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Incidence of Hyper Progressive Disease in Combination Immunotherapy and Anti-Programmed Cell Death Protein 1/ Programmed Death-Ligand 1 Monotherapy for Unresectable Hepatocellular Carcinoma

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Keywords
Anti-programmed cell death protein 1/programmed death-ligand 1 antibody · Vascular endothelial growth factor antibody · Anti-cytotoxic T-lymphocyte-associated protein 4 antibody · Hepatocellular carcinoma · Hyper progressive disease

Abstract

Introduction: Programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) signaling blockade is the most effective strategy for the treatment of immune evading hepatocellular carcinoma (HCC). While immune checkpoint inhibitor has revolutionized the concept of cancer treatment, it has also led to unexpected tumor growth. Regulatory T cells express PD-1 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) receptors, which are proliferated and activated by antibody binding, and their ratio to CD8+ T cells is altered, which is one of the causes for hyper progressive disease (HPD). We examined the frequency of HPD in anti-PD-1/PD-L1 monotherapy and combination therapy with vascular endothelial growth factor (VEGF) antibody and anti-CTLA-4 antibodies. ***Methods:*** This was a prospective and retrospective cohort study which enrolled 198 patients with unresectable HCC from January 2015 to December 2021 at the Kindai University Hospital. Fifty-eight patients received anti-PD-1/PD-L1 monotherapy, 119 patients combination with VEGF antibody, and 21 patients combination with anti-CTLA-4 antibody. We defined HPD as tumor growth rate (TGR) ratio ≥4, ΔTGR ≥40%, and tumor growth kinetics ratio ≥4. ***Results:*** The HPD rate was 10.3% (6/58) in anti-PD-1/PD-L1 monotherapy, 1.7% (2/119) in combination with VEGF antibody, and 4.8% (1/21) in combination with anti-CTLA-4 antibody ($p = 0.034$). The odds ratio for HPD in the combined anti-CTLA-4 antibody group was 0.433 (95% confidence interval [CI]: 0.05–3.83) when compared to the anti-PD-1/PD-L1 monotherapy group and 2.93 (95% CI: 0.25–33.79) when compared to the combined VEGF antibody group. ***Conclusion:*** The frequency of HPD in unresectable HCC compared to anti-PD-1/PD-L1 monotherapy was decreased with the combination with anti-VEGF antibody and

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not increased with anti-CTLA-4 antibody. Anti-PD-1/PD-L1 combined with anti-CTLA-4 antibody is now available in real-world and needs to be further validated with accumulated clinical practice.

Introduction

Considering the selection and evolution of malignant tumor, it has been suggested that cancer cells with low immunogenicity may survive and establish a strong immunosuppressive phenotype that evades antitumor immune responses [1–3]. Decreased expression of neoantigens [4–6] and major histocompatibility complex class I [7] can disturb CD8+ T cells for recognizing cancer cells, resulting in the survive of cancer cells. Another mechanism for suppressing tumor-infiltrating cytotoxic T-lymphocytes could be the acquisition of specific driver mutations that activate Wnt/β-catenin signal [8, 9]. For the establishment of immune suppressive microenvironment, it is also important to invite immunosuppressive cells such as myeloid-derived suppressor cells, tumor-associated macrophages (TAMs), regulatory T cells (Treg cells), and cancer-associated fibroblasts [10–12] and to obtain phenotype that can help cancer cells survive in the hypoxic and lactate-rich condition [13]. Among these, the expression of programmed cell death-ligand 1 (PD-L1) on cancer cells and various immunosuppressive molecules such as programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) on T cells are major components of immune escape [14, 15].

Immune checkpoint inhibitors (ICIs) including anti-CTLA-4 and anti-PD-1/PD-L1 antibodies, reactivate cytotoxic CD8+ T cells and kill cancer cells [16]. ICIs are an unpreceded therapeutic approach and have dramatically changed the treatment concept in many malignant neoplasms. In an observational study in a pooled analysis, long-term survival was observed in about 20% of melanoma patients treated with ICI [17], and the durable response is an unique characteristic in the treatment of this type of agent compared to the conventional cytotoxic anticancer agents.

On the other hand, disturbing of the host immune response without concomitant cell-killing anticancer agents may induce hyper progressive disease (HPD) by activating immunosuppressive cells as well as improving the exhaustion of effector CD8+ T cells. HPD is an oncological emergency, in which unexpected rapid tumor growth is triggered by immunotherapy compared to the growth before the treatment. Previous studies have shown that the incidence of HPD in hepatocellular carcinoma (HCC) patients treated with nivolumab or pembrolizumab, anti-PD-1 monotherapy, ranges from 8.0% to 12.7% [18–20]. On the other hand, the incidence of HPD, broadly defined, in patients carried HCC treated with combination of PD-L1 antibody and vascular endothelial growth factor (VEGF) antibody has been reported to be 4.5–10.2% [21]. Several mechanisms have been proposed for the induction of HPD for the cases of lung cancer, malignant melanoma, and gastric and colorectal cancer. One of the most possible mechanisms is inhibition of the PD-1/PD-L1 results in the proliferation and activation of Treg cells over CD8+ T cells in a Treg cell-rich tumor environment [22–24]. Contribution of the Fc region of anti-PD-1 antibodies on the induction of M2-like TAMs is, reportedly, also important for the maintenance of HPD status [12]. However, this hypothesis may not clue to the pathogenesis of HPD in HCC cases because generally, extensive infiltration of Treg is relatively rare in HCC compared to other types of malignancies including gastric cancer, whereas considerable incidence of HPD has been reported in both malignancies.

More critically, ICIs are becoming key agents for the treatment of HCC, whereas no clinical biomarkers have been identified to predict antitumor efficacy, and there is also a lack of consensus on how to deal with HPD. So far, HPD is mainly reported on ICI monotherapy; we compared the incidence of HPD among anti-PD-1 monotherapy and combination immunotherapies with intensive discussion of the possible mechanisms of HPD.

Materials and Methods

Patients

This is a single-institution prospective and retrospective cohort study which enrolled 256 patients with unresectable HCC who received ICIs from January 1, 2015, to December 31, 2021, at the Kindai University Hospital in a setting of prospective clinical trial (n = 101) and in a setting of real-world practice (n = 155). Excluding 58 cases who could not be adequately evaluated, a total of 198 cases were included in this analysis. The details were as follows: 58 patients received anti-PD-1/PD-L1 antibody monotherapy (43 nivolumab, 3 pembrolizumab, 12 durvalumab), 119 patients received anti-PD-1/PD-L1 antibody combined with VEGF antibody (119 atezolizumab and bevacizumab), and 21 patients received anti-PD-1/PD-L1 antibody combined with anti-CTLA-4 antibody (15 durvalumab and tremelimumab, 6 nivolumab and ipilimumab). In atezolizumab and bevacizumab combined group, 16 cases were entered in published clinical trials, and 103 cases were consecutively collected in prospective real-world clinical cohort study, which registered as UMIN000045557

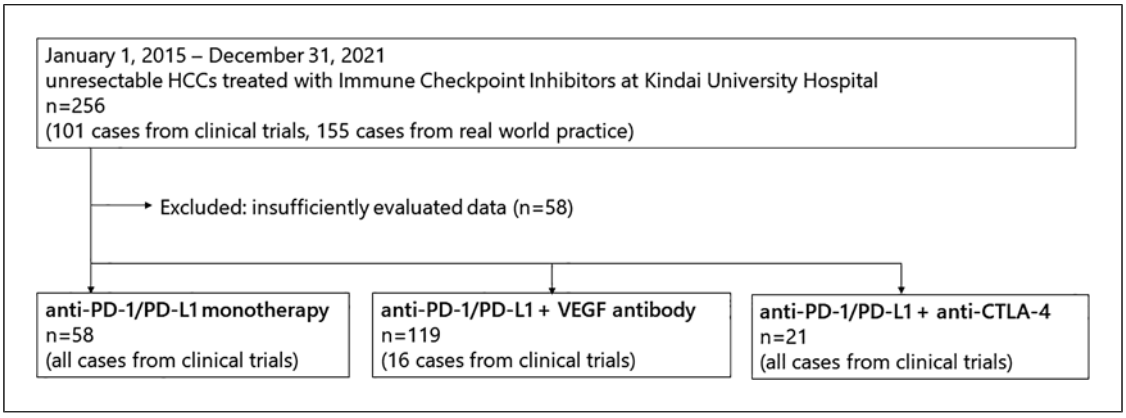


Fig. 1. Flow diagram of the objective patients with unresectable hepatocellular carcinoma. The subjects were a total of 198 out of 256 patients with unresectable HCC who underwent ICI treatment at Kinki University Hospital from January 1, 2015, to December 31, 2021. Fifty-eight patients received anti-PD-1/PD-L1 monotherapy, 119 patients received anti-PD-1/PD-L1 antibody plus VEGF antibody, and 21 patients received anti-PD-1/PD-L1 antibody plus anti-CTLA-4 antibody. ICI, immune checkpoint inhibitor; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; VEGF, vascular endothelial growth factor; CTLA4, cytotoxic T-lymphocyte-associated protein 4; ICI, immune checkpoint inhibitors; HCC, hepatocellular carcinoma.

from September 24, 2021. However, it is not a complete prospective study because some cases were included before September 24, 2021. The flow diagram for the selection of patients is shown in Figure 1.

The diagnosis of HCC was made based on the histological and/or radiological findings as recommended by the guidelines of the American Association for the Study of Liver Diseases [25]. The inclusion criteria were as follows: patients with performance status defined by the Eastern Cooperative Oncology Group [26] of zero or 1, patients with evaluable viable lesions including intermediated stage HCC treated with ICIs, patients with Child-Pugh class A liver function, and an expected survival time longer than 3 months. The exclusion criteria were as follows: patients with the high risk for esophageal and gastric variceal rupture, patients who refused to participate in this clinical research. One hundred and ninety-eight patients with HCC met the criteria and were included in the analysis. This study was approved by the Institutional Review Board and Medical Ethics Committee of the Kindai University Hospital (IRB approval R02-258).

Assessment of Tumor Response to ICIs

ICI treatment was administered according to the treatment protocol for each drug, with dose interruption or withdrawal depending on the grade of the adverse events. Treatment was discontinued in the event of unacceptable adverse events or imaging and/or clinical PD. The primary endpoints were HPD rate for each treatment group. The secondary endpoints were disease control rate (DCR) and objective response rate (ORR), progression-free survival (PFS), overall survival (OS), and analysis of risk factors contributing to HPD. Since HPD is a condition unique to ICI therapy, we adopted the definition of Kim et al. [18] that clarifies the difference between HPD and non-HPD patients. We defined HPD as patients who met all of the following criteria: tumor growth rate (TGR) [27] ratio ≥4, ΔTGR ≥40%, and tumor growth kinetics (TGK) [28] ratio ≥4. In addition, we also investigated the frequency of HPD defined by TGR ratio ≥2 and TGK ratio ≥2, as proposed by Wang et al. [29] to allow comparison with previous reports. TGR = 100 (exp [TG] – 1), where TG is the growth rate and exp (TG) represents the exponential of TG. The absolute change in TGR (ΔTGR) was defined as the difference between TGRpre and TGRpost divided by TGRpre. TGK was defined as the difference between pre- and post-baseline imaging and the sum of the largest diameters of the target lesions per unit time between baseline imaging. Tumor diameter was measured in up to two target tumors by Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1) before 1–2 months of ICI treatment, baseline of ICI treatment, and 1–2 months after ICI therapy. We complied with no local therapy such as TACE or RFA and no drug therapy between the pre- and post-baseline imaging. Dynamic computed tomography or gadolinium-ethoxybenzyl-diethylenetriamine-enhanced magnetic resonance imaging was performed every 6–8 weeks and laboratory tests were performed every 3–4 weeks. The DCR and ORR were determined based on the best response observed during the treatment and observation periods with anti-PD-1/PD-L1 antibody.

Statistical Analyses

Statistical analyses were performed using the IBM Statistical Package for the Social Sciences Statistics version 28.0 (IBM; Armonk, NY, USA). Continuous variables were expressed as median (range or interquartile range) as appropriate. For cross-tabulations, χ^2 tests were performed when the expected value was 5 or higher. The Kaplan-Meier method (log-lank test) was applied for comparison of OS and PFS. A two-tailed p value <0.05 was considered to indicate a statistically significant difference in all analyses.

Results

Patient Characteristics

The characteristics of the patients at baseline are summarized in Table 1. The median age of patients was significantly higher in the combined VEGF antibody

Table 1. Patient's characteristic at baseline

	Anti-PD-1/PD-L1 monotherapy $n = 58$ (all cases from clinical trials)	Anti-PD-1/PD-L1 + VEGF antibody $n = 119$ (16 cases from clinical trials)	Anti-PD-1/PD-L1 + anti-CTLA-4 $n = 21$ (all cases from clinical trials)	p value
Age, median (range)	69 (22, 86)	72 (35, 89)	69 (26, 80)	$p = 0.009$
Sex, male, female	46, 12	31, 88	4. 17	N.S
PS, 0, 1	54, 4	109, 10	20, 1	N.S
Etiology, HCV, HBV, NBNC	19, 13, 26 (alcohol 14, NASH 9)	38, 26, 55 (alcohol 28, NASH 18)	8, 7, 7 (alcohol 1, NASH 3)	N.S
BCLC stage, A, B, C	1, 17, 40	4, 53, 62	0, 10, 11	N.S
VI, no, yes	43, 15	94, 25	17, 4	N.S
EHS, no, yes	25, 33	69, 50	11, 10	N.S
WBC, median (IQR)	5.01 (4.01, 5.83)	5.13 (4.03, 6.51)	4.18 (3.81, 5.71)	N.S
NLR, median (IQR)	2.65 (1.72, 4.93)	3.15 (1.73, 4.61)	3.00 (2.23, 3.61)	N.S
CRP, median (IQR)	0.224 (0.073, 0.565)	0.445 (0.126, 1.478)	0.155 (0.111, 0.220)	$p = 0.030$
Platelets, median (IQR)	16.7 (11.6, 19.6)	15.5 (10.7, 21.9)	12.2 (9.9, 17.2)	N.S
Serum albumin, median (IQR)	3.75 (3.20, 4.20)	3.70 (3.25, 4.00)	3.90 (3.70, 4.28)	$p = 0.067$
Total bilirubin, median (IQR)	0.8 (0.6, 1.2)	0.8 (0.50 1.0)	0.6 (0.4, 0.9)	$p = 0.089$
ALBI score, median (IQR)	−2.48 (−2.85, −1.90)	−2.40 (−2.73, −1.93)	−2.76 (−2.96, −2.59)	$p = 0.028$
AST, median (IQR)	42.5 (32.8, 71.8)	38.0 (27.5, 53.5)	39.0 (26.0, 45.0)	N.S
ALT, median (IQR)	29.0 (21.0, 49.3)	33.0 (21.0, 45.5)	24.0 (19.0, 34.0)	N.S
Serum AFP level, median (IQR)	266.5 (5.0, 4,280.0)	137.0 (9.0, 1,778)	20.0 (4.0, 19,766)	$p = 0.095$
DCP, median (IQR)	779.5 (191.8, 4,374.3)	1,227 (79.5, 6,361.5)	1,199.5 (291, 15,172.5)	N.S

Values were expressed in median (range or IQR). PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; VEGF, vascular endothelial growth factor; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PS, performance status; HCV, hepatitis C virus; HBV, hepatitis B virus; NBNC, negative for hepatitis B surface antigen and hepatitis C antibody; NASH, nonalcoholic steatohepatitis; BCLC, Barcelona Clinic Liver Cancer; VI, vascular invasion; EHS, extrahepatic spread; WBC, white blood cell; NLR, neutrophil-lymphocyte ratio; CRP, C-reactive protein; ALBI, albumin-bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; IQR, interquartile range; N.S, not significant.

group; 72 years (range: 35–89) versus 69 in other two groups. There were no significant differences among the three groups with respect to gender, performance status, etiology, and BCLC stage. In laboratory tests, C-reactive protein was significantly higher in the combined VEGF antibody group and albumin-bilirubin (ALBI) score was significantly better in the combined anti-CTLA-4 antibody group, but there were no significant differences among the three groups in other parameters.

Administration of Anti-PD-1/PD-L1 Monotherapy/ Combination Therapy

At the data cutoff date (January 31, 2022), the median follow-up period was 26.3 months (95% confidence interval [CI]: 19.2–33.5) (Kaplan-Meier estimate). Patients received ICI treatment either as a standard of care ($n = 103$) or in clinical trials already published ($n = 95$). During the observation period, 81 patients (40.9%) died of HCC progression or other reasons, and 164 patients (82.8%) discontinued treatment due to disease progression, treatment-related adverse events, or switching to other therapies. Thirty-four of the 198 patients (17.2%) who had a partial response (PR) or stable disease (SD) were still on ICI treatment at the data cutoff.

The HPD Rate of Each ICI Therapy

The outcomes determined based on the best response observed during the treatment and observation period with ICI treatment are shown in Table 2. ORR was 19.0% (11/58) in the anti-PD-1/PD-L1 monotherapy group, 20.2% (24/119) in the combined VEGF antibody group, and 28.6% (6/21) in the combined anti-CTLA-4 antibody group, with no significant difference. DCR was 41.4% (24/58), 72.3% (86/119), and 52.4% (11/21), respectively, with significantly better response in the combined VEGF antibody group ($p < 0.001$). The HPD rate defined by TGR ratio ≥4, ΔTGR ≥40%, and TGK ratio ≥4 was 10.3% (6/58), 1.7% (2/119), and 4.8% (1/21), respectively, with significantly better results in the combined VEGF antibody group ($p = 0.034$). The HPD rate defined by TGR ratio ≥2 and TGK ratio ≥2 was 13.8% (8/58), 3.4%

Table 2. The best response per RECIST v1.1

	Anti-PD-1/PD-L1 monotherapy $n = 58$ (all cases from clinical trials)	Anti-PD-1/PD-L1 + VEGF antibody $n = 119$ (16 cases from clinical trials)	Anti-PD-1/PD-L1 + anti-CTLA-4 $n = 21$ (all cases from clinical trials)	p value
ORR (CR + PR)	19.0% (11/58)	20.2% (24/119)	28.6% (6/21)	N.S
DCR (CR + PR + SD)	41.4% (24/58)	72.3% (86/119)	52.4% (11/21)	$p < 0.001$
PDR (PD + HPD)	53.4% (31/58)	18.5% (22/119)	42.9% (9/21)	$p < 0.001$
CR per RECIST v1.1	0.0% (0/58)	0.0% (0/119)	0.0% (0/21)***	$p < 0.001$
PR per RECIST v1.1	19.0% (11/58)	20.2% (24/119)	28.6% (6/21)***	
SD per RECIST v1.1	22.4% (13/58)	52.1% (62/119)	23.8% (5/21)***	
PD per RECIST v1.1	43.1% (25/58)	16.8% (20/119)	38.1% (8/21)***	
HPD defined by Kim*	10.3% (6/58)	1.7% (2/119)	4.8% (1/21)***	**$p = 0.034$**
HPD defined by Wang**	13.8% (8/58)	3.4% (4/119)	9.5% (2/21)***	**$p = 0.015$**
NE	5.2% (3/58)	9.2% (11/119)	4.8% (1/21)	

PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; VEGF, vascular endothelial growth factor; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; ORR, objective response rate; DCR, disease control rate; PDR, progressive disease rate; RECIST v1.1, Response Evaluation Criteria in Solid Tumors version 1.1; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; HPD, hyper progressive disease; NE, not evaluated; N.S, not significant. *Tumor growth rate (TGR) ratio ≥4, ΔTGR ≥40%, and tumor growth kinetics (TGK) ratio ≥4 [18]. **TGR ≥2, and TGK ≥2 [29]. ***The expected value was less than 5, and then the χ^2 test could not be performed.

(4/119), and 9.5% (2/21) ($p = 0.015$). The intervals between imaging evaluations were as follows: median 1.06 months, mean 1.04 months (SD ± 1.43) between pre- and baseline; median 2.00 months, mean 1.28 months (SD ± 2.99) between baseline and post-. The details of HPD patients ($n = 9$, 4.5%) are summarized in Table 3. Using Fisher's exact test, the odds ratio for the occurrence of HPD in the combined anti-CTLA-4 antibody group versus the anti-PD-1/PD-L1 monotherapy group was 0.433 (95% CI: 0.05–3.83). The odds ratio for HPD in the combined anti-CTLA-4 antibody group versus the combined VEGF antibody group was 2.93 (95% CI: 0.25–33.79). The changes of TGR and TGK in HPD patients ($n = 9$) are presented in Figure 2 before and after the initiate of ICI treatment. The degree of increase in both TGR and TGK was particularly marked in the combined VEGF antibody group. Among the patients with HPD, we present 1 case of anti-PD-1/PD-L1 monotherapy (Fig. 3a) and 1 case of combined VEGF antibody are presented (Fig. 3b).

The PFS and OS according to the Best Response of ICI Therapies

The PFS for each best response was evaluated by Kaplan-Meier method (Fig. 4). The median PFS per best response was 18.7 months (95% CI: 3.8–33.5) in PR patients ($n = 42$), 7.2 months (95% CI: 4.5–9.9) in SD ($n = 80$), 1.8 months (95% CI: 1.7–2.0) in PD ($n = 54$), and 1.8 months (95% CI: 1.1–2.5) in HPD patients ($n = 9$) ($p < 0.001$). The median OS per best response was not reached in PR patients ($n = 42$), 31.3 months (95% CI: 19.4–43.3) in SD, 15.5 months (95% CI: 11.9–19.1) in PD, and 15.7 months (95% CI: 5.5–25.9) in HPD patients ($p < 0.001$) (online suppl. Fig. A; for all online suppl. material, see https://doi.org/10.1159/000531024).

The Risk Factors Contributing to HPD of ICI Therapies

The results of the univariate analysis of risk factors contributing to HPD are shown in Table 4. Total bilirubin level was a significant risk factor in the anti-PD-1/PD-L1 monotherapy group, and age was a significant risk factor in the combined VEGF antibody group. In the analysis of all patients, age and serum AFP level were significant risk factors contributing to HPD. The proportion of HPD occurring at each age-group was 12.5% (5/40) in those 60 years old (y.o.) or younger, 5.6% (3/54) in those 61–70 y.o., 1.3% (1/78) in those 71–80 y.o., and 0% (0/31) in those 81 y.o. and older ($p = 0.056$). The shortest distance method of ROC analysis showed that HPD was more likely to occur at age 68 years or younger with a sensitivity of 88.9% and specificity of 64.5% (AUROC: 0.788, $p = 0.004$) in all patients, and at age 53 years or younger with a sensitivity of 100% and specificity of 92.4% (AUROC: 0.947, $p = 0.031$) in the combined VEGF antibody group. The results of PFS by age, using 68 years as the cutoff value, showed that median PFS was significantly worse in patients younger than 68 years (3.8 vs. 6.5 months, $p = 0.024$) (online suppl. Fig. B).

Table 3. HPD patient's characteristics

	Anti-PD-1/PD-L1 monotherapy $n = 6$	Anti-PD-1/PD-L1 + VEGF antibody $n = 2$	Anti-PD-1/PD-L1 + anti-CTLA-4 $n = 1$	Total $N = 9$
Age, median (range)	53 (40, 72)	48 (43, 53)	58	53 (40, 72)
Sex, male, female	5, 1	1, 1	1, 0	7, 2
Etiology, HCV	1	0	0	1
HBV	2	1	1	4
NBNC	3 (alcohol 1, NASH 2)	1 (cryptogenic)	0	4
BCLC stage, B, C	2, 4	1, 1	0, 1	3, 6
VI, no, yes	3, 3	2, 0	1, 0	3, 6
EHS, no, yes	4, 2	1, 1	0, 1	4, 5
HCC differentiation well, moderate, poorly, ND	5, 1, 0, 0	0, 0, 0, 2	0, 0, 0, 1	5, 1, 0, 3
IHC β-catenin, P, N, ND	3, 2, 1	0, 0, 2	0, 0, 1	3, 2, 4
IHC GS, P, N, ND	3, 2, 1	0, 0, 2	0, 0, 1	3, 2, 4
IHC PD-L1, P, N, ND	0, 5, 1	0, 0, 2	0, 0, 1	0, 5, 4
WBC, median (IQR)	5.78 (2.30, 11.2)	5.34, 7.21	4.06	5.34 (2.30, 11.2)
NLR, median (IQR)	2.64 (1.93, 10.0)	1.6, 3.21	3.16	3.21 (1.6, 10.0)
CRP, median (IQR)	0.389 (0.036, 5.015)	0.469, 0.486	ND	0.469 (0.036, 5.015)
Platelets, median (IQR)	14.9 (8.4, 22.4)	16.0, 27.7	12.7	15.4 (8.4, 27.7)
Serum albumin, median (IQR)	3.4 (3.1, 4.5)	2.9, 4.3	4.3	3.4 (2.9, 4.5)
Total bilirubin, median (IQR)	1.2 (0.6, 1.9)	0.4, 0.4	0.3	0.6 (0.3, 1.9)
ALBI score, median (IQR)	−1.94 (−3.16, −1.89)	−3.1, −1.91	−3.19	−3.03 (−3.19, −1.89)
AST, median (IQR)	81 (47, 301)	38, 73	27	75 (27, 301)
ALT, median (IQR)	39 (28, 61)	29, 39	29	37 (28, 61)
Serum AFP level, median (IQR)	822 (6, 63,250)	425, 211	7	822 (6, 63,250)
DCP, median (IQR)	3,609 (136, 4,518)	1,823, 5,565	1,597	3,609 (136, 5,565)

Values were express in median (range or IQR). PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; VEGF, vascular endothelial growth factor; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; HCV, hepatitis C virus; HBV, hepatitis B virus; NBNC, negative for hepatitis B surface antigen and hepatitis C antibody; NASH, nonalcoholic steatohepatitis; BCLC, Barcelona Clinic Liver Cancer; VI, vascular invasion; EHS, extrahepatic spread; IHC, immunohistochemistry; P, positive; N, negative; ND, no data; GS, glutamine synthetase; WBC, white blood cell; NLR, neutrophil-lymphocyte ratio; CRP, C-reactive protein; ALBI, albumin-bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; IQR, interquartile range.

Discussion

HPD is a serious cancer condition characterized by a rapid acceleration of tumor growth immediately after ICI therapy, and the process is complex, with many factors speculated to be involved [27, 28]. It has been reported that immunosuppressive cells play a critical role in HPD [18, 27, 30–32]. Two main mechanisms have been proposed as the causes of HPD: one is that high expression of PD-1 in regulatory T (Treg) cells allows Treg cells to infiltrate the tumor, proliferate theirself, and promote Treg activity with the initiation of ICI therapy [22]. The other is that the Fc region of the anti-PD-1 antibodies causes M2-like differentiation of TAMs and maintains an immunosuppressive state in the tumor [33]. Increased Treg cell infiltration in tumors has been associated with poor prognosis [34]. In addition, some animal studies have shown that depletion or loss of function of Treg cells decreases tumor size [35–37]. HPD due to Treg cell expansion is expected to have a shorter time to progression and be more likely to metastasis. The prediction of HPD using peripheral blood as well as tumor tissue has been actively studied [38]. Although the copy number instability score based on cell-free DNA has been reported to be useful in predicting the development of HPD, but there is not yet enough evidence for clinical application [39].

Kim et al. [18] have reported in detail about HPD associated with anti-PD-1 monotherapy for HCC. They investigated the extent of tumor growth in regorafenib

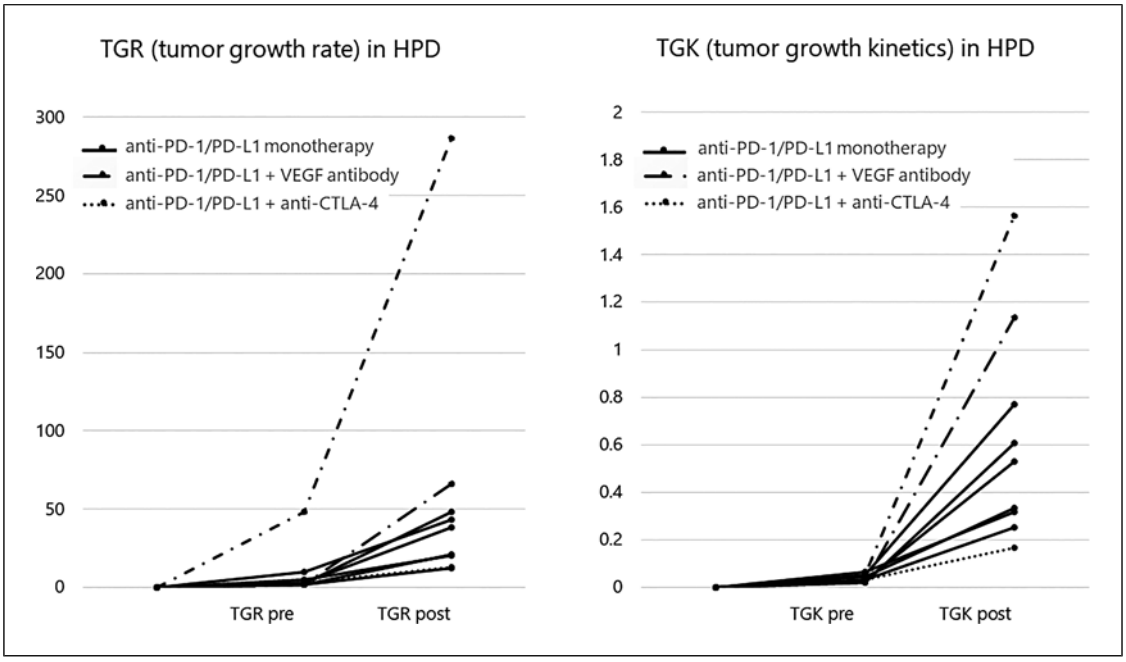
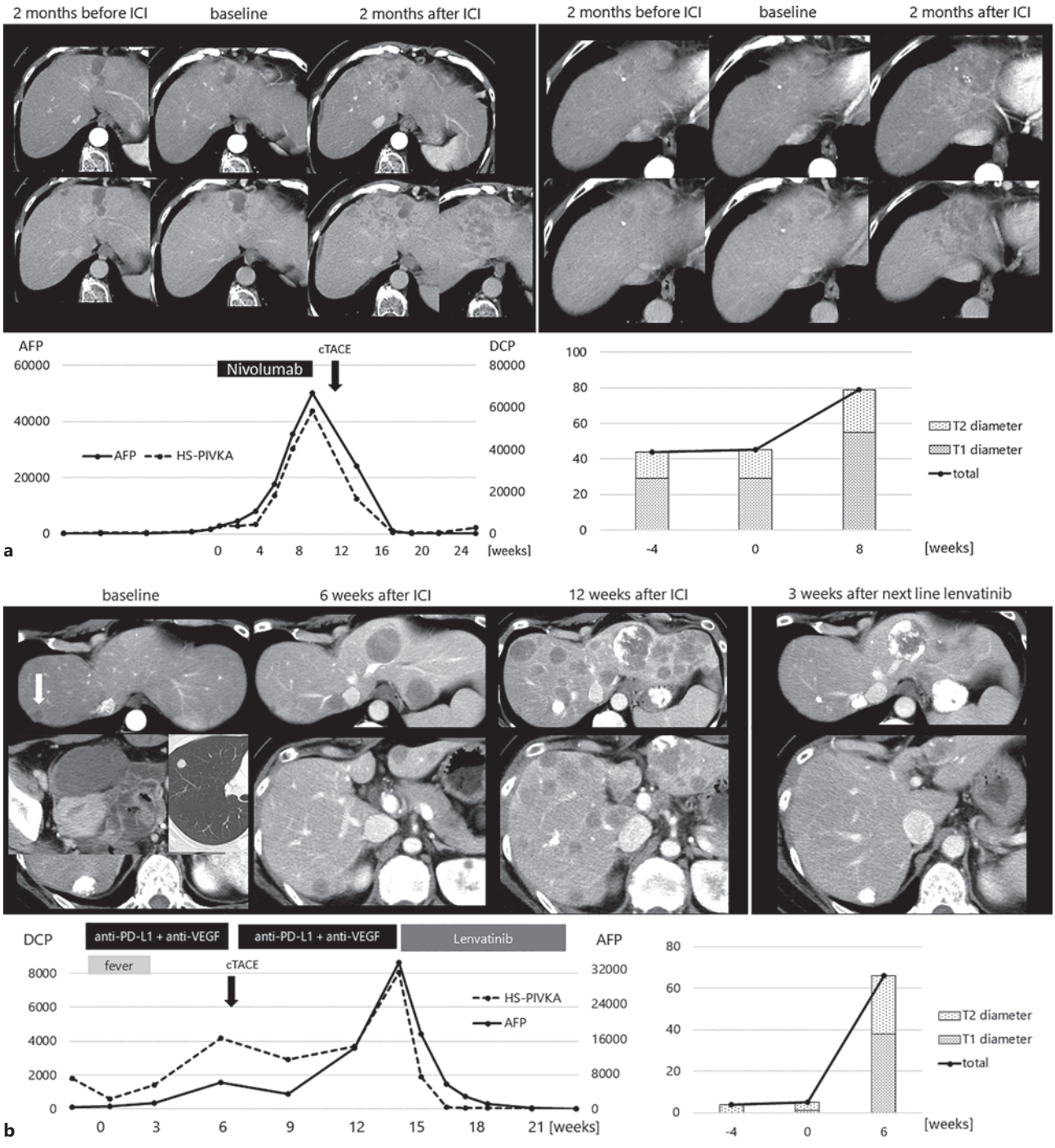


Fig. 2. Tumor growth rate and tumor growth kinetics in HPD patients TGR and TGK before and after ICI administration were shown in 9 HPD patients, and the increase in both TGR and TGK was particularly marked in the VEGF antibody group. TGR, tumor growth rate; TGK, tumor growth kinetics; HPD, hyper progressive disease; ICI, immune checkpoint inhibitors; HCC, hepatocellular carcinoma; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; VEGF, vascular endothelial growth factor; CTLA4, cytotoxic T-lymphocyte-associated protein 4.

and ICI considering that HPD is an ICI-specific event. Since this paper shows that tumor enlargement with TGR ratio ≥2 and TGK ratio ≥2 can occur even with regorafenib treatment, we adopted this reasonable definition of Kim et al. [18] for the purpose of strictly defining HPD as an ICI-specific phenomenon. Under a strict definition of HPD, HPD occurs in 12.7% (24/189) of patients with HCC as well as gastric cancer and malignant melanoma. In our study, we used the same definition to investigate HPD and found the same frequency of HPD at 10.3% (6/58).

In a mouse model of HPD, blockade of PD-1 signaling has been shown to contribute to enhanced immunosuppression of Treg cells and accelerated tumor growth [24]. Notably, a marked increase in the infiltrated effector Treg cells was found in HPD patients with gastric cancer, in contrast to a decrease in non-HPD patients [24]. A higher proportion of effector CD8+ T cells expressing PD-1 relative to PD-1+ Treg cells in tumor tissue will predict an effective response to cancer immunotherapy in malignant melanoma, gastric cancer, and lung cancer [23, 29, 40]. There is a wide spectrum of Treg cells with and without PD-1 expression, and the PD-1 and FoxP3/CTLA4 axes work together to maintain peripheral tolerance [41]. PD-1 expressed on Treg cells binds to its ligands (PD-L1 or PD-L2) [42] and recruits the tyrosine phosphatase SHP-2, leading to dephosphorylation of the antigen receptor complex [43], resulting in Treg-dependent immunosuppression. In contrast to CTLA-4, PD-1 signaling in Treg cells acts in an inhibitory manner, the mechanism of which has not been fully elucidated.

For unresectable HCC, anti-PD-1/PD-L1 combined with anti-CTLA-4 antibodies therapy is newly available. The frequency of HPD in regimens including anti-CTLA-4 antibodies is of interest because anti-CLTA-4 antibodies act directly on Treg cells which cause HPD, and on the other hand, clinical trials of anti-PD-1/PD-L1 plus anti-CTLA-4 antibody therapy have reported a high PD ratio [44]. In our study, the frequency of HPD in the combined anti-CTLA-4 antibody group was not higher than that in the monotherapy group (odds ratio was 0.433). We are interested in the reduction of Treg cells induced by anti-CTLA-4 antibodies. Treg cells account for about 5% of CD4+ T cells, whereas in many cancer microenvironments, such as malignant melanoma and lung cancer, Treg cells increase to 20–50% of CD4+ T cells. Effector Treg cells infiltrate



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tumor tissues mainly by chemoattraction via CC chemokine receptors (CCR)4-CCL22 and recognize tumor antigens and self-antigens [45]. In support of this, CCR4 is highly expressed in tumor tissues that are highly infiltrated by effector Treg cells [46]. Effector Treg cells constantly express high levels of CTLA-4,

and its expression on Treg cells downregulates the expression of CD80/CD86 (B7) costimulatory molecules on antigen-presenting cells, especially dendritic cells [47]. As a result, activation of other T cells is suppressed. In addition, CTLA-4 is a receptor that acts in a function-promoting manner on Treg cells, thus enhancing its ability to suppress immune responses to cancer cells [48]. Allison et al. [49] reported tumor regression after administration of anti-CTLA4 antibody to tumor-bearing mice [49, 50]. It was also reported that anti-CTLA-4 antibody promotes antitumor activity by a selective reduction of intratumoral Treg cells along with concomitant activation of effector T cells [48, 51, 52]. Apoptosis of Treg cells induced by CTLA-4/FasL from antigen-presenting cells may contribute to CTLA-4 antibody-specific Treg reduction [53]. Experiments using anti-CTLA-4 antibody without antibody-dependent cellular cytotoxicity activity have revealed the importance of depletion of effector Treg cells by anti-CTLA4 antibody from tumors to enhance antitumor immunity rather than direct activation of effector CD8+ T cells [51, 54, 55]. The reduction in the number of tumor-infiltrating effector Treg cells after anti-CTLA-4 antibody treatment is strongly correlated with clinical response [56], and Treg cells are expected to be a biomarker to identify patients who respond to treatment.

In our study, we adopted the very strict definition of HPD presented by Kim et al. [18] and the frequency of HPD with combined therapy of anti-PD-1/PD-L1 and VEGF antibody was 1.7% (2/119). In the study by Maesaka et al. [21], the frequency of HPD was reported as follows: 10.2% (9/88) under the definition of TGR ratio and TGK ratio ≥2, and 4.5% (4/88) for TGR ratio ≥4. Both studies showed that the frequency of HPD with combined VEGF antibody was significantly lower than that with anti-PD-1/PD-L1 monotherapy (in our study, odds ratio was 2.93). VEGF is involved not only in abnormal tumor angiogenesis but also in the development of an immunosuppressive tumor microenvironment by suppressing antigen presenting cells, antitumor TAMs, and cytotoxic T-lymphocyte while promoting Treg cells, myeloid-derived suppressor cells, and protumor TAMs. VEGFR2 is selectively expressed on effector Treg cells [57]; colorectal cancer infiltrated with VEGFR2-positive Treg cells is associated with recurrence and poor prognosis [58]. In addition, VEGF signaling pathway promotes differentiation and proliferation of Treg cells [59] and inhibits dendritic cell maturation [60]. The VEGFR2 antibody ramucirumab and lenvatinib have been shown to significantly reduce effector Treg cells when administered to human gastric cancer [61] and mouse model [62], indicating that suppression of VEGF may be a potential therapeutic target for Treg cells. The combined inhibition of VEGF and PD-1/PD-L1 leads to a decrease in Treg cells and CCR2+ monocytes, a shift of TAMs from M2 to M1 types, and enhanced CTL infiltration and activation in mouse models, and then combination with PD-1 blockade further prolonged the prognosis of HCC [63]. Kim et al. [18] also mentioned the possibility that combination therapy with VEGF inhibitor and PD-1 pathway blockade could suppress the development of HPD.

Based on the above discussion (Fig. 5), it is reasonable to conclude that the frequency of HPD is lower in the combined VEGF antibody group compared to anti-PD-1/PD-L1 monotherapy. Since there are few previous reports on the frequency of HPD in the anti-CTLA-4 antibody combination regimen and the frequency was not suppressed as much as expected in this study, and further studies are needed to elucidate the mechanisms of HPD in HCC.

Fig. 3. HPD patients with ICI therapy. **a** We present a HPD case of anti-PD-1/PD-L1 monotherapy. Female, 60s, PS1, CP7B, BCLC stage B up-to-7 criteria IN HCC. Her blood tests were negative for HBs antigen and HCV antibody, and she was diagnosed as NASH. She was treated with nivolumab as 1st line. Liver biopsy prior to anti-PD-1 antibody administration showed well-differentiated HCC, IHC showed positive nuclear β-catenin staining, positive GS, negative PD-L1 staining. In the second month after nivolumab administration, both the maximum tumor diameter and serum tumor markers increased rapidly, and the patient was judged HPD, so the ICI treatment was discontinued and the disease was controlled by TACE. **b** We present a HPD case of anti-PD-1/PD-L1 combined VEGF antibody. Female, 40s, PS0, CP5A, BCLC stage C with peritoneal dissemination and multiple lung metastases. Her blood test was negative for HBs antigen and HCV antibody. 1st line was lenvatinib, but due to PD, atezolizumab plus Bevacizumab was administered as 2nd line. New intrahepatic lesions rapidly increased at 6 weeks after initiating combination therapy with PD-L1 antibody and VEGF antibody; ICI was continued with addition of TACE, but treatment was discontinued due to HPD of intrahepatic lesions. The patient was promptly switched to lenvatinib, which resulted in prominent shrinkage of intrahepatic lesions and serum tumor markers within the normal level. ICI, immune checkpoint inhibitor; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; VEGF, vascular endothelial growth factor; HCV, hepatitis C virus; HBV, hepatitis B virus; NASH, nonalcoholic steatohepatitis; HCC, hepatocellular carcinoma; BCLC, Barcelona Clinic Liver Cancer; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; PD, progressive disease; HPD, hyper progressive disease.

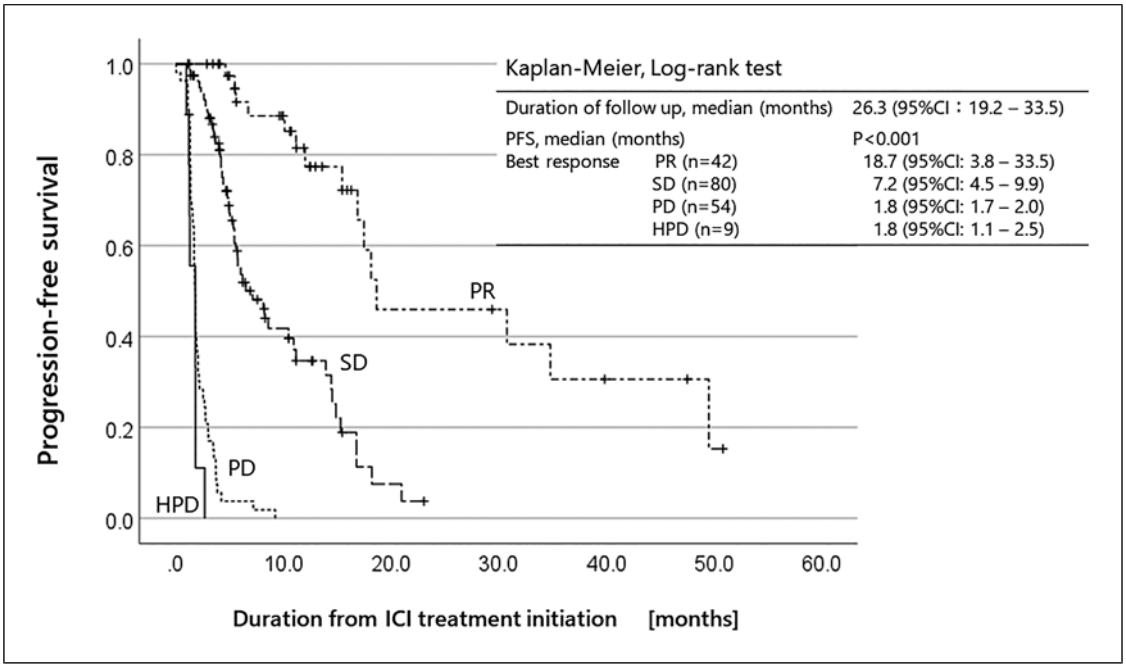

Fig. 4. Kaplan-Meier analysis of progression-free survival per best response to ICI treatment The median PFS was 18.7 months in best response PR to ICI therapy, 7.2 months in SD, and 1.8 months in PD/HPD patients ($p < 0.001$). ICI, immune checkpoint inhibitor; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; VEGF, vascular endothelial growth factor; CTLA4, cytotoxic T-lymphocyte-associated protein 4; PR, partial response; SD, stable disease; PD, progressive disease; HPD, hyper progressive disease; PFS, progression-free survival; CI, confidence interval.

Table 4. Univariate analysis of factors contributing to HPD

	Anti-PD-1/PD-L1 monotherapy $n = 6$	Anti-PD-1/PD-L1 + VEGF antibody $n = 2$	Anti-PD-1/PD-L1 + anti-CTLA-4 $n = 1$	All patients $n = 9$
Age	N.S	$p = 0.005$	N.S	$p = 0.014$
Sex	N.S	N.S	N.S	N.S
Etiology, HCV/HBV/NBNC	N.S	N.S	N.S	N.S
BCLC stage	N.S	N.S	N.S	N.S
1st line/later treatment	N.S	N.S	N.S	N.S
Platelets	N.S	N.S	N.S	N.S
WBC	N.S	N.S	N.S	N.S
NLR	N.S	N.S	N.S	N.S
CRP	N.S	N.S	N.S	$p = 0.060$
Serum albumin	N.S	N.S	N.S	N.S
Total bilirubin	$p = 0.017$	N.S	N.S	N.S
ALBI score	N.S	N.S	N.S	N.S
ALT	N.S	N.S	N.S	N.S
AST	N.S	N.S	N.S	$p = 0.054$
Serum AFP level	N.S	N.S	N.S	$p = 0.028$
DCP	N.S	N.S	N.S	N.S

PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; VEGF, vascular endothelial growth factor; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; HCV, hepatitis C virus; HBV, hepatitis B virus; NBNC, negative for hepatitis B surface antigen and hepatitis C antibody; BCLC, Barcelona Clinic Liver Cancer; WBC, white blood cell; NLR, neutrophil-lymphocyte ratio; CRP, C-reactive protein; ALBI, albumin-bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; N.S, not significant.

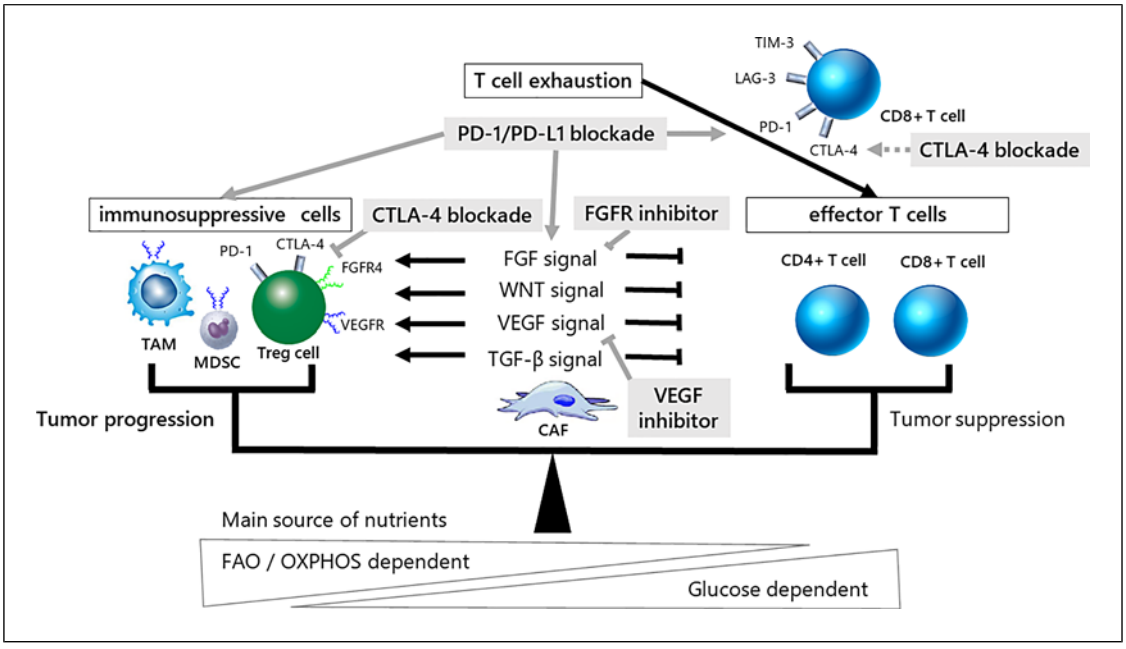

Fig. 5. Tumor immune microenvironment and drug therapy on the delicate balance. Immunosuppressive cells and effector T cells are in constant antagonism, and tumors continue to grow by overcoming nutritional competition or by taking advantage of immune escape. Tumors activate the VEGF, FGFR, and TGF-β pathways to proliferate. HPD is a syndrome that includes a variety of conditions that are very difficult to explain by a single mechanism. When immune escape is lifted by PD-1/PD-L1 signaling blockade, not only exhausted effector T cells improve, but also immunosuppressive cells proliferate and become activated. Simultaneous blockade of the VEGF or FGF pathways, and suppress of Treg cells with anti-CTLA4 antibodies may prevent the reversal of the equilibrium ratio of effector CD8+ T cells to immunosuppressive cells including Treg cells. PD-1, programmed cell death 1; PD-L1, programmed death-ligand 1; CTLA4, cytotoxic T-lymphocyte-associated protein 4; Treg cell, regulatory T cell; eTreg cell, effector Treg cell; exCD8+ T cell, exhausted CD8+ T cell; MDSCs, myeloid-derived suppressor cells; TAM, tumor-associated macrophage; CAF, cancer-associated fibroblast; APC, antigen presenting cell; DC, dendritic cell; VEGF, vascular endothelial growth factor; FGFR, fibroblast growth factor receptor; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor-β.

Limitations of this study include the following: single-center study, data only Japanese without including various ethnic groups, small sample size, prospective in anti-PD-1/PD-L1 monotherapy group and anti-CTLA-4 antibody combined group, but almost retrospective in anti-VEGF combined group. We cannot explore this further because we did not have access to pathological tissues before and after HPD. No predictive biomarkers were found. Therefore, this study contains elements of a proof-of-concept study, and future prospective multicenter studies are needed to confirm the frequency of HPD.

Conclusion

HPD is defined as unexpected rapid tumor growth specific to ICI therapy; tumor growth is triggered when blockade of the PD-1/PD-L1 signal pathway leads to increased proliferation of immunosuppressive cells such as Treg cells and TAMs rather than effector T cells. The combination of anti-VEGF antibody was shown to reduce the frequency of HPD. The frequency of HPD with anti-CTLA-4 antibodies regimen, which suppresses Treg cells and corrects the balance between Treg cells and effector T cells, will be further clarified in real-world clinical practice in the future.

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Statement of Ethics

IRB approval R02-258 (approved in 2021). This study was approved by the Institutional Review Board and Medical Ethics Committee of the Kindai University Hospital (IRB approval R02-258), and the written informed consent was obtained from all the patients.

Conflict of Interest Statement

M.K. has received grants from Taiho Pharmaceuticals, Chugai Pharmaceuticals, Otsuka, Takeda, Sumitomo Dainippon-Sumitomo, Daiichi Sankyo, AbbVie, Astellas Pharma, and Bristol-Myers Squibb. He has also received grants and personal lecture fees from Merck Sharpe and Dohme (MSD), Eisai, and Bayer, and is an adviser for MSD, Eisai, Bayer, Bristol-Myers Squibb, Eli Lilly, Chugai, AstraZeneca, and ONO Pharmaceuticals. He is an Editor-in-Chief of LIVER CANCER. K.U. has received Honoraria from: Bayer AG, Eisai Co. Ltd., Merck Sharp and Dohme, Eli Lilly and Company, Chugai Pharmaceutical Co., Ltd., Takeda Pharmaceutical Co., Ltd., Pfizer Inc., Otsuka Pharmaceutical Co., Ltd., Sumitomo Dainippon Pharma Co., Ltd., Taiho Pharmaceutical. Co. Ltd., EA Pharma Co., Ltd., AbbVie GK and Kowa Co., Ltd. He has received consulting or advisory fees from: Eisai Co. Ltd., Eli Lilly and Company, Chugai Pharmaceutical Co., Ltd., Takeda Pharmaceutical Co., Ltd., and Pfizer Inc. T.A., M.M., H.C., M.T., S.H., H.I., Y.M., A.Y., K.S., and M.T., no relevant conflicts of interest to disclose. N.N. is an Editorial Board member of liver cancer.

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Author Contributions

Conceptualization: M.K. and K.U.; methodology and supervision: M.K.; software, validation, and formal analysis: T.A.; investigation: T.A. and M.T.; data curation: A.Y., K.U., M.M., H.C., M.T., S.H., H.I, and Y.M; writing – original draft preparation: T.A. and N.N.; writing – review and editing and project administration: N.N. and M.K.; visualization: K.S. and M.T.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its supplementary material files. Further inquiries can be directed to the corresponding author.

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