Association between contrast enhancement on contrast-enhanced CT and lenvatinib effectiveness in hepatocellular carcinoma

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Abstract. The aim of the present study was to investigate whether the degree of contrast enhancement on contrast-enhanced (CE)-CT can predict the prognosis of patients with hepatocellular carcinoma (HCC) treated with lenvatinib (LEN). A total of 67 consecutive patients with LEN-treated HCC were retrospectively analysed. In the pretreatment CE-CT, the CT values were measured using a region of interest within the main nodule and the liver parenchyma in the arterial phase, and the macroscopic degree of contrast enhancement of the tumour area was quantified by calculating the enhancement ratio (ER) of the liver parenchyma. The associations of pretreatment ER with progression-free survival (PFS) and overall survival (OS) were then investigated. There were 20, 27 and 20 patients in the ER ≥1.5, 1.0≤ ER <1.5 and ER <1.0 groups, respectively. There was no significant difference in the PFS and OS among the three ER groups (PFS, P=0.63; OS, P=0.455). The ER <1.0 group had significantly more patients with larger tumour diameters, Barcelona Clinic Liver Cancer (BCLC) stage C with extrahepatic metastases, and higher des-γ-carboxy prothrombin values compared with the ER ≥1.0 group, suggesting that ER <1.0 reflected more aggressive types of HCC. The multivariate analysis revealed tumour size and α-fetoprotein as independent predictors of shorter PFS. Albumin-bilirubin grade 2 and BCLC stage C were significant predictors of poor OS, whereas the ER was confirmed as a non-significant predictor of both PFS and OS. Only non-alternating LEN and transarterial therapy (AT) were identified as independent predictors of unfavourable OS in patients with BCLC stage B HCC. Therefore, LEN has a strong therapeutic effect on HCC, regardless of the degree of contrast enhancement. Furthermore, AT may prolong the OS of LEN-treated patients with BCLC stage B HCC, regardless of tumour vascularity.

Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver and is among the leading causes of cancer-related mortality worldwide (1). Most patients with HCC are diagnosed at an advanced stage, resulting in a poor prognosis (2). Recently, several types of molecular targeted agents (MTAs) have been approved for the treatment of unresectable HCC (3,4). Lenvatinib (LEN) is an MTA approved as a first-line treatment; it targets vascular endothelial growth factor receptors (VEGFRs) 1-3, fibroblast growth factor receptors (FGFRs) 1-4, platelet-derived growth factor receptor-α, RET and KIT (5). LEN exerts strong antitumour effects by inhibiting both VEGFR- and FGFR-induced angiogenesis, as well as FGFR- and RET-induced abnormal cancer cell proliferation (6).

Several studies have reported therapeutic effect-associated imaging findings of LEN (7-10). As early imaging biomarkers, a reduction in tumour enhancement intensity in the arterial phase on contrast-enhanced (CE)-CT at 2 weeks and a decrease in the time-intensity curve in the arterial phase on contrast-enhanced ultrasound at 1 week are useful predictors of LEN effectiveness (7,8). On pretreatment imaging, the heterogeneous enhancement pattern of HCC in the arterial phase on CE-CT and ^{18}F-fluorodeoxyglucose (FDG) uptake on ^{18}F-FDG-positron emission tomography (PET)/CT for HCC may be useful predictors of early response to LEN (9,10).

MTAs exert antitumour effects mainly by inhibiting angiogenesis. Imaging of angiogenesis is best performed using CE-CT and MRI. The enhancement pattern of HCC depends on the changes in the vasculature that occur during tumour angiogenesis (3). Clinically, however, it is unclear whether strong tumour staining is associated with a high therapeutic efficacy of MTAs, and vice versa.

To the best of our knowledge, there have been no reports to date focusing on the association between the degree of contrast enhancement on pretreatment imaging and LEN

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effectiveness in HCC. The aim of the present study was to retrospectively investigate the association between the precise degree of contrast enhancement on pretreatment CE-CT and the therapeutic efficacy of LEN in patients with HCC.

Materials and methods

Patients. Between March 2018 and December 2020, 114 consecutive patients with HCC who received LEN at Kurume University Hospital (Kurume, Japan) were enrolled in the present study. The study was conducted in accordance with the principles outlined in the Declaration of Helsinki, and the protocol was approved by the Ethics Review Committee of Kurume University (approval no. 20192). An opt-out approach was used to obtain informed consent from the patients, and personal information was protected during data collection.

The inclusion criteria for this study were as follows: i) Patients with intrahepatic tumours; ii) patients taking LEN for >30 days; and iii) patients who underwent CE-CT at the scheduled time prior to LEN administration. Of the 114 patients, 67 met the inclusion criteria and were subjected to further radioclinical analysis.

Administration of LEN. LEN (Eisai Co., Ltd.) was orally administered at a dose of 12 mg for a body weight of ≥60 kg and at 8 mg for a body weight of <60 kg, once per day. Adverse events (AEs) were assessed using the National Cancer Institute's Common Terminology Criteria for Adverse Events, version 4.0 (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm). For patients with AEs of grade ≥3, either LEN dose reduction or discontinuation was allowed. If required, the dose was reduced from 12 to 8 mg, or from 8 to 4 mg. In addition, alternate-day administration or 5 days-on/2 days-off (weekends-off) were also performed according to the patient's condition. The relative dose intensity (RDI) was defined as the actual dose divided by the standard dose. The 8-week RDI (8W-RDI) was calculated as the cumulative dose within the initial 8 weeks of starting LEN treatment divided by the standard dose (11).

Evaluation of therapeutic response and follow-up schedule. Therapeutic response was evaluated using dynamic CT or MRI at 4-6 weeks after the initiation of LEN according to the Modified Response Evaluation Criteria in Solid Tumours (mRECIST) (12), and at intervals of 2-3 months thereafter, until patient death or study completion.

CT protocol and image analysis. CT examinations were performed using multi-detector raw CT scanners: Discovery, GE Healthcare (n=21); Revolution, GE Healthcare (n=20); iCT, Philips Healthcare (n=18); Aquilion, Canon Medical Systems Corporation (n=4); and SOMATOM, Siemens Healthineers (n=4). Multiphasic dynamic CT was performed with a delay of 30-40 sec (arterial phase), 60-70 sec (portal phase) and 150-180 sec (equilibrium phase) with intravenous administration of non-ionic contrast material at a rate of 3-4 ml/sec using an automated power injector. The dose of contrast medium was adjusted according to the renal function of each patient.

If patients had multiple HCC nodules, the largest representative nodule was examined per patient. On CE-CT, the CT value was measured using a region of interest (ROI) in the arterial phase with a maximum round or oval area set within the tumour in the slice where the tumour was best visualised (almost equal to the slice including the largest diameter of the tumour) using Picture Archiving and Communication System (Fig. 1A). If the lipiodol was partially deposited in the tumour to be evaluated, the ROI was carefully defined to avoid the lipiodol deposition area based on plain (non-CE) CT (Fig. 1B). Furthermore, if the main tumour exhibited a poor contrast enhancement, we referred to ^{18}F-FDG-PET/CT to confirm FDG uptake (Fig. 1C) or previous CT or MRI scans to confirm the increase in size and to distinguish necrotic tissue from viable lesions. The CE-CT imaging protocols were uniform across all cases. However, the dose of contrast medium was reduced in some cases due to renal dysfunction. This could have influenced the contrast enhancement and, thus, the absolute CT value of the nodule in the arterial phase could not be used. Therefore, the background liver was set as the object. The ROI in the liver parenchyma was placed near the nodule to be evaluated in the same slice with a size of at least 100 mm^2, excluding major vessels and artifacts. The enhancement ratio (ER) was calculated as follows: ER = CT value of ROI in the tumour/CT value of ROI in the liver parenchyma. Furthermore, the ER values were divided into three groups: ER <1.0 (low contrast enhancement compared with the liver parenchyma), 1.0≤ ER <1.5 (moderate contrast enhancement compared with the liver parenchyma) and ER ≥1.5 (high contrast enhancement compared with the liver parenchyma).

Alternating LEN and transarterial therapy (AT). In addition, as will be described later, AT has been employed for cases with progressive disease (PD) at our facility. Details of AT are provided below.

AT involves the administration of treatment using a transarterial approach, such as transcatheter arterial chemoembolization (TACE) and hepatic arterial infusion chemotherapy (HAIC), upon the development of PD, as indicated by the reappearance of contrast enhancement in the tumour or appearance of new lesions in the liver during LEN treatment.

The details of AT administration were as follows: i) LEN was discontinued 2 days prior to transarterial therapy; ii) TACE was performed, except in cases exhibiting multinodular or invasive growth, in which HAIC was performed instead; iii) within 2 weeks of transarterial therapy, depending on the condition of each patient, LEN administration was resumed at the same or half the dose as that administered prior to transarterial therapy (13).

TACE and HAIC protocol. Angiography was performed for the celiac and the common hepatic arteries using a 3- or 4-Fr catheter, and digital subtraction angiography was performed using a non-ionic iodine contrast agent. The tumour-containing segment was evaluated by imaging techniques including cone-beam CT. Subsequently, a 1.7- or 1.9-Fr microcatheter (Piolax, Inc.) was inserted into the subsegmental artery with the adapted microwire (Piolax, Inc.). The catheter was advanced towards the tumour-feeding artery. Depending on the size and number of tumours, conventional TACE (C-TACE) was performed using 20-50 mg of epirubicin (Nippon Kayaku Co., Ltd.) or cisplatin (Nippon Kayaku Co., Ltd.) with lipiodol
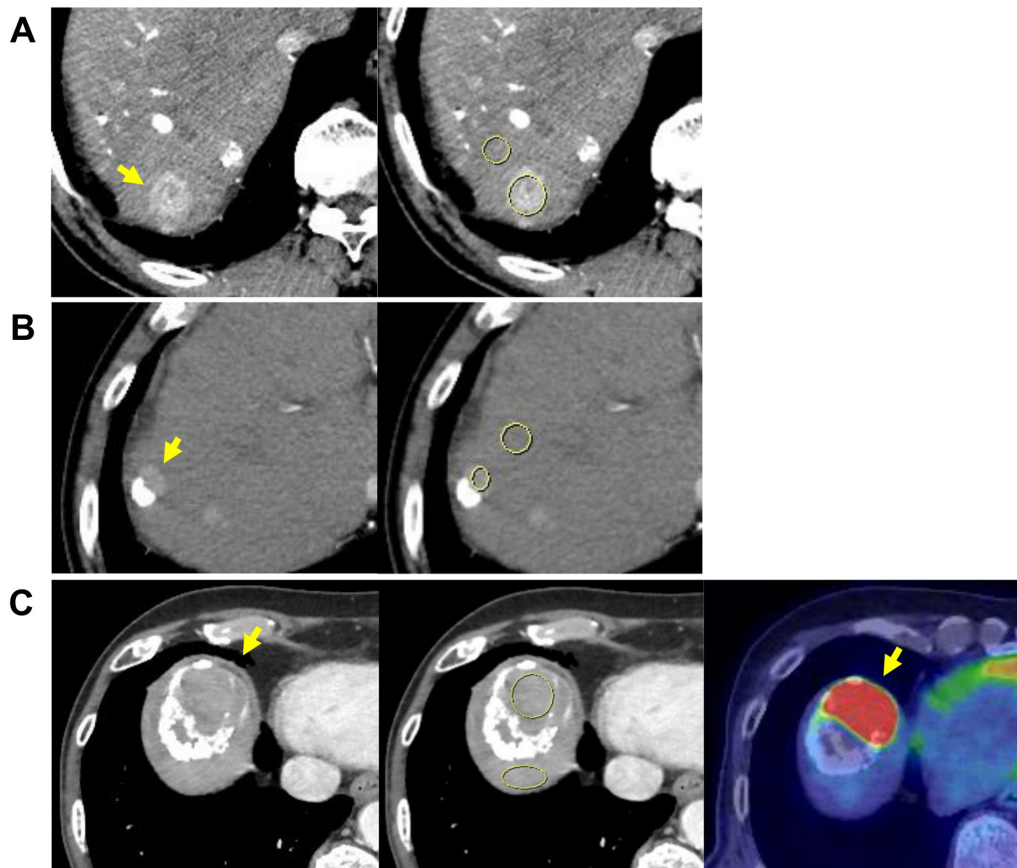
Figure 1. Method of ER measurement. (A) CT value was measured using a ROI in the arterial phase with a maximum round or oval area within the tumour in the slice where the tumour was best visualised (almost equal to the largest diameter of the tumour), and ER was calculated as follows: ER = CT value of ROI in the tumour/CT value of ROI in the liver parenchyma. (B) A case of lipiodol deposition in the main tumour. The ROI was carefully set to avoid the lipiodol deposition area. (C) A case of poor contrast enhancement in the main tumour. We referred to ^{18}F-FDG- positron emission tomography/CT to confirm FDG uptake. The ER value in the aforementioned cases was (A) 1.45, (B) 1.38 and (C) 0.93. Arrows are used to indicate the tumour; circles are used to indicate the ROI in the tumour and liver parenchyma. ER, enhancement ratio; ROI, region of interest; FDG, fluorodeoxyglucose.

(Guerbet Co., Ltd.), and was absorbed by gelatin sponge particles (Nippon Kayaku Co., Ltd.) (13,14).

HAIC was conducted via the insertion of an implanted catheter (Piolax, Inc.) An indwelling catheter (5-Fr W-Spiral catheter; Piolax, Inc.) was inserted through the right femoral artery, with the distal end of the catheter extending into the hepatic or gastroduodenal artery, and the proximal end connected to the port system (Soph-A-Port®; Sophysa). Following the inpatient regimen of HAIC, 50 mg of fine-powder cisplatin was suspended in 5-10 ml of lipiodol; the suspension volume was determined according to the tumour size [the volume (in ml) was 1-2 less than the maximum diameter (in cm)]. On day 1, the cisplatin-lipiodol suspension was injected through the implanted angiography catheter, followed by the injection of 250 mg of 5-fluorouracil (5-FU). Then, 1,250 mg of 5-FU was continuously infused for 5 days using an infu-sion balloon pump (Surefuser™+, Nipro Pharma Corporation). This regimen was administered once per week during the first 2 weeks of admission, and then a combination of 20 mg cisplatin with lipiodol and 5-FU (500-1,250 mg) was infused every 2 weeks at the outpatient department until disease progression (13,15).

Statistical analysis. Continuous variables are expressed as median (range). The χ^2 test or Fisher's exact test were used to analyse the association between categorical variables, and Wilcoxon's test was used to analyse the association between continuous variables. Progression-free survival (PFS) and overall survival (OS) were calculated from the date of initia-tion of LEN administration to tumour progression and death, respectively. A Cox proportional hazard model was used for univariate and multivariate analyses to identify any indepen-dent variables associated with PFS and OS. The PFS and OS rates were evaluated using the Kaplan-Meier method, and the log-rank test was used to compare the patient groups. P<0.05 was considered to indicate statistically significant differences. JMP software (version 15; SAS Institute, Inc.) was used for all statistical analyses.

Results

Patient and tumour characteristics. The patient and tumour characteristics are summarised in Table I. The majority of the patients had Child-Pugh class A liver cirrhosis, with the excep-tion of 2 patients with class B cirrhosis. Albumin-bilirubin (ALBI) grade 1 and 2 were observed in 30 and 37 patients, respectively. A total of 2 patients had stage A tumours, 43 had stage B tumours and 22 had stage C tumours as per the Barcelona Clinic Liver Cancer (BCLC) staging system (16). Only 1 patient had macrovascular invasion; 21 of the 22 patients

Table I. Patient and tumour characteristics.

Characteristics	n=67
Age (years)	73 (47-90)
Sex (male/female)	54/13
Aetiology (HBV/HCV/others)	9/30/28
Albumin (g/dl)	3.9 (2.9-4.7)
Total bilirubin (mg/dl)	0.7 (0.4-1.9)
Prothrombin activity (%)	98 (56-130)
Child-Pugh score (A/B/C)	65/2/0
ALBI grade (1/2/3)	30/37/0
Tumour number (<5/5-10/≥10)	30/18/19
Tumour size (mm)	25 (10-170)
Macrovascular invasion (present/absent)	1/66
Extrahepatic metastasis (present/absent)	21/46
BCLC stage (A/B/C)	2/43/22
Up-to-seven criteria (within/beyond)	25/42
AFP (ng/ml)	25.5 (1.4-118,560)
DCP (mAU/ml)	396 (12-179,531)
TACE refractoriness (yes/no)	51/16
ER	1.28 (0.01-2.30)
ER <1.0/1.0≤ ER <1.5/ER ≥1.5	20/27/20
Initial dose of lenvatinib (8/12 mg)	46/21
8W-RDI (%)	75 (15-100)

Continuous variables are expressed as median (range). HBV, hepatitis B virus; HCV, hepatitis C virus; ALBI grade, albumin-bilirubin grade; BCLC stage, Barcelona Clinic Liver Cancer stage; AFP, α-foetoprotein; DCP, des-γ-carboxy prothrombin; TACE, transcatheter arterial chemo-embolization; ER, enhancement ratio; 8W-RDI, relative dose intensity after 8 weeks of lenvatinib induction.

with BCLC stage C HCC had extrahepatic metastasis. There were 25 patients within up-to-seven criteria [defined as HCC with 7 as the sum of the size of the largest tumour (in cm) and the number of intrahepatic tumours] and 42 beyond this criteria.

Among the 67 patients, 51 had HCC that was refractory to TACE. C-TACE was performed in 46 patients and drug-eluting beads TACE (DEB-TACE) was performed in 5 patients as pretreatment of LEN. Lesions treated with DEB-TACE may display reduced enhancement and lower ER. However, since the main nodules to be evaluated in these cases were new lesions appearing after DEB-TACE (n=1), or previously existing lesions increasing in size with no therapeutic efficacy (n=2), or lesions displaying tumour angiogenesis resumption and increase in size after DEB-TACE (n=2), it was hypothesized that DEB-TACE did not affect the evaluation of the contrast enhancement in this study. Therefore, these cases were included in the present analysis. The initial dose of LEN was 8 mg for 46 patients and 12 mg for 21 patients. The RDI at 8 weeks was 75% (range, 15-100%). By the time LEN was discontinued, dose reduction had been performed in 50 (74.6%) patients. The main reasons for dose reduction were fatigue (n=14), decreased appetite (n=10), proteinuria (n=4), ascites (n=4), palmar-plantar erythrodysaesthesia syndrome (n=3), diarrhoea (n=3), elevated aspartate aminotransferase levels (n=2), high ammonia levels (n=2), hypertension (n=1), hoarseness (n=1), neutropenia (n=1), increased blood bilirubin levels (n=1), hyperthyroidism (n=1), vomiting (n=1), headache (n=1) and gingival bleeding (n=1). The contrast medium dose was reduced in 24 patients (35.8%) due to renal dysfunction. The CT values in the ROI of the tumour and liver parenchyma were 100.56 Hounsfield units (HU) (range, 0.48-169.86 HU) and 80.79 HU (range, 54.79-113.41 HU), respectively. The areas of the ROI in the tumour and liver parenchyma were 176.98 mm^2 (range, 25.51-5332.46 mm^2) and 256.68 mm^2 (range, 112.55-947.37 mm^2), respectively. The median ER was 1.28 (range, 0.01-2.3).

Evaluation using mRECIST after the initial treatment with LEN. Complete response, partial response, stable disease and PD were observed in 10.4% (7/67), 46.3% (31/67), 32.8% (22/67) and 10.4% (7/67) of the patients, respectively. The overall objective response rate (ORR) and disease control rate (DCR) were 56.7% (38/67) and 89.6% (60/67), respectively.

Comparison of PFS and OS among the ER groups. The PFS and OS curves stratified by ER in the arterial phase on CE-CT are shown in Fig. 2. The ER ≥1.5, 1.0≤ ER <1.5 and ER <1.0 groups comprised 20, 27 and 20 patients, respectively. There was no significant difference among the ER groups (PFS, P=0.63; OS, P=0.455).

Comparison of patient and tumour characteristics between the ER ≥1.0 and <1.0 groups. Next, we focused on comparing baseline patient and tumour characteristics between the ER ≥1.0 and ER <1.0 groups, based on the high or low contrast enhancement compared with that of the liver parenchyma. A comparison of patient and tumour characteristics between the two groups is shown in Table II. The ER <1.0 group comprised significantly more cases with larger tumour diameters (P<0.001), BCLC stage C with extrahepatic metastases (P=0.007) and higher des-γ-carboxy prothrombin (DCP) values (P=0.046) compared with the ER ≥1.0 group, suggesting that ER <1.0 was characteristic of aggressive types of HCC. There was no significant difference in the ORR and DCR between the ER groups (ORR, P=0.072; DCR, P=0.938).

Factors associated with PFS and OS. Independent predictors of PFS (Table III) and OS (Table IV) were investigated using Cox proportional hazard analysis in all patients. The results of the univariate analysis showed that tumour size (≥30 mm; P=0.02), α-foetoprotein (AFP) (≥100 ng/ml; P=0.04) and DCP (≥200 mAU/ml; P=0.03) were significant risk factors for PFS. The multivariate analysis revealed that tumour size (≥30 mm; HR=2.14; 95% CI: 1.18-3.85; P=0.012) and AFP (≥100 ng/ml; HR=1.98; 95% CI: 1.08-3.63; P=0.026) were independent predictors of shorter PFS. The results of the univariate analysis showed that BCLC stage C (P=0.019) was a significant risk factor for OS. The multivariate analysis revealed that ALBI grade 2 (HR=2.60; 95% CI: 1.08-6.26; P=0.034) and BCLC stage C (HR=2.86; 95% CI: 1.28-6.38; P=0.011) were independent predictors of poor OS. The ER was confirmed to be a non-significant predictor of both PFS and OS.

Factors associated with OS in patients with BCLC stage B HCC. As there were 43 HCC patients with BCLC B, among

Table II. Comparisons of patient and tumour characteristics based on the ER.

Characteristics	ER ≥1.0	ER <1.0	P-value
Age (years)	76 (54-90)	73 (47-88)	0.344
Sex (male/female)	37/10	16/4	0.906
Aetiology (HBV/HCV/others)	5/23/19	4/7/9	0.451
Albumin (g/dl)	3.8 (2.9-4.7)	4.0 (3.1-4.4)	0.287
Total bilirubin (mg/dl)	0.73 (0.4-1.9)	0.70 (0.5-1.3)	0.967
Prothrombin activity (%)	96 (56-124)	103 (76-130)	0.415
Child-Pugh score (A/B/C)	45/2/0	20/0/0	0.349
ALBI grade (1/2/3)	18/29/0	12/8/0	0.102
Tumour number (<5/5-10/≥10)	18/15/14	12/3/5	0.214
Tumour size (mm)	22 (10-170)	42 (17-122)	<0.001
Macrovascular invasion (present/absent)	1/46	0/20	0.511
Extrahepatic metastasis (present/absent)	9/38	12/8	0.001
BCLC stage (A/B/C)	2/35/10	0/8/12	0.007
Up-to-seven criteria (within/beyond)	17/30	8/12	0.767
AFP (ng/ml)	26.1 (1.6-40804)	25.0 (1.4-118560)	0.827
DCP (mAU/ml)	232 (12-15120)	1408 (22-179531)	0.046
TACE refractoriness (yes/no)	40/7	11/9	0.008
ER	1.45 (1.01-2.30)	0.84 (0.01-0.98)	<0.001
Initial dose of lenvatinib (8/12 mg)	34/13	12/8	0.319
8W-RDI (≥75%/<75%)	24/23	10/10	0.937
Objective response rate	30 (63.8%)	8 (40%)	0.072
Disease control rate	42 (89.3%)	18 (90%)	0.938

Continuous variables are expressed as median (range). HBV, hepatitis B virus; HCV, hepatitis C virus; ALBI grade, albumin-bilirubin grade; BCLC stage, Barcelona Clinic Liver Cancer stage; AFP, α-foetoprotein; DCP, des-γ-carboxy prothrombin; TACE, transcatheter arterial chemo-embolization; ER, enhancement ratio; 8W-RDI, relative dose intensity after 8 weeks of lenvatinib induction.
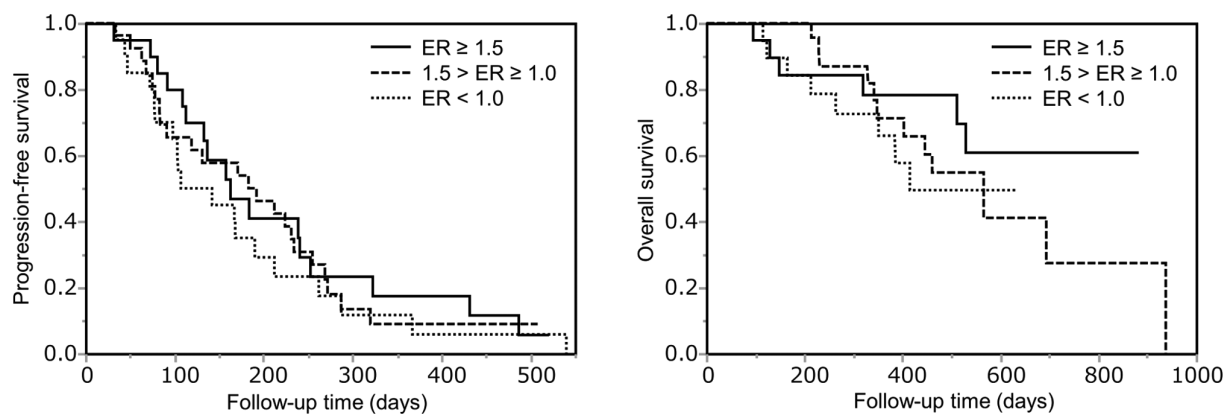

Figure 2. Kaplan-Meier curves of the estimated PFS and OS based on the differences in the ER. The groups with ER ≥1.5, 1.0 ≤ER <1.5 and ER <1.0 included 20, 27 and 20 patients, respectively. There was no significant difference among the ER groups (PFS, P=0.63; OS, P=0.455). ER, enhancement ratio; PFS, progression-free survival; OS, overall survival.

whom 23 patients (53%) underwent AT as a post-PD treatment, it was considered important that the effect of such additive AT on OS was assessed. Therefore, the OS of 43 patients with BCLC stage B was analysed, focusing on the presence or absence of AT (Table V). The univariate analysis revealed that ALBI grade 2 (HR=4.77; 95% CI: 1.05-21.55; P=0.043) and non-AT (HR=20.95; 95% CI: 2.7-162.55; P=0.004) were significant factors affecting OS. The multivariate analysis revealed only non-AT as an independent predictor of unfavourable OS (HR=16.42; 95% CI: 2.03-133.04; P=0.009). ER was not identified as a significant predictor in this analysis.

Discussion

The present study evaluated the association between the precise degree of contrast enhancement and the therapeutic

Table III. Univariate and multivariate analyses of progression-free survival of patients with hepatocellular carcinoma.

Variables	Univariate analysis		Multivariate analysis	
	HR (95% Cl)	P-value	HR (95% Cl)	P-value
Age (≥70 years)	0.96 (0.56-1.66)	0.883		
Sex (male)	0.97 (0.50-1.87)	0.916		
HCV positivity	1.62 (0.95-2.78)	0.073	1.60 (0.93-2.77)	0.091
ALBI grade 2	1.17 (0.69-1.96)	0.561		
Tumour number (≥5)	1.04 (0.62-1.75)	0.882		
Tumour size (≥30 mm)	1.85 (1.10-3.12)	0.020	2.14 (1.18-3.85)	0.012
BCLC stage C	1.12 (0.64-1.96)	0.679		
Up-to-seven criteria (beyond)	1.36 (0.79-2.33)	0.272		
AFP (≥100 ng/ml)	1.76 (1.03-3.00)	0.040	1.98 (1.08-3.63)	0.026
DCP (≥200 mAU/ml)	1.82 (1.06-3.12)	0.030	1.33 (0.75-2.36)	0.329
TACE refractoriness	1.06 (0.58-1.95)	0.839		
ER <1.0	1.30 (0.74-2.26)	0.364		
Initial dose of lenvatinib 8 mg	1.55 (0.89-2.72)	0.123		
8W-RDI (<75%)	1.53 (0.91-2.58)	0.108		

Continuous variables are expressed as median (range). HCV, hepatitis C virus; ALBI grade, albumin-bilirubin grade; BCLC stage, Barcelona Clinic Liver Cancer stage; AFP, α-foetoprotein; DCP, des-γ-carboxy prothrombin; TACE, transcatheter arterial chemoembolization; ER, enhancement ratio; 8W-RDI, relative dose intensity after 8 weeks of lenvatinib induction.

Table IV. Univariate and multivariate analyses of overall survival of patients with hepatocellular carcinoma.

Variables	Univariate analysis		Multivariate analysis	
	HR (95% Cl)	P-value	HR (95% Cl)	P-value
Age (≥70 years)	0.98 (0.42-2.28)	0.966		
Sex (male)	1.06 (0.40-2.84)	0.908		
HCV positivity	1.15 (0.52-2.52)	0.729		
ALBI grade 2	2.34 (0.98-5.61)	0.057	2.60 (1.08-6.26)	0.034
Tumour number (≥5)	1.06 (0.48-2.37)	0.884		
Tumour size (≥30 mm)	1.71 (0.78-3.77)	0.181		
BCLC stage C	2.58 (1.17-5.69)	0.019	2.86 (1.28-6.38)	0.011
Up-to-seven criteria (beyond)	1.18 (0.51-2.75)	0.694		
AFP (≥100 ng/ml)	1.70 (0.77-3.73)	0.186		
DCP (≥200 mAU/ml)	1.82 (0.79-4.19)	0.157		
TACE refractoriness	1.09 (0.41-2.94)	0.859		
ER <1.0	1.47 (0.63-3.46)	0.373		

Continuous variables are expressed as median (range). HCV, hepatitis C virus; ALBI grade, albumin-bilirubin grade; BCLC stage, Barcelona Clinic Liver Cancer stage; AFP, α-foetoprotein; DCP, des-γ-carboxy prothrombin; TACE, transcatheter arterial chemoembolization; ER, enhancement ratio.

efficacy of LEN. Through various analyses, it was observed that there was no significant difference in PFS and OS among the ER groups; therefore, ER was not found to be a significant predictor in LEN-treated patients with HCC.

On dynamic CT, hypoattenuation in the arterial phase is frequently observed in both well-differentiated and poorly differentiated HCCs (17,18). Furthermore, sarcomatous hepatic tumours generally exhibit hypovascularity, which is seen as rim enhancement or non-enhancement on arterial phase imaging (19). Thus, nodules with poor contrast enhancement in the arterial phase are considered as either less or highly malignant. The present study included two cases of BCLC stage A (both in the ER >1.5 group); however, advanced or unresectable HCCs, for which LEN treatment is usually recommended, are in BCLC stage B or C (2). Although this could not be ascertained in the present study, as histopathological

Table V. Univariate and multivariate analyses of overall survival of patients with BCLC stage B hepatocellular carcinoma (n=43).

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Age (≥70 years)	1.33 (0.36-4.86)	0.670		
Sex (male)	0.97 (0.27-3.53)	0.963		
HCV positivity	0.85 (0.28-2.53)	0.769		
ALBI grade 2	4.77 (1.05-21.55)	0.043	2.12 (0.45-9.99)	0.341
Tumour number (≥5)	1.06 (0.33-3.53)	0.892		
Tumour size (≥30 mm)	1.08 (0.38-3.66)	0.770		
Up-to-seven criteria (beyond)	0.96 (0.29-3.11)	0.943		
AFP (≥100 ng/ml)	1.37 (0.46-4.07)	0.576		
DCP (≥200 mAU/ml)	2.16 (0.69-6.72)	0.180		
ER <1.0	0.91 (0.20-4.11)	0.901		
Non-AT	20.95 (2.7-162.55)	0.004	16.42 (2.03-133.04)	0.009

Continuous variables are expressed as median (range). HCV, hepatitis C virus; ALBI grade, albumin-bilirubin grade; BCLC stage, Barcelona Clinic Liver Cancer stage; AFP, α-foetoprotein; DCP, des-γ-carboxy prothrombin; TACE, transcatheter arterial chemoembolization; AT, alternating lenvatinib and transarterial therapy; ER, enhancement ratio.

examinations were not performed immediately prior to LEN administration, it is unlikely that well-differentiated HCCs were included. In fact, in the present study, the number of patients with aggressive HCC, characterized by large size, extrahepatic metastases and high DCP values, was significantly higher in the ER <1.0 group compared with the other groups. Although such HCCs are refractory to various treatments and have a poor prognosis (20,21), no significant difference in PFS has been reported. Previously, Kawamura et al (9) examined the association between PFS and the pretreatment enhancement patterns of HCC on CE-CT in LEN-treated patients. The patterns were defined as homogeneous or heterogeneous, and it was concluded that there was no difference in PFS between the patterns, despite the heterogeneity indicating the highly malignant nature of HCC (9,22). Although that evaluation method was quite different from the one used in the present study, the findings suggest that LEN exerts a strong therapeutic effect regardless of the degree of HCC differentiation.

In human HCC, CTNNB1 and TP53 mutations define two distinct tumour phenotypes, and histological subtypes are associated with clinical and molecular characteristics (23). The CTNNB1 mutation is frequent, even in well-differentiated HCC, whereas TP53 mutations are more frequent in poorly differentiated HCCs and those with foci of sarcomatous change (23,24). Recently, Rodríguez-Hernández et al (25) reported that LEN was more effective in moderately-to-poorly differentiated liver cancer cells with the p53 mutation. Thus, these reports support our finding that LEN is effective against moderately-to-poorly differentiated HCC.

In the present study, the ER was not identified as a significant predictor of PFS or OS. The multivariate analyses demonstrated that ALBI grade 2 and BCLC stage C were independent predictors of poor OS. ALBI grade is useful in predicting prognosis after HCC treatment, such as liver resection, radiofrequency ablation and TACE (26-28). Regarding the predictive potential of the ALBI grade in LEN treatment,

Ueshima et al (29) reported that HCC patients with ALBI grade 1 presented a higher response rate and lower frequency of treatment discontinuation due to severe AEs. Furthermore, Hiraoka et al (30) reported that modified ALBI grade 2b or 3 was superior to Child-Pugh classification in predicting poor prognosis of patients with HCC receiving LEN treatment. Although the modified ALBI grading system was not used in the present study, classical ALBI grade 2 was found to be a prognostic factor in patients with HCC receiving LEN treatment.

As there were 23 patients (53%) with BCLC stage B HCC in whom AT was performed after PD, a sub-analysis of OS in BCLC stage B patients was performed. Although 38 of the 43 patients (88%) treated with LEN had TACE-refractory HCC, the additive effect of AT significantly prolonged OS in this study. Previously, Kudo et al (31) reported that TACE plus sorafenib significantly improved PFS compared with TACE alone in patients with unresectable HCC. They considered that sorafenib-induced normalisation of tumour blood vessels leads to increased accumulation of TACE-derived mixture of lipiodol and anticancer agent compared with TACE alone. LEN is also an angiogenesis inhibitor, similar to sorafenib; therefore, it was suggested that a similar mechanism was involved in the limited number of patients with BCLC stage B disease. Accordingly, normalisation of the tumour vasculature may increase responsiveness to AT in hypovascular HCCs, which are known to hardly respond to TACE (32,33).

Recently, Shimose et al (13) reported that AT prolonged OS in LEN-treated patients with BCLC stage B HCC in the propensity score matching (PSM) analysis of their multicentre study. They deduced the mechanism underlying the efficacy of AT as follows: Transarterial therapy-induced intratumour ischaemia upregulates hypoxia-inducible factor 1-α expression, which leads to increased expression of its downstream angiogenic factors for tumour growth, and LEN administration after transarterial therapy suppresses the effects of these

angiogenic factors (34,35). Similarly, it was considered reasonable that the AT strategy contributed to the improved OS of the LEN-treated patients with BCLC stage B HCC in the present study.

There were certain limitations to the present study. First, this was a retrospective study that involved a small number of patients from a single centre; thus, there was a possibility of selection bias. Furthermore, the ER <1.0 group with larger tumour diameters, BCLC stage C with extrahepatic metastases and higher DCP values, consisted of only 20 patients, which may represent possible confounding factors and lead to underestimation of the impact of ER on PFS and OS. This may be resolved by matching tumour factors with PSM analysis in a larger number scale. Second, only one case of macrovascular invasion was enrolled. Thus, PFS and OS could not be analysed in HCC patients with macrovascular invasion. Third, a histological examination was not performed immediately prior to LEN administration. Therefore, the result of the present study was only the association between the contrast enhancement and the therapeutic effect. However, procedures like aspiration biopsy are invasive and have drawbacks, including sampling errors. Therefore, histological examination is often not performed immediately prior to LEN administration in the clinical setting. Therefore, we believe that this result may have potential benefits for LEN treatment. Finally, the ER used in the present study may be affected by the haemodynamic condition of organs, the types of CT devices and the types of contrast media, regardless of their superiority over absolute CT values in assessing the contrast enhancement on the tumour. Further studies are needed to determine whether pretreatment degree of contrast enhancement is important through prospective analyses in a larger number of cases with a unified CT device.

In conclusion, the lower pretreatment contrast enhancement in HCC did not contribute to the prediction of the PFS or OS in patients with LEN-treated HCC. The results of the present study suggested that LEN exerted a strong therapeutic effect on HCC, regardless of the degree of contrast enhancement in the tumour. In addition, AT may prolong the OS of patients with LEN-treated BCLC stage B HCC, regardless of the tumour vascularity.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SO participated in the conception and design of study, acquisition and interpretation of data, and drafting of the manuscript. SS participated in the conception and design of the study, acquisition and interpretation of data. SO and SS have seen and can confirm the authenticity of the raw data. TN, NK, YN, TS, HI, MN and RK participated in the acquisition of data. HK and TT participated in the interpretation of data and critical revision of the manuscript for important intellectual content. All the authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Review Committee of Kurume University (approval no. 20192). An opt-out approach was used to obtain informed consent from the patients, and personal information was protected during data collection.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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