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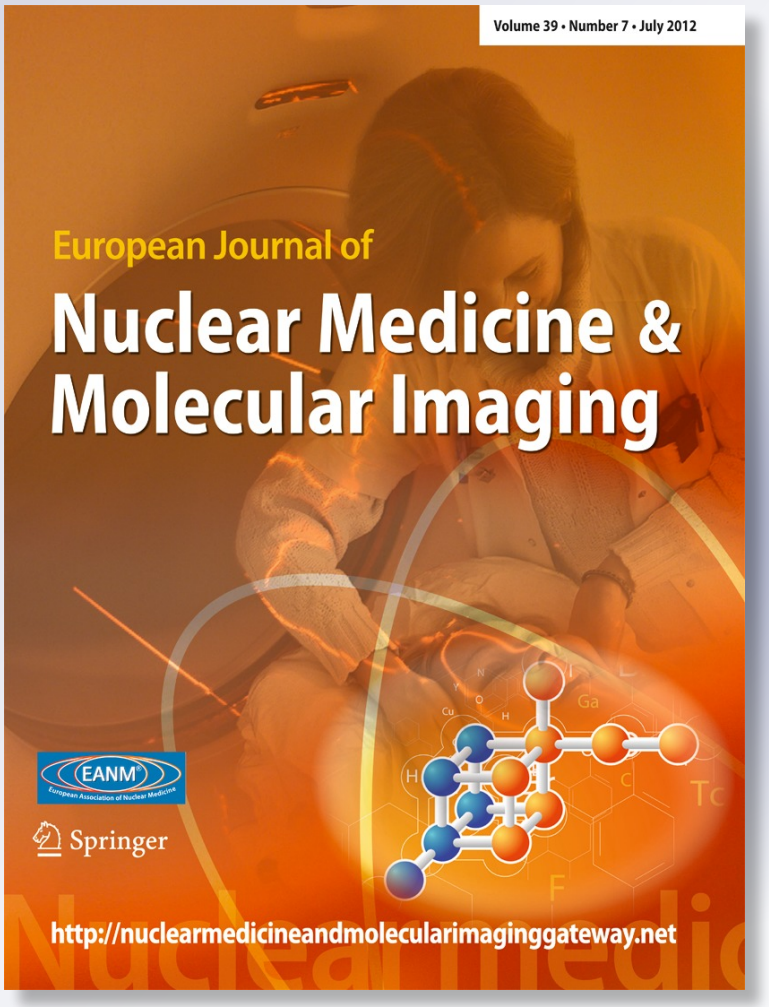

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ORIGINAL ARTICLE

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Abstract
Purpose Hepatocellular carcinoma (HCC) is the fifth and seventh most common cause of cancer in men and women, respectively. Transcatheter arterial chemoembolization (TACE) is the standardized therapy for the intermediate stage of HCC. However, the 3-year overall survival remains low (<30 %) in these patients. Thus, there is a critical need for the development of treatment modalities to improve the survival rate. This study aimed to evaluate whether the combination of ^{131}I-metuximab with chemoembolization could improve treatment efficiency.
Methods Between January 2009 and January 2010, a prospective two-arm nonrandomized study was performed in patients with intermediate HCC. Of 138 patients, 68 (combination therapy group) received 132 courses of intraarterial ^{131}I-metuximab injections combined with chemoembolization (mean 1.94 per patient, median 2, range 1–2), followed by 152 sessions of TACE (mean 2.24 per patient, median 2, range 0–4). The remaining 70 patients (monotherapy group) received 296 sessions of TACE (mean 4.23 per patient, median 4, range 1–7).
Results The overall median survival times for the combination therapy group and the group treated only with TACE were 26.7 months (95 % CI 20.7–31.3 months) and 20.6 months (95 % CI 15.3–24.7 months), respectively. The combination therapy group had a significantly higher survival rate than the TACE-only group (P=0.038). Age ≥65 years, serum albumin ≤35 g/l, and treatment category (combination therapy or TACE only) were independent prognostic factors for survival according to multivariate analysis.
Conclusion The combination of ^{131}I-metuximab and chemoembolization extended survival in patients with intermediate HCC compared with TACE only, and was well tolerated by patients with Child-Pugh class A or B disease. This combination seems to be a promising treatment modality for patients with intermediate HCC.

Keywords Radioimmunotherapy · Cancer · Antibody · Radiopharmaceuticals · Iodine-131

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Introduction

Liver cancer ranks as the fifth most common cancer in men and is the second leading cause of cancer-related death. In women, it is the seventh most frequent cancer and sixth leading cause of cancer death. An estimated 748,300 new liver cancer cases and 695,900 cancer deaths occurred worldwide in 2008. Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver [1]. Transplantation,

hepatectomy, and local ablative therapy remain the only potentially curative options for HCC [2, 3]. However, only 30 % of patients diagnosed with HCC are candidates for curative therapy [2]. Transcatheter arterial chemoembolization (TACE) has an established palliative role in selected patients with multinodular and large HCC tumours without portal vein invasion [4]. However, most multiple HCCs are due to intrahepatic metastases and spread of micrometastases from the primary lesion [5]. TACE alone fails to provide a complete response (CR) in the majority of these patients since the therapy occludes only satellite nodes that rely on an arterial blood supply. Extensive or complete necrosis is rarely observed following TACE when the tumour exceeds 5 cm because larger tumours usually receive arterial and portal blood supplies that allow the tumour to remain viable [6]. Accordingly, patients with large tumours have significantly worse survival rates than those with smaller tumours following TACE therapy. These limitations of TACE therapy for HCC indicate the need to evaluate its use in combination with other therapies.

HCC has traditionally been regarded as a radioresistant tumour due to its limited ability to take up a lethal dose for the successful use of external beam techniques [7, 8]. Improvements in the application of focused external beam radiation through the use of conformal radiation therapy have increased the delivery of higher doses directly to unresectable HCCs with a low risk of complications [7]. However, the benefits of conformal radiation therapy have not yet been evaluated in randomized controlled trials [9].

In the last decade, radioimmunotherapy (RAIT) has become a promising treatment modality for tumours, due to the specificity of the antibodies and the improved cancer-eradication power of the radionuclides, resulting in improved clinical efficacy with few side effects [10, 11]. Radiolabelled antibodies injected via the hepatic artery can target and concentrate in tumour tissues, thereby killing more tumour cells, while causing as little injury as possible to normal tissues [10]. RAIT might therefore provide a new therapeutic approach for patients with HCC [6, 10]. The China State Food and Drug Administration (registration no. S20050039) has approved a therapeutic anti-HCC radioimmunological agent, ^{131}I-metuximab (Licartin, Chengdu Huasun Bio-Tech), for the treatment of unresectable HCC. ^{131}I-Metuximab is generated by ^{131}I-labelling of the murine monoclonal antibody (mAb) fragment HAb18 F(ab')2 derived from HAb18G/CD147. In phase I/II trials, ^{131}I-metuximab was found to concentrate in tumours, and is safe and effective for the treatment of patients with HCC [7, 12]. Additionally, life-threatening toxic effects were not found. In a phase I trial, 28 patients were randomly assigned to receive hepatic artery infusion at doses of 9.25, 18.5, 27.75 or 37 MBq/kg [10]. On the basis of dose-limiting toxicity and pharmacokinetics, 27.75 MBq/kg was set as both the safe and the optimal dosage for clinical use, and was subsequently used in a phase II study [10]. Blood clearance was consistent with a biphasic model and the half-life was 90.56–63.93 h [10].

In the phase II trial, which included 106 HCC patients, 27.75 MBq/kg was found to be an effective therapeutic dose for HCC [10]. The ^{131}I-metuximab was found to concentrate in tumour tissues. By radioimmunodetection, 97 of 103 patients (94.17 %) showed hot regions on SPECT scans following ^{131}I-metuximab injection that were consistent with defects shown on ^{99m}Tc-sodium phytate colloid scans. These findings suggest that ^{131}I-metuximab specifically bound to tumour tissue [10]. Of the 73 patients completing two cycles of therapy, 6 (8.22 %) had a partial response (PR), 14 (19.18 %) had a minor response, and 43 (58.90 %) showed stable disease (SD). Although no significant difference in antitumour effect was observed between one cycle and two cycles (P=0.0507), the response rate after two cycles was higher than that after one cycle. The 21-month survival rate was 44.54 %, and progression-free survival was significantly higher than that of patients with progressive disease (PD) after either one or two cycles (P<0.0001 and P=0.0019).

HAb18G/CD147 expression has been shown to be associated with tumour recurrence and has been found to be a significant independent predictor of a poor prognosis in HCC patients after tumour resection or orthotopic liver transplantation [13–15]. Blocking HAb18G/CD147 with mAb HAb18 or ^{131}I-metuximab has been reported to inhibit HCC growth and metastasis in vivo [10]. ^{131}I-Metuximab exhibits stronger inhibitory effects than the mAb, due to the improved cancer-eradication power of ^{131}I [10]. Furthermore, ^{131}I-metuximab has been found to be able to deliver a sufficient radiation dose to the tumour lesion with relative sparing of normal surrounding liver parenchyma, which permits the delivery of higher doses of radiation directly to the tumour when compared with an external beam technique [7, 10]. ^{131}I-Metuximab monotherapy has been shown to be effective in the treatment of HCC and in the prevention of HCC recurrence after orthotopic liver transplantation [10, 16]. TACE is an established treatment for patients with intermediate HCC (Barcelona Clinic Liver Cancer, BCLC, stage B), which is defined by preserved liver function (Child-Pugh class A or B) with large or multifocal tumours without vascular invasion or extrahepatic spread, and absence of symptoms [2, 3]. The combination of RAIT and TACE may enhance anti-tumour effects and reduce toxicity [17].

Thus, a prospective nonrandomized comparative study that thoroughly evaluated repeated combination therapy of ^{131}I-metuximab and TACE was conducted to evaluate the efficacy and safety of using these combined therapies.

Materials and methods

Patient cohort

Between January 2009 and January 2010, a prospective, nonrandomized, comparative study was performed in patients with unresectable HCC at a single institution. In one arm, 68 patients were treated with the combination therapy of ^{131}I-metuximab and chemoembolization. In another arm, 70 patients with HCC were treated with repeated conventional TACE. We comprehensively evaluated toxicity, imaging and survival outcomes, and all data were collected prospectively. Survival was compared between these two groups. The Institutional Review Board of the Eastern Hepatobiliary Hospital approved the study. All patients in the study gave written informed consent. The design of the study was in accordance with Good Clinical Practice, the Helsinki Declaration, laws in China, and the requirements of the Medical Ethics Committee of the Eastern Hepatobiliary Surgery Hospital, Second Military Medical University of Shanghai, China.

Pretreatment evaluation and staging

Pretreatment assessments included history, physical examination, laboratory tests, chest radiography, abdominal ultrasonography, CT, and/or MRI of the abdomen. The diagnosis of HCC was based on the diagnostic criteria used by the European Association for the Study of the Liver (EASL), and required at least two radiological imaging modalities showing characteristic features of HCC, or one radiological imaging modality showing characteristic features of HCC associated with an α-fetoprotein (AFP) level greater than 400 ng/ml, or cytological/histological evidence from an ultrasound-guided biopsy [18]. Inclusion criteria included a histopathological or imaging diagnosis of HCC based on accepted guidelines [18], an Eastern Cooperative Oncology Group performance status [19] of 0, and Child-Pugh class A or B at enrolment. Exclusion criteria included anaphylactic reactions to biological products or allergic diathesis, portal vein thrombosis and/or extrahepatic metastases, advanced liver disease (Child-Pugh class C), encephalopathy, refractory ascites, portosystemic shunt, hepatofugal blood flow, active gastrointestinal bleeding, any contraindication to an arterial procedure such as impaired clotting tests (platelet count below 50×10^9/l or prothrombin activity below 50 %), renal failure, severe atheromatosis, or any contraindication to epirubicin (leucocyte count less than 3×10^9/l, cardiac ejection fraction less than 50 %). In addition, female patients who were pregnant or lactating were excluded from the study.

On dynamic CT, HCC usually appeared hyperattenuated in the arterial phase or washed out in the delayed phase (approximately 3 min after contrast agent injection) [20]. On the other hand, HCC typically appeared as a hypervascular lesion by hepatic arteriography on dynamic MRI. Extrahepatic metastases were routinely examined by ultrasonography, CT and chest radiography. Bone metastases were evaluated by scintigraphy if clinically suspected [21, 22]. Patients were evaluated by Child-Pugh scoring, Okuda staging, and BCLC staging (A early, B intermediate, C advanced, D end-stage) [19]. Lymph nodes >2.0 cm were defined as extrahepatic metastases [23].

After the pretreatment examination, a full discussion by our multidisciplinary treatment team, which included radiologists, surgeons, hepatologists and oncologists, was conducted to evaluate the clinical diagnosis and staging. Subsequently, patients who met the inclusion criteria were informed about the details of the combination therapy by their physicians, which included information on possible benefits and risks of complications. All patients were informed regarding the details of TACE treatment as the standard treatment for multiple HCC according to the BCLC proposal. The treatment choice was made by the patients. After the patients had made their decision, therapy was arranged within 1 week.

Therapy and follow-up

The mAb HAb18 (immunoglobulin G1) was obtained by preparing a hybridoma from BALB/c mice immunized with a cell suspension extracted from fresh human HCC tissues [10, 24]. The antigen, HAb18G/CD147, a member of the CD147 family, is highly expressed on HCC cells [13, 14]. Immunohistochemistry performed with HAb18 showed that 39 of 52 HCCs (75 %) stained positively, with no cross-reaction with normal tissues [24].

Technical and dosimetry considerations for ^{131}I-metuximab have been described previously [10]. The target dose was 27.75 MBq/kg (0.75 mCi/kg) [10]. Lugol's liquid was administered from 3 days before treatment to 7 days after treatment (ten drops daily for 10 days in total) to block thyroid uptake of ^{131}I. Following confirmation of a negative response to a subcutaneous injection of metuximab, the appropriate dose of ^{131}I-metuximab was administered over 5–10 min into the proper hepatic artery, followed by embolization of the tumour-feeding arteries using lipiodol (Lipiodol Ultra-Fluide; Guerbet Laboratories, Aulnay-Sous-Bois, France) and sponge gelatin (Gelfoam; Hangzhou Alc, China; 350–560 μm in diameter), accompanied by chemotherapy with epirubicin (Pharmorubicin; Pfizer, Wuxi, China). Patients were routinely injected with 2–20 ml of lipiodol (1–2 ml/cm diameter of the tumour). Lipiodol and gelatin sponges were used, alone or in combination, to occlude the tumour-feeding artery, according to individual conditions. Embolization was performed with

absorbable gelatin sponge particles to achieve stasis in the tumour-feeding artery.

According to our institutional protocol, each patient in the combination therapy group was treated with a first course of ^{131}I-metuximab and chemoembolization, followed by an enhanced abdominal CT scan 4–6 weeks later. In the absence of a CR according to World Health Organization (WHO) [25] or EASL [18] criteria, patients received the second course of treatment, consisting of chemoembolization alone, 4–6 weeks after the first course. CT scans were performed 4–6 weeks after the second course of treatment during the first 5 months. Patients requiring a third course of treatment received combination therapy. Each patient in the TACE-only group was treated with repeated chemoembolization every 4–6 weeks in the first 6 months and every 3–6 months thereafter, except when a CR was achieved according to WHO or EASL criteria.

Treatment response was assessed by contrast-enhanced spiral CT 1 month later after the third course. After three courses of treatment, patients were assessed every 3 months for 2 years, and every 6 months thereafter by spiral CT, ultrasonography, serum biochemistry, and clinical examination. Chemoembolization alone was performed in both groups after 6 months unless CR was sustained or any contraindication developed. Additionally, chemoembolization alone or combination therapy was discontinued in any patient who developed a limiting toxicity or at the request of the patient. Symptomatic treatment or traditional Chinese therapy was used when possible. Two radiologists who were unaware of the patients' clinical data and treatment assignments reviewed the CT scans. The magnitude of treatment response was defined by WHO or EASL criteria.

Primary and secondary endpoints

The primary endpoint of this study was overall survival (OS) and the secondary endpoints included safety, response rate and time-to-progression (TTP) [19]. OS was calculated from the date of assignment to the time of death or the end of follow-up [19]. Clinical and laboratory adverse events were recorded using Common Terminology Criteria for Adverse Events v3.0 (CTCAE v3.0; National Cancer Institute, Bethesda, MD) at any time during follow-up. Response rate was calculated based on WHO criteria and EASL criteria [18, 19, 25]. TTP was calculated from the date of assignment to the time of radiological progression [19].

Clinical and laboratory toxicities

Clinical follow-up for adverse events was recorded according to CTCAE v3.0. Toxicities were recorded at any time during follow-up. Conservatively, preexisting laboratory toxicities were counted as toxicities at follow-up, even if there was no change in grade.

Sample size

Sample size was computed using OS as the main endpoint. The assumptions were 2-year survival of 49 % in the combination therapy group and 26 % in the TACE-only group (these data were from the update in our cohort study). Using a two-sided test with 80 % power at a significance level of 5 %, the minimal sample size needed to detect a significant difference was calculated to be 66 patients in each treatment group.

Statistical analysis

Survival was analysed by the Kaplan-Meier method. Survival probabilities were estimated using the life-table method, and differences in survival rate between the groups were evaluated using the log-rank test. Baseline patient characteristics between the groups were compared using a *t*-test (means) or a Mann-Whitney *U* test (median) for continuous variables, while the chi-squared or Fisher's exact test was used (for small *n* or highly imbalanced table cells) for categorical data. Data were summarized using descriptive statistics (mean and standard deviation for continuous variables; count and frequency for categorical variables). Univariate and multivariate analyses were used to examine the factors associated with survival with the Cox proportional hazards model. The following variables were entered into the multivariate model: age, sex, baseline bilirubin, baseline albumin, ascites, AFP, maximum baseline dimension, solitary/multifocal, and treatment received (combination therapy or TACE only). The stepwise method was chosen for variable selection in the multivariate model fitting, where the inclusion criterion for entry of the variable into model was $P \leq 0.25$, and the exclusion criterion for removal of the variable from the model was $P > 0.15$.

Composite variables, such as Child-Pugh class and Okuda stage, were excluded from the multivariate analyses since the majority of the variables in the Child-Pugh class and Okuda stage had already been included (Table 1). Hazard ratio estimates were based on simultaneous analysis of all predicated variables. Statistical analyses were carried out with SAS software, version 9.1(SAS Institute, Cary, NC). All reported *P* values are two-sided, and $P < 0.05$ was considered statistically significant.

Results

Patient population

Patient demographics, tumour characteristics and stages are presented in Table 2. Patients in the combination therapy group were older, but not significantly (50 vs. 46 years,

Table 1 Univariate and multivariate analyses for survival

Predictor	Category	Univariate analysis (Cox proportional hazards model)		Multivariate analysis (Cox proportional hazards model)	
		Hazard ratio (95 % CI)	*P* value	Hazard ratio (95 % CI)	*P* value
Age (years)	<65	1.000		1.000	
	≥65	2.239 (1.023–4.898)	0.044	2.770 (1.237–6.204)	0.013
Sex	Male	1.000		–	–
	Female	1.107 (0.627–1.952)	0.726	–	–
Child-Pugh class	A	1.000		–	–
	B	1.651 (1.018–2.678)	0.042	–	–
Baseline bilirubin (μmol/l)	≤34	1.000		–	–
	>34	1.751 (0.801–3.826)	0.160	–	–
Baseline albumin (g/l)	>35	1.000		1.000	
	≤35	1.961 (1.209–3.182)	0.006	1.997 (1.228–3.248)	0.005
HBV DNA (copies/ml)	$\leq 10^4$	1.000		–	–
	$>10^4$	1.194 (0.612–2.330)	0.614	–	–
Ascites	Absent	1.000		–	–
	Present	1.729 (0.789–3.788)	0.171	–	–
AFP (ng/ml)	≤200	1.000		–	–
	>200	1.421 (0.899–2.246)	0.132	–	–
Maximum baseline dimension (cm)	≤5	1.000		1.000	
	>5	0.678 (0.386–1.192)	0.177	0.562 (0.314–1.007)	0.053
Solitary/multifocal	Solitary	1.000		–	–
	Multifocal	0.453 (0.182–1.126)	0.088	–	–
Okuda stage	I	1.000		–	–
	II	1.814 (1.012–3.250)	0.046	–	–
Treatment	Combination therapy	1.000		1.000	
	TACE only	1.489 (0.947–2.340)	0.084	1.575 (1.000–2.281)	0.049

respectively; $P=0.06$). Patients in both groups were treatment-naive, and had comparable rates of portal hypertension, ascites, cirrhosis, tumour distribution and cancer stage (BCLC stage, Okuda stage, and Child-Pugh class).

Treatment

The median number of treatment courses was four in the combination therapy group (mean 4.18 per patient, range 1–6) and five in the TACE-only group (mean 4.23 per patient, range 1–7; $P=0.813$). In the combination therapy group, 68 patients received a total of 132 courses of hepatic arterial injections of ^{131}I-metuximab combined with chemoembolization (mean 1.94 per patient, median 2, range 1–2), followed by 152 sessions of TACE (mean 2.24 per patient, median 2, range 0–4). The total number of treatments was 284 (mean 4.18 per patient, median 4, range 1–6). The median dose per treatment was 1,702 MBq (95 % CI, 1,332–1,850 MBq). In the TACE-only group, 70 patients were treated with 296 sessions of TACE (mean 4.23 per patient, median 4, range 1–7). The total number of courses in the combination therapy group was slightly less than in the TACE-only group, but without statistical significance ($P=0.813$). Three patients (one in the combination therapy group, two in the TACE-only group) were lost to follow-up, and their data were censored at the time of the last visit. Two patients in the TACE-only group refused to continue according to the treatment protocol and their data were censored at the initiation of the new therapy. One received combination therapy with ^{131}I-metuximab and chemoembolization and the other received combination therapy with percutaneous ethanol injection and chemoembolization.

Imaging outcomes

Response rate Assessment of response was possible in 131 patients (94.9 %) who survived for at least 6 months. CT was also performed at least every 8 to 12 weeks in progression-free patients. In the combination therapy group, the numbers of patients assessed as achieving CR, PR, SD and PD were 16 (23.5 %), 31 (45.6 %), 10 (14.7 %) and 8 (11.8 %), respectively, according to EASL criteria, and 0,

Table 2 Baseline patient characteristics

Parameter		Combination therapy (n=68)		Conventional TACE (n=70)		P value
		n (%)	Median (range)	n (%)	Median (range)	
Demographics						
Age (years)		–	51 (27–86)	–	46 (23–82)	0.06
Sex	Male	54 (79)	–	56 (80)	–	0.93
	Female	14 (21)	–	14 (20)	–	
Aetiology	HBV	57 (84)	–	64 (91)	–	0.17
	HBV + alcohol	10 (15)	–	4 (6)	–	
	Unknown	1 (1)	–	2 (3)	–	
Imaging findings						
Cirrhosis	Present	46(88)	–	47 (87)	–	0.95
	Absent	22 (12)	–	23 (13)	–	
Portal hypertension	Present	41 (60)	–	37 (53)	–	0.38
	Absent	27 (40)	–	33 (47)	–	
Ascites	Present	5 (7)	–	2 (3)	–	0.23
	Absent	63 (93)	–	68 (97)	–	
Tumour characteristics						
Lobar distribution	Unilobar	47 (69)	–	56 (80)	–	0.14
	Bilobar	21 (29)	–	14 (20)	–	
Distribution	Solitary	3 (4)	–	4 (6)	–	0.73
	Multifocal	65 (96)	–	66 (94)	–	
Largest tumour size (cm)		–	4.1 (1.2–8.1)	–	3.8 (1–7.8)	0.43
Laboratory data						
AFP (μg/l)	≤200	33 (49)	–	37 (53)	–	0.61
	>200	35 (51)	–	33 (47)	–	
Total bilirubin (μmol/l)	<34	63 (93)	–	66 (94)	–	0.70
	34–50	5 (7)	–	4 (6)	–	
Albumin (g/l)	>35	53 (78)	–	55 (79)	–	0.93
	28–35	15 (22)	–	15 (21)	–	
HBV DNA (copies/ml)	$\leq 10^4$	33 (49)	–	39 (56)	–	0.40
	$>10^4$	35 (51)	–	31 (44)	–	
Staging						
Child-Pugh class	A	54 (79)	–	57 (82)	–	0.77
	B	14 (21)	–	13 (18)	–	
Okuda stage	I	58 (85)	–	62 (89)	–	0.57
	II	10 (15)	–	8 (11)	–	

HBV hepatitis B virus, *AFP* alpha-fetoprotein

32 (47.1 %), 27 (39.7 %) and 6 (8.9 %), respectively, according to WHO criteria. In the TACE-only group, the numbers of patients assessed as achieving CR, PR, SD and PD were 17 (24.3 %), 27 (38.6 %), 11(15.7 %) and 11 (15.7 %), respectively, according to EASL criteria, and 0, 28 (40 %), 30 (42.9 %) and 8 (11.4 %), respectively, according to WHO criteria. The objective response rates were similar according to both EASL criteria (combination therapy 69.1 %, TACE only 62.8 %; P=0.483) and WHO criteria (combination therapy 47.1 %, TACE only 40 %; P=0.434).

Time-to-progression Progression occurred in 46 patients (combination therapy group 18, TACE-only group 28), among whom 8 patients developed extrahepatic progression (combination therapy group 2, TACE-only group 6). In patients with Child-Pugh class A, the median TTP was 22.9 months (95 % CI, 11.7–28.1 months) in the combination therapy group, and 14.8 months (95 % CI, 9.8–24.6 months) in the TACE-only group (P=0.061). In patients with Child-Pugh class B, the median TTP was 15.8 months (95% CI, 9.5–25.3 months) in the combination therapy group, and 10.3 months (95% CI, 8.3–17.5 months) in the TACE-only group (P=0.124). Combination therapy outperformed TACE only as shown by the median TTP (18.6 vs. 12.5 months, respectively; P=0.046).

Survival

At the time of this report, post-therapy survival in the 138 patients who participated in the study was as long as 31 months. At a median follow-up time of 22 months (95 % CI, 6–31 months), 75 patients (54.3 %) were

censored. In the combination therapy group, the survival rates at 6, 12, 18 and 24 months were 95.6 %, 80.9 %, 69.1 % and 53.6 %, respectively, and the median OS was 26.7 months (95 % CI, 20.7 to 31.3 months). In the TACE-only group, the survival rates at 6, 12, 18 and 24 months were 94.3 %, 72.9 %, 52.9 % and 37.9 %, respectively, and the median OS was 20.6 months (95 % CI, 15.3 to 24.7 months). Survival was significantly longer in the combination therapy group than in the TACE-only group (P=0.038; Fig. 1).

Univariate and multivariate analyses

The results of the univariate and multivariate analyses are presented in Table 1. In the multivariate analysis, significant risk factors for survival were age ≥65 years (hazard ratio, HR, 2.24; 95 % CI 1.02–4.50), performance status 0 (HR 0.57, 95 % CI 0.37–0.87), Child-Pugh class B (HR 1.65, 95 % CI 1.02–2.68), baseline albumin ≤35 g/l (HR 1.961, 95 % CI 1.21–3.18), and Okuda stage II (HR 1.81, 95 % CI 1.01–3.25). In the multivariate analysis, the independent risk factors for survival were age ≥65 years (HR 2.77, 95 % CI 1.24–6.20), baseline albumin ≤35 g/l (HR 1.99, 95 % CI 1.23–3.25), treatment received (combination therapy or TACE only) (HR 1.58, 95 % CI 1.00–2.28).

Causes of death

At the time of analysis, 31 (45.6 %) patients in the combination therapy group had died, and 39 (55.7 %) in the TACE-only group. The causes of death included tumour progression in 46 (65.7 %) patients, liver failure with SD in 19 (27.1 %), and other causes in 5 (7.1 %), including two in whom death was possibly related to treatment. One patient suffered a stroke 5 weeks after the third course of therapy, and the other patient suffered a myocardial infarction 7 weeks after the second course of therapy. The deaths of both patients were considered to be possibly related to treatment because of the lack of direct evidence on the basis of a conservative approach.

Clinical and laboratory toxicities

The clinical and laboratory toxicities observed during the study are summarized in Table 3. Although anorexia and fatigue were more common in the combination therapy group, fever/chills and nausea/vomiting were more common in the TACE-only group. However, the differences in these variables were not statistically significant. The frequencies of grade 1/2 platelet toxicity (P<0.0001), grade 3/4 platelet toxicity (P=0.004), grade 1/2 white blood cell count (WBC) toxicity (P<0.0001) and grade 3/4 WBC toxicity (P=0.036) were significantly higher in the combination therapy group. Ten patients with grade 2 WBC toxicity and all the patients with grade 3/4 WBC toxicity were treated with recombinant human granulocyte colony-stimulating factor injection. All the patients with grade 3/4 platelet toxicity and 13 patients with grade 2 platelet toxicity were treated with recombinant human interleukin-11 injection. Additionally, two patients with grade 4 platelet toxicity in the combination therapy group received platelet transfusions. Most of

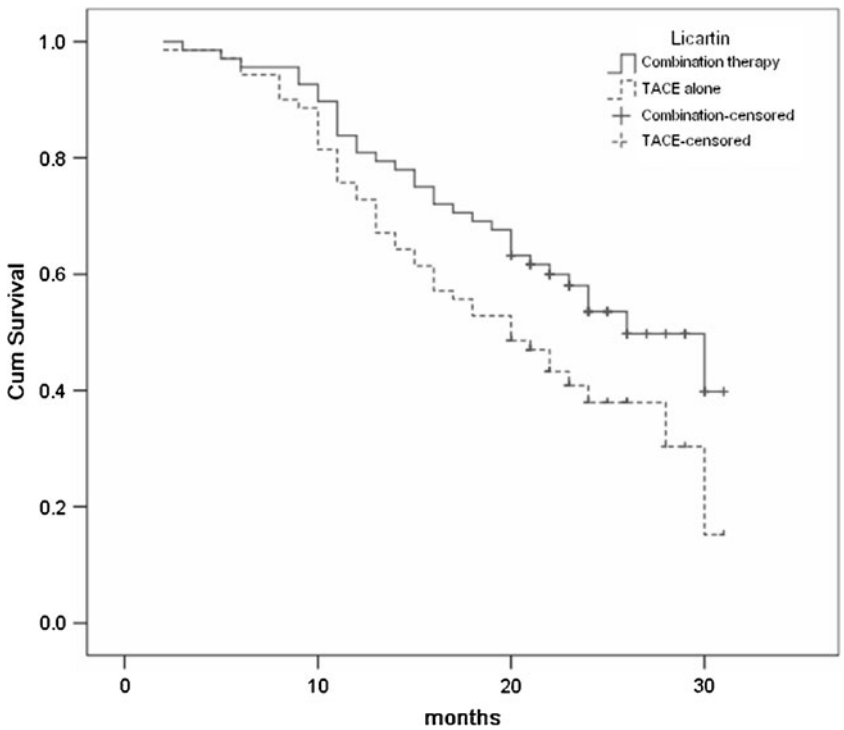

Fig. 1 Kaplan-Meier curves of survival of patients receiving combination therapy and those receiving TACE only (P=0.038)

Table 3 Clinical and biochemical toxicities in patients receiving combination therapy or TACE only

Toxicity		Statistical analysis	Combination therapy (n=68)	TACE only (n=70)	P value
Clinical					
Grade 1/2	Anorexia	Chi-squared test	35 (51)	33 (47)	0.611
	Fever/chills	Chi-squared test	28 (41)	31 (44)	0.712
	Nausea/vomiting	Chi-squared test	22 (32)	24 (34)	0.810
	Pain	Chi-squared test	30 (44)	34 (49)	0.600
	Fatigue	Chi-squared test	36 (53)	27 (39)	0.090
	Diarrhoea	Fisher's exact test	3 (4)	2 (3)	0.678
Grade 3/4	Anorexia	Chi-squared test	19 (17)	17 (13)	0.625
	Fever/chills	Fisher's exact test	2 (3)	2 (3)	1.000
	Nausea/vomiting	Fisher's exact test	1 (1)	2 (3)	1.000
	Pain	Chi-squared test	13 (12)	12 (9)	0.763
	Fatigue	Fisher's exact test	1 (1)	2 (3)	1.000
	Diarrhoea	–	0	0	
Biochemical					
Grade 1/2	Bilirubin	Chi-squared test	31 (46)	33 (48)	0.855
	Albumin	Chi-squared test	30 (44)	29 (41)	0.750
	ALT	Chi-squared test	47 (70)	50 (71)	0.766
	AST	Chi-squared test	48 (71)	51 (74)	0.767
	WBC	Chi-squared test	46 (68)	14 (20)	<0.0001
	Platelets	Chi-squared test	35 (51)	6 (9)	<0.0001
Grade 3/4	Bilirubin	Chi-squared test	8 (12)	9 (13)	0.845
	Albumin	–	0	0	–
	ALT	Chi-squared test	13 (20)	15 (21)	0.736
	AST	Chi-squared test	17 (25)	13 (18)	0.360
	WBC	Chi-squared test	10 (15)	3 (4)	0.036
	Platelets	Chi-squared test	12 (17)	2 (3)	0.004

these haematological toxicities were relatively mild and manageable. None of these patients exhibited any signs of life-threatening toxicities.

Discussion

Although the combination therapy of ^{131}I-metuximab injection and chemoembolization has been shown to extend OS compared with TACE only in historical controls in a cohort study with 110 patients [17], the advantage of this therapy has not yet been demonstrated in a prospective comparative study. In this study, we demonstrated that combination therapy with RAIT and chemoembolization involving injections of ^{131}I-metuximab integrated with epirubicin chemotherapy and the use of embolization agents extended median survival compared with TACE only. The response rate was higher in the combination therapy group than the TACE-only group, but did not reach statistical significance. Additionally, the TTP was significantly higher in the combination therapy group than in the TACE-only group. Patients in the combination therapy group were more likely to experience decreases in WBC and platelet count. Although patients in the combination therapy group received fewer courses of treatments, this parameter did not reach statistical significance. In addition, repeated combination therapy was well tolerated with little toxicity.

In this study, we did not use ^{131}I-metuximab alone for comparison because of ethical considerations. However, we compared our present study data with those from the clinical phase I/II trials of ^{131}I-metuximab treatment. Treatment of HCC patients with ^{131}I-metuximab alone resulted in survival rates at 6, 12, 18 and 21 months of 82.6 %, 58.7 %, 52.0 % and 44.5 %, respectively, with a median survival time of 19 months, and a tumour response rate of 8.22 % according to WHO criteria [10]. In comparison, patients receiving combination therapy in our study showed survival rates at 6, 12, 18 and 21 months of 95.6 %, 80.9 %, 69.1 % and 61.7 %, respectively, with a median survival time of 26.7 months, and a tumour response rate of 47.1 % according to WHO criteria and 69.1 % according to EASL criteria. Since both studies were performed in patients staged as Okuda stage I/II and Child class A/B and had similar baseline characteristics, we postulate that the

combination of ^{131}I-metuximab injections plus chemoembolization exhibited better clinical efficacy than ^{131}I-metuximab injections alone.

The mechanism by which ^{131}I-metuximab may benefit HCC patients has been investigated both in vitro and in vivo, as well as in clinical trials. Metuximab is specific and has high affinity for a target antigen highly expressed on HCC cells. Specific concentrations of the conjugated ^{131}I in HCC tissues allows the tumour cells to be killed both directly and indirectly. Thus, injection of ^{131}I-metuximab can eradicate tumour cells without internalization of the mAb, making it more effective than mAb-conjugated chemicals and toxins. In addition, the target antigen, HAb18G/CD147, is a cell adhesion molecule with multiple functions, and is closely related to tumour metastasis [26]. Injection of ^{131}I-metuximab into HCC cells and xenografts has been found to inhibit oncogenesis and metastasis in the liver and other organs, blocking and destroying cells carrying HAb18G/CD147 and inhibiting HCC metastasis [10].

The response rate tended to be higher in the combination therapy group, although without a significant difference. And this tendency translated into a significantly longer TTP in this study. There are several possible explanations for this finding. First, the trial was possibly not powered to assess differences in response rate. Second, tumour progression may be delayed, since ^{131}I-metuximab has been shown to be a promising antimetastasis agent with the ability to specifically bind to HAb18G/CD147 and effectively inhibit HCC metastasis [16]. It is generally believed that the high risk of HCC metastasization is from tumour cells that have escaped to the circulation [16]. ^{131}I-Metuximab may efficiently target and bind to escaped HCC cells in the circulation or to liver-homing cells by specific antigen–antibody recognition allowing the bound radionuclide to destroy the target site and eradicate the tumour cells [16]. This mechanism could also partly explain the result that there tended to be fewer extrahepatic metastasis in the combination therapy group than in the TACE-only group. The antimetastasis ability of ^{131}I-metuximab would not contribute greatly to the improvement in response rate, which was assessed early after combination therapy, but would participate in prolonging TTP by delaying tumour progression.

The combination of RAIT plus chemoembolization may have a synergistic effect in the treatment of HCC. First, TACE permits the radiation dose to be reduced to a safe level while achieving a higher response rate by decreasing the tumour burden attributed to the ischaemic necrosis achieved. Second, TACE may enhance the efficacy of ^{131}I-metuximab due to its arterial embolization effect, substantially reducing blood flow to the HCC and resulting in tumour retention of ^{131}I-metuximab. Third, retention of the anticancer drug in the tumour may have a radiosensitizing effect on RAIT. Moreover, RAIT is a form of continuous low dose-rate radiation within the tolerance levels of normal tissues, which can translate into a high biologically effective dose to the tumour. ^{131}I-Metuximab injection did not cause any severe side effects when combined with chemoembolization [17]. Additionally, ^{131}I-metuximab can eliminate residual cancer cells after TACE. Taken together, these mechanisms may explain, at least in part, the ability of combination therapy to enhance survival, compared with conventional TACE only in patients with intermediate HCC.

This comparative trial offered the opportunity to distinguish adverse events specific to RAIT with ^{131}I-metuximab from those associated with chemoembolization. In both groups, the majority of non-haematological adverse events were grade 1 or 2, and without significant differences. The increased frequency of haematological toxicities observed in patients receiving combination therapy might have been due to the use of radiolabelled ^{131}I. The majority of haematological toxicities associated with ^{131}I-metuximab have consisted of reversible depletion of one or more haematological cell lines [10, 17].

Building on the success of ^{131}I-metuximab combined with chemoembolization for intermediate HCC, further clinical trials are required. Also, further trials will need to explore repeated courses of combination therapy and combined modality dose and schedule. The combination therapy with the RAIT drug Licartin and chemoembolization is safe and effective and may be a clinically meaningful advance in the treatment of intermediate HCC. The improved TTP and OS found in this comparative study of combination therapy versus TACE only laid the foundation for the next generation of clinical trials of RAIT.

The principal limitation of this study was its nonrandomized nature. A cautious attitude was adopted so a nonrandomized study was carried out to evaluate the availability and tolerability of repeated combination therapy. The positive results of this study provide strong support for a randomized controlled trial comparing combination therapy and TACE only. To further clarify the efficacy of the combination therapy of ^{131}I-metuximab and chemoembolization in patients with intermediate HCC, a prospective, randomized trial comparing ^{131}I-metuximab plus chemoembolization with chemoembolization alone is being conducted. In conclusion, our findings indicate that the combination of the RAIT drug ^{131}I-metuximab with chemoembolization may represent a promising treatment modality for patients with intermediate HCC.

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