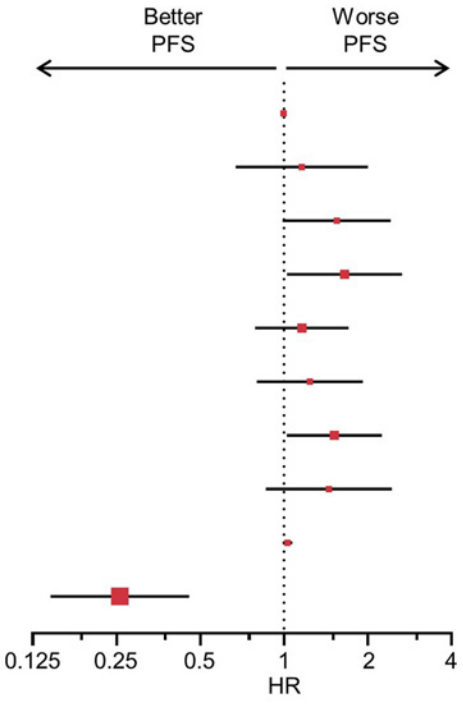
In this study, we found that thyroid AEs occurred in 19.7% of patients (17.3% with hypothyroidism and 5.8% with thyrotoxicosis) after Ate/Bev treatment, with a median follow-up of 11.0 months. Patients with thyroid AEs exhibited favorable clinical outcomes with Ate/Bev treatment compared with those without thyroid AEs. Through the analysis of circulating cytokine levels and early T-cell responses in patients with and without thyroid AEs, we demonstrated that patients with thyrotoxicosis showed a significantly lower level of baseline IL-6.

In a systematic review and meta-analysis of patients treated with cytotoxic T-lymphocyte-associated protein-4 inhibitors (ipilimumab), PD-1 inhibitors (nivolumab or pembrolizumab), or PD-L1 inhibitors (atezolizumab), the incidence of hypothyroidism was 3.9%, 7.0%, and 3.9%, and that of thyrotoxicosis was 1.7%, 3.2%, and 0.6%, respectively. Although the specific tumor type was not significantly associated with the incidence of hypothyroidism or thyrotoxicosis, patients treated with combination immunotherapy were significantly more likely to experience thyroid dysfunction [11]. In the IMbrave150 trial with a median follow-up of 8.9 months, 10.9% and 4.6% of patients treated with Ate/Bev experienced hypothyroidism and thyrotoxicosis, respectively [12], and this incidence was higher than atezolizumab alone in a previous meta-analysis. In the present study, the incidence of thyroid dysfunction, especially hypothyroidism, was higher than that reported in the IMbrave150 trial. A possible explanation is that we included patients with subclinical thyroid dysfunction and had a longer median follow-up duration.

The median time to onset of hypo- and hyperthyroidism after Ate/Bev treatment was 3.5 and 1.3 months, respectively. This finding is consistent with that reported by Ikeda et al. in the IMbrave150 analysis (3.5 months of hypothyroidism and 2.7 months of hyperthyroidism) [12]. These results indicate that thyroid AEs are relatively early events after Ate/Bev treatment rather than a consequence of chronic exposure to Ate/Bev treatment. The clinical course of thyroid AEs in the present study was consistent with that of previous ICI treatments for other solid tumors [14, 20, 21]. Most patients with thyroid AEs progressed to hypothyroidism with or without a prior hyperthyroidism period.

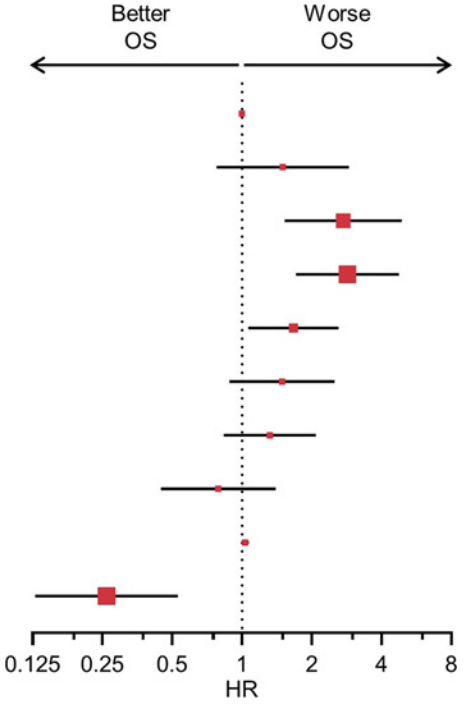
Patients with thyroid AEs demonstrated significantly better clinical outcomes in terms of PFS, OS, and ORR than those without thyroid AEs. These findings were consistent even after adjusting for confounding factors, such as age, sex, ECOG performance status, Child-Pugh class, alpha-fetoprotein level, macroscopic vascular invasion, extrahepatic spread, etiology of HCC, and NLR. Furthermore, favorable survival outcomes in patients with thyroid AEs were also externally validated in a cohort of IMbrave150 patients treated with Ate/Bev. In a further evaluation conducted after excluding patients with progressive disease to minimize short-term exposure to Ate/Bev, thyroid AEs still showed favorable survival outcomes, indicating that this subset of patients received significant clinical benefit from Ate/Bev. Furthermore, both overt and subclinical

Variable		Multivariate HR (95% CI)	*P*
Age		0.995 (0.976, 1.015)	0.630
Sex	Male vs. Female	1.158 (0.671, 2.001)	0.598
ECOG	1 vs. 0	1.546 (0.988, 2.418)	0.056
Child-Pugh class	B vs. A	1.649 (1.025, 2.653)	0.039
AFP	≥400 vs. <400	1.159 (0.787, 1.706)	0.455
PVT	Yes vs. No	1.238 (0.798, 1.920)	0.342
Extra-hepatic	Yes vs. No	1.518 (1.024, 2.250)	0.038
Etiology	Viral vs. Non-viral	1.449 (0.860, 2.441)	0.164
NLR		1.030 (0.991, 1.071)	0.134
Thyroid AE	Yes vs. No	0.257 (0.145, 0.456)	< 0.001



a

Variable		Multivariate HR (95% CI)	*P*
Age		0.997 (0.974, 1.020)	0.786
Sex	Male vs. Female	1.497 (0.777, 2.886)	0.228
ECOG	1 vs. 0	2.730 (1.526, 4.883)	0.001
Child-Pugh class	B vs. A	2.847 (1.708, 4.746)	< 0.001
AFP	≥400 vs. <400	1.662 (1.062, 2.602)	0.026
PVT	Yes vs. No	1.485 (0.881, 2.503)	0.137
Extra-hepatic	Yes vs. No	1.315 (0.832, 2.078)	0.242
Etiology	Viral vs. Non-viral	0.789 (0.446, 1.395)	0.415
NLR		1.031 (0.995, 1.068)	0.089
Thyroid AE	Yes vs. No	0.260 (0.128, 0.528)	< 0.001



b

Fig. 2. Multivariable analysis of PFS and OS. Forest plots showing multivariate HRs and 95% CIs for PFS (**a**) and OS (**b**). HR, hazard ratio; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group performance status; AFP, alpha-fetoprotein; MVI, macrovascular invasion; NLR, neutrophil-lymphocyte ratio; AE, adverse event; PFS, progression-free survival; OS, overall survival.

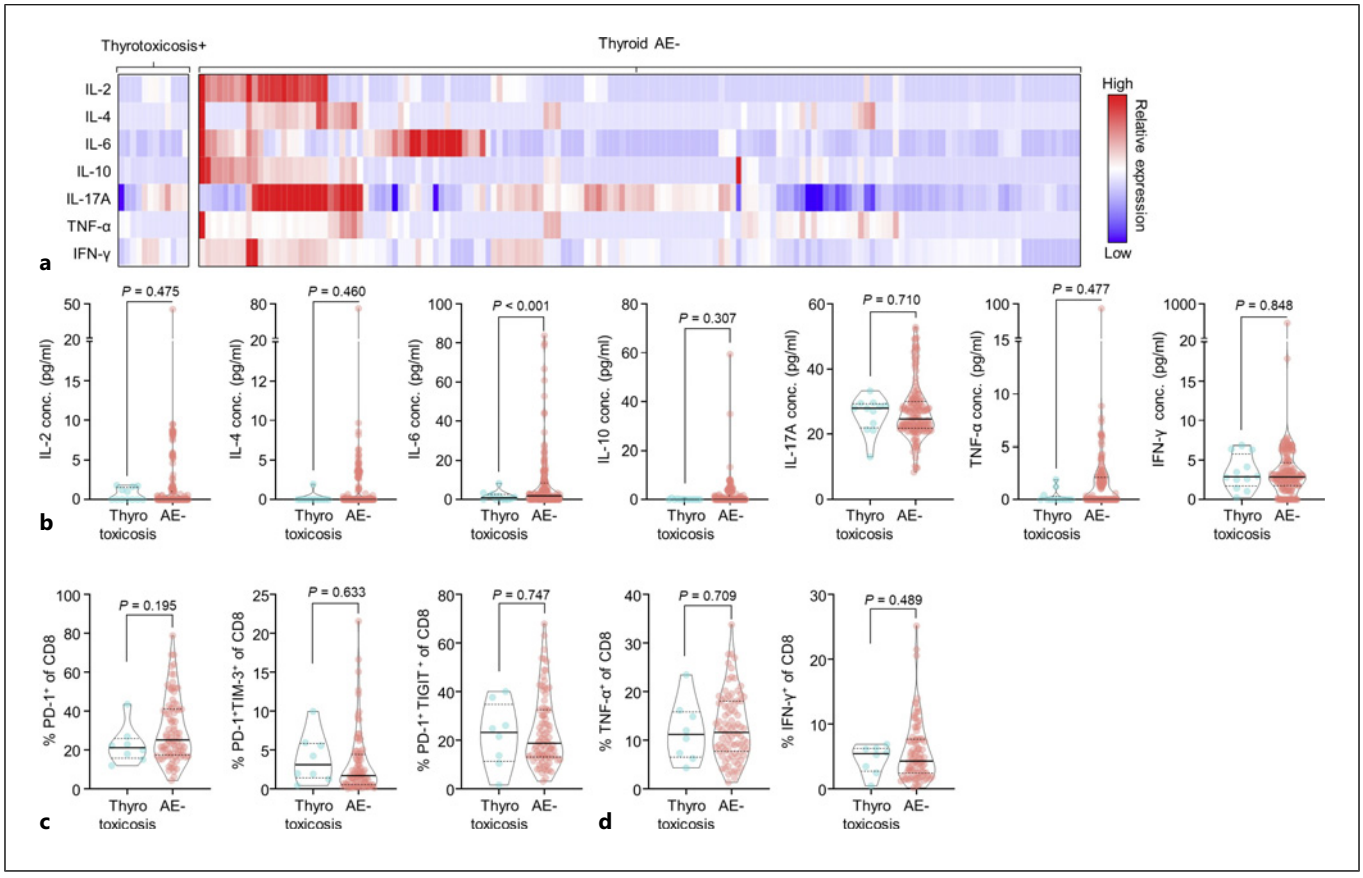


Fig. 3. Comparison of baseline cytokine levels and T-cell fractions between patients with thyrotoxicosis and those without thyroid AEs. Baseline cytokine levels (**a**, **b**), $CD8^+$ cell subset fractions (**c**), and effector cytokines in $CD8^+$ T cells (**d**).

thyroid dysfunction were associated with prolonged PFS; however, only overt thyroid dysfunction was confirmed as a prognostic factor for OS. This result was consistent with a previous study on thyroid AEs after nivolumab treatment, which found that better OS was only associated with overt thyroid dysfunction and not subclinical thyroid dysfunction [22]. Meanwhile, the survival outcomes of patients with early- and late-onset thyroid AEs were comparable. This indicates that the favorable prognostic impact of thyroid AEs was maintained regardless of the time of onset.

Many studies have reported the association between thyroid irAEs and improved outcomes in other cancer types [10, 23, 24]. A recent meta-analysis showed that thyroid irAE development during ICI therapy was associated with improved PFS and OS (hazard ratios, 0.58 and 0.52, respectively) [24]. However, the majority of the study population consisted of patients with non-small cell lung cancer, and the vast majority of patients received PD-1 inhibitors, making it difficult to extrapolate the study's results to the broader cancer population treated with any ICIs. Therefore, our study stands out from previous studies as it involves the concurrent administration of bevacizumab, an angiogenesis inhibitor, along with ICI. Furthermore, our study has the strength of being the first to report the correlation between thyroid irAEs and Ate/Bev treatment in patients with HCC.

Notably, this immunological side effect of immunotherapy is closely related to the antitumor mechanism. In this study, patients with thyroid AEs had a lower NLR and a higher incidence of dermatological AEs and adrenal insufficiency, suggesting that T-lymphocyte activation might be involved in the occurrence of thyroid AEs and a favorable response to immunotherapy. Thus, we attempted to elucidate the association between thyroid AEs, T-cell immunity, and various cytokines using multiplex flow cytometric analyses. We were able to identify that patients with thyrotoxicosis had a lower level of baseline IL-6. This is

consistent with our previous finding that high baseline IL-6 levels can be associated with reduced clinical outcomes and impaired T-cell function in patients with unresectable HCC after Ate/Bev treatment [25].

We found a favorable survival outcome in patients with thyroid AEs through univariate and multivariate analyses in a large multicenter cohort and IMbrave150 validation cohort. Therefore, thyroid AEs after Ate/Bev treatment may provide a clue for favorable efficacy as an early on-treatment pharmacodynamic marker.

However, this study had some limitations. As Ate/Bev treatment is a combination therapy of anti-PD-L1 and anti-vascular endothelial growth factor, bevacizumab may affect the development of thyroid AEs through nonimmune mechanisms. However, we were unable to separately evaluate the effects of Ate/Bev on thyroid dysfunction. Therefore, further research is required to elucidate the precise mechanisms underlying Ate/Bev-induced thyroid dysfunction. In addition, although we elucidated the association between thyrotoxicosis and low level of IL-6, we were unable to identify the association between specific immunological phenotypes and hypothyroidism. In the present study, we only examined the immune profiles at baseline, but long-term serial monitoring of peripheral blood cytokines may be required to further elucidate immunologic changes that precede or accompany thyroid dysfunctions during Ate/Bev treatment. Moreover, there is a possibility that the background liver function might affect the baseline immune profile, but the significance was unclear because of the limited number of enrolled patients.

This study demonstrated that a fraction of patients with HCC experienced thyroid dysfunction relatively early after Ate/Bev treatment. Patients with thyroid AEs during Ate/Bev treatment demonstrated favorable clinical outcomes compared with those without thyroid AEs. Therefore, thyroid AEs may provide early clinical clues for treatment outcomes in patients with unresectable HCC treated with Ate/Bev.

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Statement of Ethics

This study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and was approved by the participating hospitals' Institutional Review Boards (CHA Bundang Medical Center, CHA-2017-11-052, CHA-2017-11-054; Ulsan University Hospital, 2020-12-006; Haeundae Paik Hospital, 2020-12-019-001). Written informed consent was obtained from all the patients.

Conflict of Interest Statement

Hong Jae Chon has a consulting or advisory role at Eisai, Roche, Bayer, ONO, MSD, BMS, Celgene, Sanofi, Servier, AstraZeneca, SillaJen, Menarini, and GreenCross Cell and has received research grants from Roche, Dong-A ST, and Boryung Pharmaceuticals. Chan Kim has a consulting or advisory role at Roche, ONO, MSD, BMS, Oncocross, and Virocure and has received research grants from Boryung Pharmaceuticals, Oncocross, SillaJen, and Virocure. Ho Yeong Lim has a consulting or advisory role at Eisai, Roche, Bayer, ONO, MSD, BMS, and AstraZeneca. Vincent E. Gaillard is a full-time employee of F. Hoffmann-La Roche Ltd. The other authors have no potential conflicts of interest to disclose.

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Author Contributions

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Data Availability Statement

Data are not publicly available due to ethical reasons. Further inquiries can be directed to the corresponding author.

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