Safety evaluation of lenvatinib treatment after atezolizumab plus bevacizumab therapy for patients with unresectable liver cancer: A comparison of lenvatinib as 1st- or 2nd-line treatment

MICHIO KIMURA[1], SHIORI YAMADA[1], MAKIKO GO[1], SATOSHI YASUDA[2], HIDENORI TOYODA[2] and EISEKI USAMI[1]

[1]Department of Pharmacy, Ogaki Municipal Hospital, Ogaki, Gifu 503-8502, Japan; [2]Department of Gastroenterology and Hepatology, Ogaki Municipal Hospital, Ogaki, Gifu 503-8502, Japan

Received February 20, 2024; Accepted May 7, 2024

DOI: 10.3892/ol.2024.14464

Abstract. Atezolizumab plus bevacizumab (Atez/BV) as first-line therapy and lenvatinib (LEN) as second-line therapy are the recommended treatments for patients with unresectable hepatocellular carcinoma. Adverse immune events caused by immune checkpoint inhibitors (such as Atez) generally only occur several months after administration; therefore, the potential influence of the first-line treatment on second-line treatment is not clear. The present study investigated the safety of second-line LEN treatment (2nd LEN) by comparing the adverse events (AEs) of 2nd LEN after first-line Atez/BV treatment for unresectable liver cancer, with those of first-line LEN treatment (1st LEN). Patients who received Atez/BV as first-line therapy and 2nd LEN, or those who received 1st LEN at Ogaki Municipal Hospital (Ogaki, Japan) between April 2018 and September 2023 were retrospectively evaluated for treatment duration and AEs. The median treatment duration for patients in the 1st LEN (n=39) and 2nd LEN (n=13) groups was 151.0 days [95% confidence interval (CI) 77-303 days] and 128.5 days (95% CI 68-270 days), respectively (P=0.385). A greater proportion of patients showed elevated aspartate aminotransferase/alanine aminotransferase levels in the 2nd LEN group (76.9%) compared with those in the 1st LEN group (46.2%) (P=0.016). Hypothyroidism was more common in those receiving 2nd LEN (46.2%) than 1st LEN (12.8%) (P=0.016). In addition, grade 1 (three patients) and grade 2 (three patients) hypothyroidism was observed in patients receiving 2nd LEN. For these six patients, during first-line Atez/BV treatment, four patients had grade 0 hypothyroidism and two patients had grade 1 hypothyroidism (P=0.025). In conclusion, patients receiving 2nd LEN after treatment with Atez/BV are at an increased risk of hypothyroidism.

Introduction

The combination of atezolizumab (Atez), a humanized immunoglobulin G1 monoclonal antibody targeting programmed death-ligand 1 (PD-L1), and bevacizumab (BV), a monoclonal antibody that targets vascular endothelial growth factor (VEGF) and inhibits angiogenesis and tumor growth, is the recommended first-line treatment for patients with unresectable hepatocellular carcinoma (HCC) with Child-Pugh A (CP-A) liver function (1-4). Lenvatinib (LEN), a multitarget tyrosine kinase inhibitor, is often used as second-line treatment. LEN targets vascular endothelial growth factor receptors 1, 2, and 3, fibroblast growth factor (FGF) receptors 1 through 4, platelet-derived growth factor receptor α, rearranged during transfection, and stem cell factor receptor. Moreover, LEN also inhibits the formation of vessel-like luminal structures by vascular endothelial cells induced by VEGF and FGF.

Atez is an immune checkpoint inhibitor (ICI) that binds to cell surface PD-L1 and suppresses its function. ICIs produce unique side effects of immune-related adverse events in various organs. These include interstitial lung disease, colitis, hypothyroidism, liver damage, skin rash, vitiligo, hypophysitis, type I diabetes, renal dysfunction, myasthenia gravis, peripheral neuropathy, myositis, and uveitis. Adverse immune events generally occur several months after drug administration; however, the timing of their appearance varies widely. For example, regarding Atez/BV, the onset of thyroid dysfunction was reported to be 93.5 (range 13-419) days in the IMbrave150 study (1). Therefore, the possibility of the influence of secondary treatment on first-line treatment cannot be

Correspondence to: Mr. Michio Kimura, Department of Pharmacy, Ogaki Municipal Hospital, 4-86 Minaminokawa-cho, Ogaki, Gifu 503-8502, Japan
E-mail: kimkim0305nao@yahoo.co.jp

Abbreviations: 1st LEN, first-line LEN treatment; 2nd LEN, second-line LEN treatment; AE, adverse event; AST/ALT, aspartate aminotransferase/alanine aminotransferase; Atez/BV, atezolizumab plus bevacizumab; CI, confidence interval; CP-A, Child-Pugh A; FGF, fibroblast growth factor; HCC, hepatocellular carcinoma; ICI, immune checkpoint inhibitor; LEN, lenvatinib; PD-L1, programmed death-ligand 1; RDI, relative dose intensity; VEGF, vascular endothelial growth factor

Key words: hypothyroidism, AE, duration of treatment, HCC, LEN, Atez/BV

ruled out. When LEN is selected as a second-line treatment in patients with CP-A liver function, it is important to maintain its safety and continue treatment.

LEN is a standard therapeutic agent for HCC, but the high incidence of adverse events (AEs) is a problematic aspect of LEN treatment (5-8), as these AEs may necessitate treatment discontinuation. The most common any-grade AEs of LEN were hypertension (42%), diarrhea (39%), decreased appetite (34%), decreased weight (31%), and fatigue (30%) during first-line LEN treatment (1st LEN) (6). The incidence of hypothyroidism was 16.4% (6). Regarding the safety of using LEN as a second-line treatment (2nd LEN), Yoo *et al* (9), Aoki *et al* (10), and Hiraoka *et al* (11) reported significant antitumor efficacy with acceptable safety. However, they did not compare the AEs of LEN when used as first- or second-line treatment. Comparing the frequency and appearance of AEs in first- and second-line treatments, will contribute to managing AEs in the 2nd-line treatment.

In this study, we investigated the safety of LEN in second-line therapy by comparing the AEs of LEN in second-line therapy after first-line Atez/BV treatment for unresectable liver cancer, with those of LEN in first-line therapy.

Patients and methods

Patients and evaluations. A total of 53 patients with CP-A unresectable liver cancer treated at the Ogaki Municipal Hospital between April 2018 and September 2023 were retrospectively evaluated. Patients who received Atez/BV as first-line therapy and LEN as second-line therapy or those who received LEN as first-line therapy were included. Patient characteristics, treatment duration, AEs, and relative dose intensity (RDI) were analyzed. Data were analyzed using electronic and pharmacy service records. AEs were evaluated according to the Common Terminology Criteria for Adverse Events, version 5.0 (12), and the most severe grades during chemotherapy were reported. Personal information was protected in the aggregated data. This study was approved by the Institutional Review Board of the Ogaki Municipal Hospital (Ogaki, Japan; approval number: 20220728-17-h). The need for informed consent was waived due to the retrospective nature of the study.

Treatment protocol. Atez was administered intravenously at 1,200 mg for 60 min on the first day. If the first dose was well-tolerated, the duration of the second infusion was shortened to 30 min. BV was administered intravenously at a dose of 15 mg/kg for 90 min on the first day. If no problems were encountered, the durations of the second and third infusions were shortened to 60 and 30 min, respectively. This procedure was repeated every 21 days.

The LEN dose was based on body weight; the initial dose was 12 mg/day for those weighing ≥60 kg and 8 mg/day for those weighing <60 kg. During the 28-day cycle, dose adjustment, including reduction to 8 or 4 mg/day, 4 mg every other day, or interruption, was allowed for LEN treatment based on AEs (6,13). In patients who experienced unacceptable drug-related AEs, the LEN dose was reduced or treatment was interrupted according to the manufacturer's instructions. Dose reduction or temporary interruption of LEN was maintained until the AE severity dropped to grade 1 or 2. In cases where dose reduction was maintained, the reduced doses administered were 20, 14, 10, 8, or 4 mg once daily.

Statistical analysis. To test whether the variances of two populations were equal regarding patient characteristics and RDI the F-test was performed. The Mann-Whitney U test or Fisher's exact probability test was used to compare patient characteristics, AEs, and RDI. The change in AE grade from first line to second line treatment was compared with the Wilcoxon signed-rank test. Kaplan-Meier and log-rank tests were used to compare treatment durations. Differences were considered statistically significant at $P<0.05$. All analyses were performed using EZR software (version 1.30, Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R software (The R Foundation for Statistical Computing, Vienna, Austria) (14).

Results

Patient characteristics. The 1st LEN and 2nd LEN groups comprised 39 and 13 patients, respectively. Patient characteristics are summarized in Table I. The median age of the patients in the 1st LEN and the 2nd LEN group was 77 (range=58-88) years and 73 (range=64-85) years, respectively. Patients differed significantly in terms of sex and history of transcatheter arterial chemoembolization between the 1st LEN and 2nd LEN groups.

Adverse events. The proportion of patients showing an increase in aspartate aminotransferase/alanine aminotransferase (AST/ALT) levels was greater in the 2nd LEN (76.9%) than in the 1st LEN (46.2%) group (P=0.016). Hypothyroidism was more common in the 2nd LEN (46.2%) than in the 1st LEN (12.8%) group (P=0.011). The major AEs for the 1st LEN and 2nd LEN groups are summarized in Table II. No differences were observed in other AEs between 1st LEN and 2nd LEN groups. The major AEs of 1st-line Atez/BV treatment in patients who were able to transition to LEN as 2nd-line treatment are summarized in Table III.

Hypothyroidism was observed in 30.8% of these patients. In comparison, hypothyroidism was seen in 11.1% (4/36) of all patients who received 1st-line Atez/BV, including the patients who were able to transition to LEN as 2nd-line treatment and those who were not. AEs with an incidence of more than 50% were reduced platelet count (76.9%) and increased AST/ALT (61.5%) in 1st-line Atez/BV treatment in patients who were able to transition to LEN as 2nd-line treatment.

Subgroup analysis of adverse events by sex. Table IV shows subgroup analysis of AEs by sex. In 2nd LEN, hypothyroidism was more common in females (75%) than in males (0%). There were no differences by sex in other AEs.

The effects of lenvatinib treatment on the treatment duration. Fig. 1 shows Kaplan-Meier survival curves of the duration of treatment with LEN for all patients. The median treatment durations for patients in the 1st LEN and 2nd LEN groups were 151.0 [95% confidence interval (CI), 77-303] days, and 128.5 (95% CI, 68-270) days, respectively (log-rank test, P=0.385).

Table I. Patient characteristics.

Characteristic	1st LEN	2nd LEN	P-value
Patients, n	39	13	
Age, years			
Median (range)	77 (58-88)	73 (64-85)	0.547[a]
Sex, n			
Male/female	36/3	5/8	<0.005[b,c]
Height, cm			
Median (range)	161 (127-171)	155 (145-169)	0.063[a]
Weight, kg			
Median (range)	60 (40-88)	58 (52-90)	0.726[a]
Body surface area, kg/m^2			
Median (range)	1.66 (1.17-2.01)	1.58 (1.45-2.01)	0.505[a]
Creatinine clearance, ml/min			
Median (range)	61.9 (40.9-134.0)	77.8 (28.4-133.0)	0.369[a]
Cause of hepatocellular carcinoma, n			
Hepatitis B virus	6	4	0.183[b]
Hepatitis C virus	15	2	0.125[b]
Non-B non-C	18	3	0.142[b]
Performance status, n			
0	31	10	0.845[b]
1	7	3	0.685[b]
2	1	0	0.559[b]
Post history of transcatheter arterial chemoembolization, n			
Yes	34	8	0.042[b,c]

[a]Mann-Whitney U test. [b]Fisher's exact probability tests. [c]P<0.05. Atez/BV, atezolizumab/bevacizumab; LEN, lenvatinib. 1st LEN is a group in which lenvatinib was used as the first-line treatment. 2nd LEN is a group in which atezolizumab plus bevacizumab was used as the first-line treatment and lenvatinib was used as the second-line treatment. Anti-cancer agent used in transcatheter arterial chemoembolization is Epirubicin.

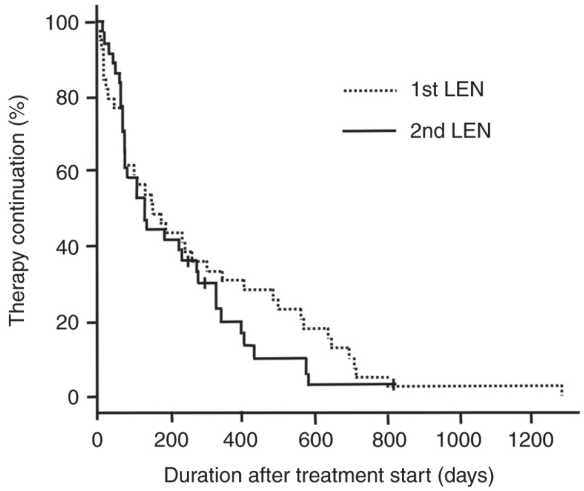

Figure 1. Kaplan-Meier survival curves showing the duration of treatment with lenvatinib for all patients. LEN, lenvatinib.

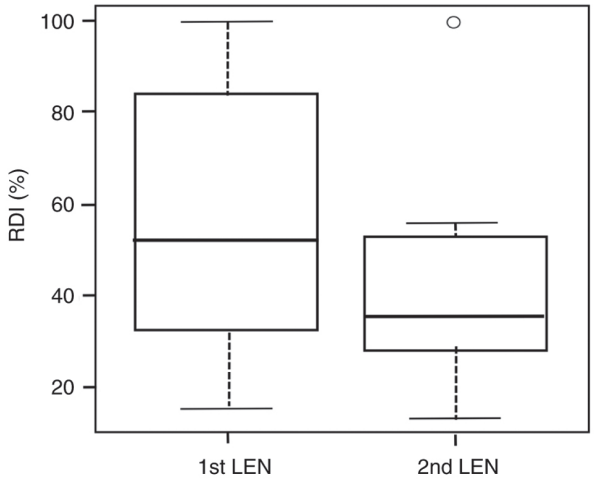

Figure 2. Relative dose intensity of lenvatinib for first-line and second-line treatment. 1st LEN: lenvatinib used as the first-line treatment. 2nd LEN: atezolizumab plus bevacizumab used as first-line treatment, and lenvatinib used as second-line treatment. In the boxplot, the bottom represents the first quartile, the horizontal line in the middle represents the second quartile (median), and the top represents the third quartile. The upper and lower whiskers represent the maximum and minimum values, respectively. Circles indicate outliers. LEN, lenvatinib; RDI, relative dose intensity.

RDI of lenvatinib in the treatment groups. The RDIs of LEN for the 1st LEN and 2nd LEN groups are shown in Fig. 2.

Table II. Adverse events.

	1st LEN (n=39)					2nd LEN (n=13)					
	Grade, n					Grade, n					
Events	1	2	3	4	All grades (%)	1	2	3	4	All grades (%)	P-value
Leucopenia	5	7	1	0	13 (33.3)	2	1	1	0	4 (30.8)	0.864
Neutropenia	2	6	3	0	11 (28.2)	3	1	0	0	4 (30.8)	0.860
Platelet count decreased	17	10	1	0	28 (71.8)	5	3	1	0	9 (69.2)	0.860
Aspartate aminotransferase/alanine aminotransferase increased	17	1	0	0	18 (46.2)	7	0	3	0	10 (76.9)	0.016[a]
Blood bilirubin increased	10	6	2	0	18 (46.2)	2	3	0	0	5 (38.5)	0.629
Anemia	13	3	2	0	18 (46.2)	4	3	0	0	7 (53.8)	0.631
Diarrhea	5	0	1	0	6 (15.4)	3	1	0	0	4 (30.8)	0.244
Nausea	3	2	0	0	5 (12.8)	1	0	0	0	1 (7.7)	0.616
Vomiting	2	0	0	-	2 (5.1)	0	0	0	-	0	0.405
Fatigue	11	4	1	-	16 (41.0)	6	2	0	-	8 (61.5)	0.199
Proteinuria	0	11	4	-	15 (38.5)	2	0	3	-	5 (38.5)	1
Anorexia	11	2	1	0	14 (35.9)	5	0	0	0	5 (38.5)	0.868
Edema in limbs	7	0	0	-	7 (17.9)	5	0	0	-	5 (38.5)	0.128
Hypertension	8	4	2	0	14 (35.9)	1	0	0	0	1 (7.7)	0.052
Hand-foot-syndrome	4	4	0	-	8 (20.5)	4	1	0	-	5 (38.5)	0.196
Hoarseness	1	0	0	-	1 (2.6)	0	0	0	-	0	0.560
Rash	1	0	0	0	1 (2.6)	0	0	0	0	0	0.560
Musculoskeletal and connective tissue disorder	2	0	0	0	2 (5.1)	0	0	0	0	0	0.405
Hypothyroidism	2	3	0	0	5 (12.8)	3	3	0	0	6 (46.2)	0.011[a]
Epistaxis	1	0	0	0	1 (2.6)	0	0	0	0	0	0.560
Oral pain	2	0	0	-	2 (5.1)	0	0	0	-	0	0.405
Dizziness	1	0	0	-	1 (2.6)	0	0	0	-	0	0.560
Abdominal pain	2	0	0	-	2 (5.1)	0	0	0	-	0	0.405

Fisher's exact probability tests. [a]P<0.05. Atez/BV, atezolizumab/bevacizumab; LEN, lenvatinib; CPK, creatine kinase. 1st LEN is a group in which lenvatinib was used as the first-line treatment. 2nd LEN is a group in which atezolizumab plus bevacizumab was used as the first-line treatment and lenvatinib was used as the second-line treatment.

The median RDIs in the 1st LEN and 2nd LEN groups were 52.0 and 35.5%, respectively. There was no significant difference in the median RDI between 1st LEN and 2nd LEN (P=0.081).

Grade change of adverse events from first-line atezolizumab plus bevacizumab to second-line lenvatinib. In patients with hypothyroidism or elevated AST/ALT levels with second-line LEN, changes in the grade of AEs from first-line treatment to second-line treatment are shown in Fig. 3. Grade 1 (three patients) and grade 2 (three patients) hypothyroidism was observed with the 2nd LEN. All six patients with hypothyroidism receiving 2nd LEN were women. In these six patients, during the first-line Atez/BV treatment, the AE grade was 0 in four patients and grade 1 in two patients. There was a significant increase in the hypothyroidism grade from Atez/BV as the first-line treatment to 2nd LEN (P=0.025). Grade 1 (seven patients) and grade 3 (two patients) elevated AST/ALT levels were observed in the 2nd LEN. For these nine patients, when receiving Atez/BV as the first-line treatment the AE grade was 0 in one patient, 1 in seven, and 2 in one. The change in the AE grade for increased AST/ALT levels was not significantly different between first-line Atez/BV and 2nd LEN treatment (P=0.427).

Discussion

In this study, the safety of using LEN as second-line treatment was clarified by comparing the AEs of the 2nd LEN administered after the first-line Atez/BV treatment, with the AEs of 1st LEN in patients with unresectable liver cancer. While 2nd LEN may be as effective as 1st LEN, the incidence of hypothyroidism was higher with 2nd LEN compared to 1st LEN.

In this study, when comparing 1st LEN and 2nd LEN, elevated AST/ALT levels and hypothyroidism were more

Table III. Adverse events of atezolizumab plus bevacizumab as 1st-line.

Events	Grade, n				All grades (%)
	1	2	3	4	
Leucopenia	2	1	1	0	4 (30.8)
Neutropenia	3	1	0	0	4 (30.8)
Platelet count decreased	5	4	1	0	10 (76.9)
Aspartate aminotransferase/alanine aminotransferase increased	5	0	3	0	8 (61.5)
Blood bilirubin increased	2	4	0	0	6 (46.2)
Anemia	4	2	0	0	6 (46.2)
Diarrhea	3	1	0	0	4 (30.8)
Nausea	1	0	0	0	1 (7.7)
Fatigue	6	1	0	-	7 (53.8)
Proteinuria	2	1	1	-	4 (30.8)
Anorexia	6	0	0	0	6 (46.2)
Edema in limbs	5	0	0	-	5 (38.5)
Hypertension	2	0	0	0	2 (15.4)
Hand-foot-syndrome	3	1	0	-	4 (30.8)
Stomatitis	1	0	0	0	1 (7.7)
Hypothyroidism	4	0	0	0	4 (30.8)

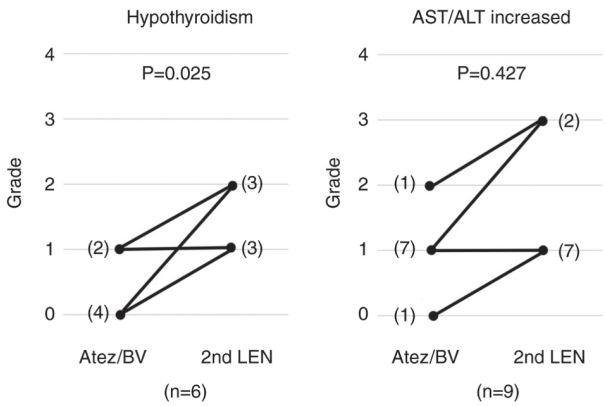

Figure 3. Grade change for adverse events from first-line atezolizumab plus bevacizumab treatment to second-line lenvatinib treatment. Atez/BV: atezolizumab plus bevacizumab used as the first-line treatment. 2nd LEN: atezolizumab plus bevacizumab used as first-line treatment, and lenvatinib used as second-line treatment. In patients with hypothyroidism or elevated AST/ALT levels during the second-line treatment, there was a change in the grade of adverse events from the first-line treatment to the second-line treatment. Numbers in brackets indicate the number of patients. Atez/BV, atezolizumab/bevacizumab; LEN, lenvatinib; AST/ALT, aspartate aminotransferase/alanine aminotransferase.

common in the 2nd LEN group. The incidences of hypothyroidism were 12.8% in the 1st LEN and 46.2% in the 2nd LEN groups. Kudo *et al* reported that the incidence of hypothyroidism when LEN was used as first-line treatment was 16% (6), while Ogushi *et al* reported that it was 25.4% (7). Thus, for 1st LEN, the incidence rate in this study was similar to or lower than that previously reported.

Lee *et al* reported the risk of developing hypothyroidism using ICIs (15). The incidence of hypothyroidism in patients who received both an angiogenesis inhibitor (AI) and an ICI was 4.4 times higher than that in patients treated with ICI alone. In other words, they showed a synergistic effect in patients who received multiple doses, which may be associated with thyroid dysfunction. Therefore, when using agents such as AIs in conjunction with ICI treatment, special attention should be paid to treatment-related side effects (15). The timing of onset of adverse immune events caused by ICIs is highly variable (1). Tyrosine kinase inhibitors such as LEN have been reported to cause thyroid dysfunction by acting on the hypothalamic-pituitary-thyroid system (16,17). Therefore, it is predicted that after Atez/BV, LEN will also be expected to exacerbate the incidence of hypothyroidism in patients treated with drugs that are potentially associated with thyroid dysfunction.

In this study, when Atez/BV was used as the 1st-line treatment and LEN as the 2nd-line treatment, the incidence of hypothyroidism in 2nd LEN was 46.2%. According to Finn *et al*, the incidence of hypothyroidism when Atez/BV was used as the first-line treatment was 10.6% (1). Although not shown in the results, the rate in the present study was 11.1%, which is similar. In other words, in this study, the incidence of hypothyroidism was not high, even when Atez/BV was used as first-line treatment. Furthermore, the incidence during 1st LEN was 12.8 and 46.2% with 2nd LEN, which was high. In contrast, Muto *et al* (18) reported that when Atez/BV was used as the 1st-line treatment and LEN was used as the 2nd-line treatment, the incidence of hypothyroidism in patients receiving 2nd LEN was 15%. There is a clear difference to the results of this study. However, it should be noted that the number of patients in both studies was small (Muto *et al* n=20, the current study n=13). Furthermore, in this study, there were differences in sex between patients in the 1st LEN and 2nd LEN groups. In this study, all six patients with hypothyroidism receiving 2nd LEN were women. Hypothyroidism is more common in women than men. Therefore, women receiving treatment with 2nd LEN should be closely monitored for hypothyroidism.

In this study, four of the six patients (66.7%) who had hypothyroidism with 2nd LEN did not have hypothyroidism during the Atez/BV first-line treatment. In addition, one of the two patients with grade 1 hypothyroidism during first-line Atez/BV treatment showed deterioration to grade 2. Thus, hypothyroidism increased with 2nd LEN after first-line Atez/BV treatment. These results suggest that tyrosine kinase inhibitors may cause thyroid dysfunction when the hypothalamus-pituitary-thyroid system is damaged by the synergistic effect from the combined use of angiogenesis inhibitors and ICIs.

The incidence of increased AST/ALT levels was 46.2% with 1st LEN and 76.9% with 2nd LEN. In contrast, eight of the nine patients (88.9%) who had an increase in AST/ALT during 2nd LEN already had an increase in AST/ALT during the first-line Atez/BV treatment. In addition, 3/9 patients (33.3%) showed worsening from the first-line treatment or a new increase in AST/ALT with the 2nd LEN. Thus, no significant increase in AST/ALT levels was observed from first-line Atez/BV treatment to 2nd LEN. Of the 13 patients receiving 2nd LEN, 7 out of 12 patients (58.3%) who discontinued treatment did so due to decreased performance status (results not

Table IV. Subgroup analysis of adverse events by sex.

	1st LEN										2nd LEN									
	Male (n=36)					Female (n=3)					Male (n=5)					Female (n=8)				
	Grade, n				All grades	Grade, n				All grades						Grade, n				
Events	1	2	3	4	(%)	1	2	3	4	(%)	P-value	1	2	3	4	All grades (%)	1	2	3	4	All grades (%)	P-value
Leucopenia	4	6	1	0	11 (30.6)	1	1	0	0	2 (66.7)	0.253	0	0	0	0	0	3	1	0	0	4 (50.0)	0.105
Neutropenia	2	6	2	0	10 (27.8)	0	0	1	0	1 (33.3)	1.253	0	0	0	0	0	3	1	0	0	4 (50.0)	0.105
Platelet count decreased	16	8	1	0	25 (69.4)	1	2	0	0	3 (100)	0.545	2	0	1	0	3 (60.0)	3	3	0	0	6 (75.0)	1
Aspartate aminotransferase/alanine aminotransferase increased	17	1	0	0	18 (50.0)	0	0	0	0	0	0.235	4	0	1	0	5 (100)	3	0	2	0	5 (62.5)	0.231
Blood bilirubin increased	9	6	2	0	17 (47.2)	1	0	0	0	1 (33.3)	1	0	1	0	0	1 (20.0)	2	2	0	0	4 (50.0)	0.565
Anemia	12	2	2	0	16 (44.4)	1	1	0	0	2 (46.2)	0.586	2	1	0	0	3 (60.0)	2	2	0	0	4 (50.0)	1
Diarrhea	5	0	1	0	6 (16.7)	0	0	0	0	0	1	0	1	0	0	1 (20.0)	3	0	0	0	3 (37.5)	1
Nausea	3	2	0	0	5 (13.9)	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1 (12.5)	1
Vomiting	2	0	0	-	2 (5.6)	0	0	0	-	0	1	0	0	0	-		0	0	0	-		1
Fatigue	11	4	0	-	15 (41.7)	0	0	1	-	1 (33.3)	1	2	1	0	-	3 (60.0)	4	1	0	-	5 (62.5)	1
Proteinuria	0	9	4	-	13 (36.1)	0	2	0	-	2 (46.2)	0.547	1	0	2	-	3 (60.0)	1	0	1	-	2 (25.0)	0.293
Anorexia	11	2	1	0	14 (38.9)	0	0	0	0	0	0.540	1	0	0	0	1 (20.0)	4	0	0	0	4 (50.0)	0.565
Edema in limbs	7	0	0	-	7 (19.4)	0	0	0	-	0	1	2	0	0	-	2 (40.0)	3	0	0	-	3 (37.5)	1
Hypertension	8	3	2	0	13 (36.1)	0	1	0	0	1 (33.3)	1	0	0	0	0	0	1	0	0	0	1 (12.5)	1
Hand-foot-syndrome	4	4	0	-	8 (22.2)	0	0	0	-	0	1	1	1	0	-	2 (40.0)	3	0	0	-	3 (37.5)	1
Hoarseness	1	0	0	-	1 (2.8)	0	0	0	-	0	1	0	0	0	-	0	0	0	0	-	0	1
Rash	1	0	0	0	1 (2.8)	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
Musculoskeletal and connective tissue disorder	2	0	0	0	2 (5.6)	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
Hypothyroidism	2	3	0	0	5 (13.9)	0	0	0	0	0	1	0	0	0	0	0	3	3	0	0	6 (75.0)	0.021[a]
Epistaxis	1	0	0	0	1 (2.8)	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
Oral pain	2	0	0	-	2 (5.6)	0	0	0	-	0	1	0	0	0	-	0	0	0	0	-	0	1
Dizziness	1	0	0	-	1 (2.8)	0	0	0	-	0	1	0	0	0	-	0	0	0	0	-	0	1
Abdominal pain	2	0	0	-	2 (5.6)	0	0	0	-	0	1	0	0	0	-	0	0	0	0	-	0	1

Fisher's exact probability tests. [a]P<0.05. LEN, lenvatinib.

shown). Therefore, the reason AST/ALT increased more with 2nd LEN than 1st LEN was considered to be the worsening of the patient's condition.

In this study, the treatment duration was similar for both 1st LEN and 2nd LEN, and the RDI was also statistically similar. Therefore, the onset of side effects was not affected by the administration period or RDI.

The limitations of this study include the limited sample size and retrospective nature of the study, which was conducted at a single institution. High hypothyroidism during LEN use after Atez/BV treatment may be due to immune-related factors or anti-VEGF therapy. The onset timing and risk factors for immune-related AEs are not clear, and cases have been reported where they occurred several months after the end of treatment (19). The median (range) first onset of hypothyroidism has been reported as 93.5 (13-419) days, with considerable variation (1,20). Furthermore, it has also been reported that patients with HCC may have a higher incidence of thyroid dysfunction when using both LEN and Atez/BV than those treated with just one of the drugs (21). However, due to the small sample size, a final conclusion could not be drawn. Hypothyroidism may also be influenced by factors other than treatment. Future studies should address these limitations. In particular, it is necessary to examine sex-based differences in incidence and severity. Additionally, durvalumab plus tremelimumab combination therapy and durvalumab monotherapy have been approved in Japan for the treatment of unresectable HCC. It is worth investigating whether similar results will be found when using these ICIs.

In clinical practice, patients receiving LEN as second-line therapy after treatment with Atez/BV are at increased risk of hypothyroidism. However, further research is required to confirm this hypothesis.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

MK contributed to the study design, collected and provided the data, was the principal author of the report, and is the guarantor of this article. MK and MG confirm the authenticity of all the raw data. ShY, MG, SaY, HT and EU contributed to the study design, reviewed the manuscript, and supervised the drafting of the report and submission process. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The study protocol was approved by the Institutional Review Board of the Ogaki Municipal Hospital (Ogaki, Japan; approval number 20220728-17-h). The requirement for informed consent was waived because of the retrospective study design.

Patient consent for publication

Written informed consent for publication was obtained from each patient.

Competing interests

The authors declare that they have no competing interests.

References

1. Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, Kim TY, Kudo M, Breder V, Merle P, Kaseb AO, *et al*: atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. N Engl J Med 14: 1894-1905, 2020.
2. Sonbol MB, Riaz IB, Naqvi SAA, Almquist DR, Mina S, Almasri J, Shah S, Almader-Douglas D, Uson Junior PLS, Mahipal A, *et al*: Systemic therapy and sequencing options in advanced hepatocellular carcinoma: A systematic review and network meta-analysis. JAMA Oncol 6: e204930, 2020.
3. Takeda S, Namisaki T, Tsuji Y, Fujimoto Y, Murata K, Enomoto M, Fujinaga Y, Nishimura N, Kitagawa K, Takaya H, *et al*: Initial experience with atezolizumab plus bevacizumab for unresectable hepatocellular carcinoma: A real-world retrospective study. Anticancer Res 42: 5465-5473, 2022.
4. Kobayashi K, Nagai H, Matsui T, Matsuda T and Higai K: Importance of Atezolizumab plus bevacizumab combination treatment as first-line therapy for immunological changes in patients with unresectable hepatocellular carcinoma. Anticancer Res 43: 4601-4609, 2023.
5. Tohyama O, Matsui J, Kodama K, Hata-Sugi N, Kimura T, Okamoto K, Minoshima Y, Iwata M and Funahashi Y: Antitumor activity of lenvatinib (e7080): An angiogenesis inhibitor that targets multiple receptor tyrosine kinases in preclinical human thyroid cancer models. J Thyroid Res 2014: 638747, 2014.
6. Kudo M, Finn RS, Qin S, Han KH, Ikeda K, Piscaglia F, Baron A, Park JW, Han G, Jassem J, *et al*: Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: A randomised phase 3 non-inferiority trial. Lancet 24: 1163-1173, 2018.
7. Ogushi K, Chuma M, Uojima H, Hidaka H, Numata K, Kobayashi S, Hirose S, Hattori N, Fujikawa T, Nakazawa T, *et al*: Safety and Efficacy of lenvatinib treatment in Child-Pugh A and B patients with unresectable hepatocellular carcinoma in clinical practice: A multicenter analysis. Clin Exp Gastroenterol 13: 385-396, 2020.
8. Iwamoto H, Suzuki H, Shimose S, Niizeki T, Nakano M, Shirono T, Okamura S, Noda Y, Kamachi N, Nakamura T, *et al*: weekends-off lenvatinib for unresectable hepatocellular carcinoma improves therapeutic response and tolerability toward adverse events. Cancers (Basel) 12: 1010, 2020.
9. Yoo C, Kim JH, Ryu MH, Park SR, Lee D, Kim KM, Shim JH, Lim YS, Lee HC, Lee J, *et al*: Clinical outcomes with multikinase inhibitors after progression on first-line atezolizumab plus bevacizumab in patients with advanced hepatocellular carcinoma: A multinational multicenter retrospective study. Liver Cancer 10: 107-114, 2021.
10. Aoki T, Kudo M, Ueshima K, Morita M, Chishina H, Takita M, Hagiwara S, Ida H, Minami Y, Tsurusaki M and Nishida N: Exploratory analysis of lenvatinib therapy in patients with unresectable hepatocellular carcinoma who have failed prior PD-1/PD-L1 checkpoint blockade. Cancers (Basel) 12: 3048, 2020.
11. Hiraoka A, Kumada T, Tada T, Hirooka M, Kariyama K, Tani J, Atsukawa M, Takaguchi K, Itobayashi E, Fukunishi S, *et al*: Lenvatinib as second-line treatment after atezolizumab plus bevacizumab for unresectable hepatocellular carcinoma: Clinical results show importance of hepatic reserve function. Oncology 101: 624-633, 2023.
12. National Cancer Institute: US Department Of Health And Human Services: Common terminology criteria for adverse events (CTCAE). Version 5.0, 2017. (https://ctep. Cancer. Gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf).

13. Tamai T, Hayato S, Hojo S, Suzuki T, Okusaka T, Ikeda K and Kumada H: Dose finding of lenvatinib in subjects with advanced hepatocellular carcinoma based on population pharmacokinetic and exposure-response analyses. J Clin Pharmacol 57: 1138-1147, 2017.
14. Kanda Y: Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant 48: 452-458, 2013.
15. Lee SE, Kim KA, Lee H and Park J: Risk of developing hypothyroidism with the use of tyrosine kinase inhibitors and immune checkpoint inhibitors. Cancer Epidemiol 81: 102265, 2022.
16. Makita N and Iiri T: Tyrosine kinase inhibitor-induced thyroid disorders: A review and hypothesis. Thyroid 23: 151-159, 2013.
17. Ahmadieh H and Salti I: Tyrosine kinase inhibitors induced thyroid dysfunction: A review of its incidence, pathophysiology, clinical relevance, and treatment. Biomed Res Int 2013: 725410, 2013.
18. Muto H, Kuzuya T, Kawabe N, Ohno E, Funasaka K, Nagasaka M, Nakagawa Y, Miyahara R, Shibata T, Hashimoto S, *et al*: Clinical outcomes with lenvatinib in patients previously treated with atezolizumab/bevacizumab for advanced hepatocellular carcinoma. Anticancer Res 43: 4673-4682, 2023.
19. Otsubo K, Nakatomi K, Furukawa R, Ashida K, Yoneshima Y, Nakanishi Y and Okamoto I: Two cases of late-onset secondary adrenal insufficiency after discontinuation of nivolumab. Ann Oncol 28: 3106-3107, 2017.
20. Muir CA, Clifton-Bligh RJ, Long GV, Scolyer RA, Lo SN, Carlino MS, Tsang VHM and Menzies AM: Thyroid immune-related adverse events following immune checkpoint inhibitor treatment. J Clin Endocrinol Metab 18: e3704-e3713, 2021.
21. Ichimura T, Ichikura D, Hinata M, Hida N and Baba T: Thyroid dysfunction with atezolizumab plus bevacizumab after Lenvatinib in hepatocellular carcinoma: A case series. SAGE Open Med Case Rep 11: 2050313X231164488, 2023.