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CLINICAL INVESTIGATION **Liver**

TARGETING RADIOIMMUNOTHERAPY OF HEPATOCELLULAR CARCINOMA WITH IODINE (^{131}I) METUXIMAB INJECTION: CLINICAL PHASE I/II TRIALS

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Purpose: HAb18G/CD147 is a hepatocellular carcinoma (HCC)-associated antigen. We developed iodine (^{131}I) metuximab injection (Licartin), a novel ^{131}I-labeled HAb18G/CD147-specific monoclonal antibody F(ab')$_2$ fragment, and evaluated its safety, pharmacokinetics, and clinical efficacy on HCC in Phase I/II trials.
Methods and Materials: In a Phase I trial, 28 patients were randomly assigned to receive the injection in 9.25-, 18.5-, 27.75-, or 37-MBq/kg doses by hepatic artery infusion. In a multicenter Phase II trial, 106 patients received the injection (27.75 MBq/kg) on Day 1 of a 28-day cycle. Response rate and survival rate were the endpoints.
Results: No life-threatening toxic effects were found. The safe dosage was 27.75 MBq/kg. The blood clearance fitted a biphasic model, and its half-life was 90.56–63.93 h. In the Phase II trial, the injection was found to be targeted and concentrated to tumor tissues. Of the 73 patients completing two cycles, 6 (8.22%) had a partial response, 14 (19.18%) minor response, and 43 (58.90%) stable disease. The 21-month survival rate was 44.54%. The survival rate of progression-free patients was significantly higher than that of patients with progressive disease after either one or two cycles ($p < 0.0001$ or $p = 0.0019$).
Conclusion: Iodine (^{131}I) metuximab injection is safe and active for HCC patients. © 2006 Elsevier Inc.

Radioimmunotherapy, Hepatocellular carcinoma, HAb18G/CD147, Monoclonal antibody, Clinical trial.

INTRODUCTION

Primary liver cancer ranks second among cancers as a cause of death, owing to its high incidence rate, rapid progression, poor prognosis, and tumor recurrence (1). The mean natural survival time of patients with hepatocellular carcinoma (HCC), the most common type of primary liver cancer, is only 3.2 months, and the 5-year survival rate is 5% (1). Less than 20% of HCC patients are fit for traditional therapies, such as surgery or chemotherapy (1, 2). In addition, at least

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85% of HCC patients in China also have liver cirrhosis, which makes therapy more difficult (1). Since the 1980s, radioimmunotherapy (RIT) has become a promising therapy for tumors, because the specificity of the antibodies and the killing power of the radionuclides tend to bring about better clinical efficacy with fewer side effects.

Iodine (^{131}I) metuximab injection (brand name, Licartin; Chengdu Hoist Hitech Co., Ltd., Chengdu, China) is an iodine ^{131}I-labeled murine monoclonal antibody (mAb) HAb18 F(ab')$_2$ fragment against the HCC-associated antigen HAb18G/CD147. The mAb HAb18 (immunoglobulin G1) was obtained by using a cell suspension extracted from fresh human HCC tissues to immunize BALB/c mice and to prepare hybridoma (3–5). Its antigen, HAb18G/CD147, a member of the CD147 family, was highly expressed on HCC cells (6, 7). The binding rate of HAb18 to human 7721 hepatoma cells, determined by flow cytometry, was 99.55%, and the mean fluorescent intensity was 171.31. Immunohistochemistry performed with HAb18 showed that the positive rate of HCC staining was 75% (39 of 52) without cross-reaction to normal tissues (5). Metuximab was prepared by pepsin digestion of HAb18 to remove the Fc fragment. Its molecular weight ranged from 94 kd to 98 kd by analysis of nonreductive sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and the molecular weight of the heavy chain and light chain ranged 26–28 kd and 21–23 kd, respectively, by reductive SDS-PAGE analysis. Its titer was >1:8000 by immunofluorescence staining analysis on HCC cells, and its affinity constant to the recombined HAb18G/CD147 molecule was determined to be 6.32×10^{-10} mol/L by surface plasmon resonance analysis. A pharmacokinetics study conducted in BALB/c mice showed that the blood clearance of the iodine (^{131}I) metuximab injection fitted a biphasic model, and the half life ($t_{1/2}$) in blood was 34.61 h (8). Biologic distribution analysis of metuximab in a human HCC xenografts model in BALB/c nude mice showed that the tumor/nontumor radiation absorbed dose ratio ranged from 2.51 ± 0.69 to 18.60 ± 2.05 (4, 9). The 30% and 50% effective doses of the iodine (^{131}I) metuximab injection were, respectively, 133.2 and 370 MBq/kg for mice, equal to 15.54 and 43.29 MBq/kg for humans. The nontoxic dose in the long-term toxicity test was 277.5 MBq/kg for rats, equal to 45.88 MBq/kg for humans. The results of safety pharmacology studies showed that iodine (^{131}I) metuximab injection caused no impairment to cardiovascular, respiratory, or nervous systems. The above results have all been sanctioned by the China State Food and Drug Administration (SFDA).

In the preliminary clinical study (approved by the Health Bureau of Shaanxi Province, China, 1999), the evidence of anti-HCC effects in 7 of 9 assessable HCC patients (77.78%) was observed, of whom 1 had complete response (CR), 1 partial response (PR), 1 minor response (MR), and 4 stable disease (SD). After the preliminary clinical study, we conducted Phase I and Phase II clinical trials to assess the safety, pharmacokinetics, and efficacy of iodine (^{131}I) metuximab injection in HCC patients.

METHODS AND MATERIALS

Patient selection

Eligible subjects for this study were patients aged 18–75 years, who had been clinically definitely diagnosed with HCC but who had no indication for surgical treatment, who had an anticipated survival time of >3 months, and whose Karnofsky Performance Score (KPS) was >60. The diagnoses were made mainly by computed tomography (CT) scans, alpha fetoprotein level detection, and analysis of clinical history and symptoms. The possible candidates who were excluded from our study were patients who had a definite diagnosis of diffuse HCC, severe diseases of the heart, kidney, or blood system, a bilirubin level >2.5 times the upper limit of normal, a serum albumin level <32 g/L (0.48 mmol/L), or other therapies within the previous 4 weeks. Patients who were ever anaphylactic to biologic products or who experienced allergic diathesis were also excluded. Female patients had to be neither pregnant nor lactating.

Study design and treatment

A dose-escalating Phase I trial was conducted to determine the dose-limiting toxicity, safe dosage, and pharmacokinetics of iodine (^{131}I) metuximab injection. As mentioned previously, the nontoxic dose in the long-term toxicity test was 277.5 MBq/kg for rats, equal to 45.88 MBq/kg for humans. Beginning from one fifth of the converted nontoxic dose for humans (45.88 MBq/kg), 9.25 MBq/kg and then two, three, and four times that dosage were established as the escalated doses in the Phase I trial (10). Twenty-eight patients (divided into four groups) were randomly assigned to receive 9.25, 18.5, 27.75, or 37 MBq/kg of iodine (^{131}I) metuximab injection, and the total dose for each patient contained 5 mg metuximab. Lugol's liquid was given to block the thyroid uptake of ^{131}I 3 days before the treatment (10 drops daily for 10 days). When negative response to a subcutaneous injection of metuximab was confirmed, 18.5 MBq of ^{99m}Tc-sodium phytate colloid was administered intravenously, and single photon emission computed tomography (SPECT) scanning was performed, which was followed by hepatic artery infusion of iodine (^{131}I) metuximab injection within 5–10 min. Radioimmunodetection of iodine (^{131}I) metuximab injection was conducted on Day 7 by SPECT. The study started with the 9.25-MBq group (4 patients) and moved in sequence to the 18.5-MBq group (8 patients), 27.75-MBq group (8 patients), and 37 MBq group (8 patients) if no severe toxic effects were observed in the following 4 weeks after each administration. When 50% of the patients in one group were found to have severe toxicity responses (≥Grade 3 by World Health Organization [WHO] criteria), the study was stopped.

Based on the results of Phase I, a multicenter Phase II trial was conducted to evaluate the efficacy of iodine (^{131}I) metuximab injection at the determined safe dosage. Fourteen patients were randomly selected to preliminarily evaluate the safety of iodine (^{131}I) metuximab injection, which was given twice at an interval of 28 days. Human antimouse antibody (HAMA) was examined on Days 7 and 35. After safety was confirmed, other patients were enrolled in the study.

All patients in our study gave written informed consent, and the clinical trials were approved by the China SFDA (no. 1999XL0140). The design of our study was in accordance with Good Clinical Practice, the Helsinki Declaration, laws in China, and the requirements of the Medical Ethics Committee of West China Hospital, Sichuan University. The clinical trials were conducted in two national bases for drug clinical trials (one of which,

West China Hospital, was also the supervisor of the trial) and in two other provincial tumor hospitals. In the present study, the mAb HAb18 was prepared and purified from the supernatant of the H18 hybridoma cell culture. Metuximab was prepared by pepsin digestion and purified by hydrophobic interaction chromatography. Its purity was >95% by analysis with high performance liquid chromatography. Iodine (^{131}I) metuximab injection, the final product, tested for sterility, pyrogen content, and safety by the National Institute for the Control of Pharmaceutical and Biologic Products and approved by the China SFDA, was prepared and supplied by the Fourth Military Medical University (11, 12). Iodine (^{131}I) metuximab injection was approved to be marketed by the SFDA on January 23, 2005. "Iodine (^{131}I) metuximab injection" was designated as the generic name by the State Pharmacopeia Committee of China (no. 262 [2004]). "Licartin" was registered as the brand name by the Trademark Office of the State Administration for Industry and Commerce of China (no. ZC3988714SL).

Toxicity determination

All toxic effects were recorded, and the severity was graded according to the WHO toxicity scale. In the Phase I study, blood and urine samples were obtained every 7 days for the detection of blood toxicity, tumor marker, and hepatic, renal, and thyroid function. Blood ion assay, myocardium zymogram, and immunologic examination were performed before treatment and 8 and 14 days after treatment.

In the Phase II study, blood toxicity and hepatic and renal function were examined before and 7 and 28 days after treatment. Thyroid function and tumor marker were detected before and every 4 weeks after treatment. Human antimouse antibody was examined 7 and 35 days after each administration. Other examinations for toxicity determination were performed as described in Phase I.

Pharmacokinetic sampling and analysis

Counts per minute (cpm) of each blood and urine sample collected from the 18.5-, 27.75-, and 37-MBq/kg patient groups at different time points were measured in a well-style scintillometer and were decay corrected to the time of sampling. Then the curve of blood radioactivity vs. time was drawn, and blood clearance rate and $t_{1/2}$ were calculated. The urine counts were expressed as the percentage of infused radioactivity, and the curve of urine radioactivity vs. time was drawn.

Antitumor effect evaluation

In the Phase II study, the patients were evaluated by reinforced scans of the abdomen with CT taken before and 28 days after treatment. Computed tomography was performed every 4 weeks in the progression-free patients. According to the WHO criteria, responses from the patients were classified as CR (absence of all evidence of disease for ≥4 weeks), PR (a decrease of ≥50% in the sum of the products of the maximal perpendicular diameters of measurable lesions lasting for ≥4 weeks, without appearance of new lesions), MR (>50% decrease of the product lasting for <4 weeks, or 25%–50% decrease of the product lasting >4 weeks), SD (change of the product in the range of 25% lasting for >4 weeks and without appearance of new lesions), and progressive disease (PD, an increase of >25% in the product or the unequivocal appearance of new tumor lesions, amounting to a shrinking rate of tumor size of >1.25). In our study, PD was set to be the threshold for tumor progression.

Statistical analysis

In the Phase I trial, 28 patients were successively assigned to receive iodine (^{131}I) metuximab injection at the different doses, and the patients in each group were enrolled in the study by simple random sampling from the patient population that met the eligibility criteria. Mathematical models were developed to fit the pharmacokinetics curves of iodine (^{131}I) metuximab injection, and the correlation of metabolism and dosage was analyzed. R-square, goodness-of-fit test, Akaike's Information Criterion (AIC), and the weighted sum of squared residuals (WSS) were chosen to judge which was the optimal model.

In the Phase II trial, the needed sample size was estimated by considering both the survival rate and clinical efficacy. We assumed the confidence level ($1-\alpha$) to be 95% and the estimate accuracy (i.e., the half width of the confidence intervals [CI, δ]) to be 10%. For the survival rate, referencing to the previously reported 2-year survival rates of transarterial chemoembolization (TACE)-treated HCC patients, which were both lower than 40% (13, 14), we assumed that the 2-year survival rate in the present study was 40% or 50% and calculated the needed sample size respectively by the sample size formula [$n = (z_{\alpha}/\delta)^2 \times p \times (1-p)$]. The results showed that 96 or 100 patients were required. As for the efficacy of iodine (^{131}I) metuximab injection, we estimated the necessary sample size referencing to the data of the preliminary clinical trial, in which the clinical effect (CR + PR + MR) of iodine (^{131}I) metuximab injection was 33.33%. On the basis of this, we assumed the clinical effect in the present study to be 33% or 50% and calculated the needed sample size respectively by the sample size formula. The results showed that 89 or 100 patients were required for the study. Considering the possibility of missing data, we finally chose 106 patients.

Response rate was one endpoint; survival rate, the other endpoint, was assessed with Kaplan-Meier curves and was tested for significance by log–rank test. All p values were two-tailed.

RESULTS

Patients characteristics

At the four centers mentioned above, 134 eligible patients were randomly enrolled. In the Phase I study, 22 men and 6 women, aged 24–75 years (mean ± standard deviation: 53 ± 10 years), were enrolled between February and June 2001. In Phase II, 97 men and 9 women, aged 27–75 years (51 ± 12 years), were enrolled between March 2002 and August 2003. The median follow-up was 15 months (range, 2–33 months). The majority of the patients enrolled in the present study were male; men are more susceptible to the hepatitis viruses than women, most of the HCC patients in China have a viral hepatitis history, and the proportion between male and female HCC patients in China is approximately 3:1 (1). All the patients received at least 1 dose of the assigned drug. Three patients in Phase II were excluded from the study after the first administration, either because they voluntarily quit the treatment or because they had therapies other than our assigned drugs. In the Phase II study, 100 patients (97.1%) had advanced HCC (Stage III or IV, tumor-node-metastasis [TNM] classification, International Union Against Cancer, version 5, 1997). The mean of tumor size for all 103 patients was 7.85 ± 3.82 cm × 6.44 ± 2.97

Table 1. Baseline characteristics

Characteristic	All 103 patients	First 14 patients	89 patients
Age (y)			
≥65	13 (12.6)	2 (14.29)	11 (12.36)
<65	90 (87.4)	12 (85.71)	78 (87.64)
Sex			
Male	94 (91.3)	13 (92.85)	81 (91.01)
Female	9 (8.7)	1 (7.14)	8 (8.99)
Tumor size (cm)			
$d \leq 3$	8 (7.8)	2 (14.29)	6 (6.74)
$3 < d \leq 5$	14 (13.6)	4 (28.57)	10 (11.24)
$5 < d \leq 10$	53 (51.5)	5 (35.71)	48 (53.93)
$d > 10$	28 (27.2)	3 (21.43)	25 (28.09)
Child-Pugh class			
A	93 (90.29)	12 (85.71)	81 (91.01)
B	10 (9.71)	2 (14.29)	8 (8.99)
Okuda stage			
I	60 (58.25)	8 (57.14)	52 (58.43)
II	43 (41.75)	6 (42.86)	37 (41.57)
AFP (μg/L)			
≥400	56 (54.37)	4 (28.57)	52 (58.43)
20–400	29 (28.16)	7 (50.0)	22 (24.72)
<20	18 (17.48)	3 (21.43)	15 (16.85)
TNM stage			
I and II	3 (2.91)	1 (7.14)	2 (2.25)
III	34 (33.01)	6 (42.86)	28 (31.46)
IVa	63 (61.17)	7 (50.00)	56 (62.92)
IVb	3 (2.91)	0	3 (3.37)
Therapy history*			
Chemotherapy	9 (8.74)	2 (14.29)	7 (7.86)
Surgery	17 (16.5)	0	17 (19.1)
TACE	18 (17.48)	3 (21.43)	15 (16.85)
No therapy	59 (57.28)	5 (35.71)	50 (56.18)

Abbreviations: AFP = alfa fetoprotein; TNM = tumor-node-metastasis; TACE = transarterial chemoembolization.

Data are given as *n* (%).

* Four weeks before beginning of study.

cm. The mean KPS was 84.14 ± 8.91. Approximately 40% of the patients were in Stage II according to the Okuda classification. Most of them were in this stage because of tumor size: most of them were in class A according to the Child-Pugh classification. The baseline characteristics of the 103 patients enrolled in Phase II are shown in Table 1.

Dose-limiting toxicity

No life-threatening toxic effects or deaths were found to be related to iodine (^{131}I) metuximab injection.

In the Phase I study, the hematologic and hepatic toxicity showed a tendency to increase with elevated dosage, but no obviously hematologic toxicity (≥Grade 3) was observed. Overall, 4 of the 28 patients (14.3%) showed aggravated hematologic damage (but still below Grade 2) after treatment, and 13 of the 28 patients (46.4%) showed aggravated hepatic function damage. No dose-limiting toxicity was found in the 9.25-MBq/kg group. In the 18.5-MBq/kg group, 3 of the 8 patients (37.5%) presented Grade 3 toxicity of liver enzymes or bilirubin. In the 27.75-MBq/kg group, 1 of the 8 patients (12.5%) presented Grade 3 toxicity of liver enzymes. In the 37-MBq/kg group, 4 of the 8 patients (50%) were found to have Grade 3 toxicity of liver enzymes. On the basis of these results, 27.75 MBq/kg was set to be the safe dosage and was used in the Phase II study (Table 2).

In the Phase I study, most of the enrolled HCC patients already had abnormal hematologic or hepatic function indexes before the treatment. However, after the treatment, some indexes of those patients were improved. Among all 28 patients, the hematologic indexes were improved in 5 of 8 patients (62.5%) with hematologic damage before treatment, and the hepatic function damage was improved in 8 of 22 patients (36.4%).

In the Phase II study, Grade 3 hematologic toxicity was observed in 1 of the 14 patients (7.14%) studied for the preliminary safety evaluation of the two administrations, whereas no Grade 3 toxicity of hepatic and renal function was observed. For the other 89 patients, Grade 3 hematologic toxicity was found in only 4 patients (4.49%), and aggravated hepatic function damage (≥Grade 3), compared

Table 2. Toxic effects of treatment

	Phase I trial				Phase II trial	
Parameter	9.25 MBq	18.5 MBq	27.75 MBq	37 MBq	First 14 patients	89 patients
White blood cell	0 (0)	0 (0)	0 (0)	3 (0)	4 (1)	15 (0)
Platelet	0 (0)	0 (0)	1 (0)	0 (0)	2 (1)	24 (4)
Serum glutamate pyruvate transaminase (SGPT)	0 (0)	2 (0)	2 (1)	4 (2)	5 (0)	14 (7)
Serum glutamate oxaloacetate transaminase (SGOT)	1 (0)	3 (3)	1 (0)	3 (2)	8 (0)	17 (2)
Total bilirubin	0 (0)	1 (1)	0 (0)	1 (0)	0 (0)	9 (2)
Direct bilirubin	0 (0)	1 (1)	0 (0)	2 (0)	2 (0)	13 (1)
Blood urea nitrogen (BUN)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Creatinine	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)

The table lists all toxic events possibly related to iodine (^{131}I) metuximab injection. The number of patients with Grade 3 and 4 toxic effects after treatment is shown in parentheses. Grade 3 toxicity: WBC count $<1.0–1.9 \times 10^6$/mL; PLT count $<2.5–4.9 \times 10^7$/mL; SGPT, SGOT, and bilirubin: >5.1–10.0 × normal value (N); BUN and creatinine: >5.0–10.0 × N (1 mCi = 37 MBq).

with that seen before treatment, was observed in <10% of the patients. According to the Child-Pugh classification, 85 patients were in Class A (82.52%), and 18 patients were in Class B (17.48%) after treatment. Compared with their before-treatment class, eight patients (7.77%) had their Child-Pugh class changed from A to B because of changes in bilirubin and/or albumin levels: 6 had bilirubin criteria changed from A to C and 1 from A to B; 1 had albumin criteria changed from A to C and 6 from A to B or from B to C.

One patient showed renal function toxicity under Grade 2; no toxicity to renal function was observed in the other 130 patients. No obvious changes of thyroid function and myocardial zymogram were found. No patient was found to have a positive HAMA response during the 4 weeks after the first treatment, and only 4 patients were found to have HAMA response at Day 7 after the second treatment (i.e., Day 35 after the first treatment), and they were excluded from the study.

Pharmacokinetics

After the cross-analysis of the three dose groups, no significant difference in catabolism of the iodine (^{131}I) metuximab injection vs. time was found between the 18.5- and 27.75-MBq/kg groups ($p = 0.2291$), whereas significant differences in that parameter were found between the 18.5- and 37-MBq/kg groups ($p = 0.0266$) and between the 27.75- and 37-MBq/kg groups ($p = 0.0155$). Blood clearance of iodine (^{131}I) metuximab injection in the three groups all accorded with a biphasic model (Fig. 1a). The half-time of distribution ($T_{1/2}\alpha$) in the 18.5-, 27.75-, and 37-MBq/kg groups was 5.94, 6.83, and 5.40 h, respectively, and the half-time of removal ($T_{1/2}\beta$, $t_{1/2}$) was 90.56, 80.98, and 63.93 h, respectively. These results suggest that the blood clearance rate was negatively correlated with the given dosage and that the 27.75-MBq/kg dose was the optimal dose for clinical use.

In all three groups, radioactivity intensity in urine decreased gradually. By 120 h, more than 47.7% of the infused radioactivity had been evacuated in urine, which indicated that this drug was mainly evacuated by the urinary system (Fig. 1b). No significant difference of the radioactivity intensity in urine vs. time was found between any two groups ($p > 0.5$).

Antitumor effect evaluation

In the Phase II study, 103 patients completed one cycle, and of these, 73 completed two cycles. After the first cycle, the numbers of patients with PR, MR, SD, and PD were 5 (4.85%), 11 (10.68%), 66 (64.08%), and 21 (20.39%), respectively. Sixty-nine progression-free patients and 4 patients with PD, who had completed the first cycle, were given the second treatment, and 30 patients were excluded because of PD (17 patients), high HAMA titer (4 patients), toxic effects (4 patients), loss to follow-up (2 patients), and other reasons (3 patients). After the two cycles, the numbers of patients with PR, MR, SD, and PD were 6, 14, 43, and 10, respectively. The objective response (CR + PR) and clinical effect (CR + PR + MR) rates after the second course were 8.22% and 27.40%, respectively. Although no significant difference of antitumor 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. Two of the 11 patients (18.18%) with MR after the first course had PR after the second course, and 6 of the 66 patients (9.09%) with SD after the first course had MR after the second. This was consistent with the typical results of mAb therapy, which is that if the drug has an effect on the tumor, another therapy will usually achieve a better effect.

A significant association was found between the TNM stage and the antitumor effect ($p = 0.0074$): the lower the TNM, the better the antitumor effect.

No correlation was found between pretreatment tumor size and the clinical efficacy by Pearson correlation ($r = 0.094$, $p = 0.346$ after one cycle; $r = 0.005$, $p = 0.965$ after two cycles). By one-way analysis of variance, although no significant difference ($p = 0.075$ after one cycle, $p = 0.647$ after two cycles) was found between the pretreatment tumor size and the tumor shrinking rate (the quotient obtained by dividing the products of the pretreatment maximal perpendicular diameters of measurable lesions by the products of the posttreatment ones) in the four groups (the maximal perpendicular diameter ≤3, 3–5, 5–10, and >10 cm), which was possibly caused by the small sample size, the shrinking rate in the first group was found to be lower than that of the other three groups, suggesting that iodine (^{131}I) metuximab injection had a better effect on small tumors than on larger ones (Table 3).

No significant differences in KPS were found between pretreatment scores and scores after one (83.5 ± 10.64, $p = 0.1792$) or two treatment cycles (84.93 ± 9.45, $p = 0.1275$).

In radioimmunodetection, it was observed in 97 of 103 patients (94.17%) that the defect shown in the ^{99m}Tc-sodium phytate-colloid scan was consistent with the hot region shown in SPECT of iodine (^{131}I) metuximab injection, suggesting that iodine (^{131}I) metuximab injection had specific binding to tumor tissues (Fig. 2). Of the other 6 patients with poor radioimmunoimaging, 3 had SD and 3 PD, suggesting that the clinical efficacy was in some way related to the antibody targeting.

The survival rate was also an endpoint. The survival rate at 6, 12, 18, and 21 months was, respectively, 82.63%, 58.68%, 51.98%, and 44.54% (by log–rank test), and the median survival time was 19 months. The survival rate of progression-free patients was significantly higher than that of patients with progressive disease after either one or two cycles ($p < 0.0001$ or $p = 0.0019$ by log–rank test) (Fig. 3), which suggested that the clinical efficacy of iodine (^{131}I) metuximab injection was positively correlated with the survival rate.

DISCUSSION

Radionuclide in radiolabeled-antibody, using antibody as the carrier, can be targeted to and concentrated in tumor

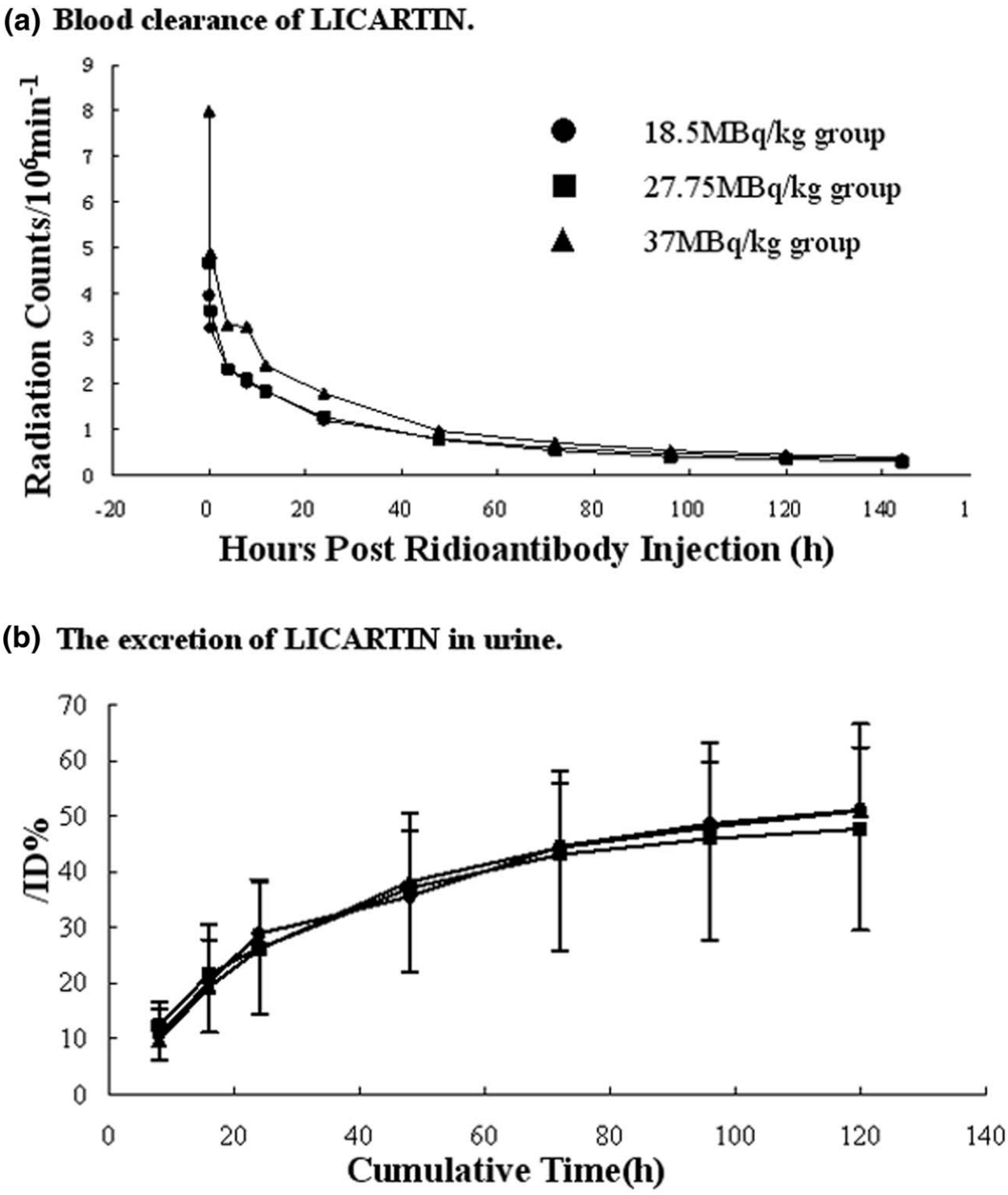

Fig. 1. Pharmacokinetics study of iodine (^{131}I) metuximab injection (Licartin).

tissues to kill more tumor cells while causing as little injury as possible to normal tissues. Thus, compared with chemotherapy or radiotherapy, RIT has few toxic effects. The effective killing range of radionuclide can reach the path length of 830 μm, which amounts to more than 50 tumor cells, and the surrounding tumor cells, which are not bound with antibody, can also be killed. Thus, RIT is a more effective therapy than that with nude antibody. For example, Zevalin, the ^{90}Y-anti-CD20 murine antibody conjugate, achieved an overall response rate of 80% with an antibody dosage of 250 mg/m^2, whereas nude antibody, Rituximab, achieved only 56% with a higher dosage (375 mg/m^2) in non-Hodgkin's lymphoma patients (15, 16). In RIT, labeling antibody with radionuclide can reduce the quantity of

Table 3. Descriptive variance of the tumor shrinking rate in different groups

	Tumor size (cm)	No. of patients	Tumor shrinking rate		95% confidence interval for mean	
			Mean	SD	Lower bound	Upper bound
After one cycle	$d \leq 3$	8	0.8554	0.2959	0.6437	1.0670
	$3 < d \leq 5$	14	1.2724	0.7229	0.8355	1.7093
	$5 < d \leq 10$	53	1.0344	0.3293	0.9462	1.1226
	$d > 10$	28	1.1024	0.3005	0.9755	1.2293
	Total	103	1.0629	0.3983	0.9850	1.1407
After two cycles	$d \leq 3$	8	0.7730	0.2478	0.5658	0.9802
	$3 < d \leq 5$	10	1.0575	0.2885	0.8511	1.2639
	$5 < d \leq 10$	39	1.0919	0.8260	0.8241	1.3596
	$d > 10$	16	1.0326	0.2693	0.8891	1.1761
	Total	73	1.0392	0.6332	0.8915	1.1870

Abbreviation: SD = standard deviation.

antibody. In our trials, the quantity of the antibody Metuximab was only 5 mg per patient, which might greatly decrease the risk of HAMA response and other toxic effects.

The selection of antibody form and radionuclide plays a vital role in RIT. For solid tumors, $F(ab')_2$ was reported to be superior to intact immunoglobulin G owing to its smaller molecular weight, stronger penetrating ability, lower immunogenicity (without the Fc fragment), faster blood clearance rate, and the maintenance of appropriate conformation (17). In our work, the target antigen HAb18G/CD147 was highly expressed on HCC cells. HAb18 could specifically bind to HCC cells with few cross-reactions with normal tissues (5, 18). The high specificity and affinity of its $F(ab')_2$ fragment (metuximab) were first revealed by our previously basic and preclinical studies, and the biological distribution analysis already reported in Phase I, and now they were confirmed by radioimmunoimaging in the present work (18, 19). Iodine (^{131}I) metuximab injection was concentrated in the HCC region. The tumor/nontumor ratio was >1 (1.04–3.79) (19). The smaller molecular weight of iodine (^{131}I) metuximab injection, together with its high specificity and the removal of the Fc fragment, might lead to the faster blood clearance that fits the biphasic model. The lower immunogenicity caused by the removal of the Fc fragment and by the lower antibody dosage probably contributes to their being fewer HAMA responses. The hepatic artery infusion of labeled antibody avoided the loss of iodine (^{131}I) metuximab injection compared with intravenous administration and facilitated the specific binding of iodine (^{131}I) metuximab injection to the HCC tissues. The characteristics mentioned above make iodine (^{131}I) metuximab injection safer and more controllable, which further confirms that $F(ab')_2$ is a better form of antibody used in RIT for solid tumors.

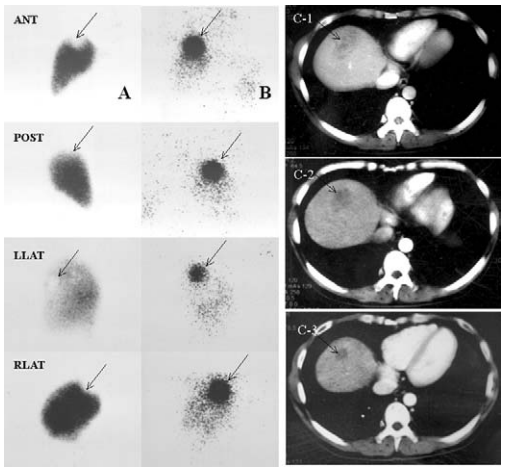

Fig. 2. Immunoscintigraphy and computed tomography scans of hepatocellular carcinoma (HCC) in the right hepatic lobe of a patient. (A) Defect in the upper region of the right lobe of the liver (*arrow*) in ^{99m}Tc-sodium phytate-colloid scan. (B) Hot region of HCC (*arrow*) in SPECT 7 days after treatment. (C) CT scan results of the same patient: (C-1) pretreatment assessment (5.8 × 4.5 cm, *arrow*); (C-2) 1 month after the first administration (4.5 × 3.5 cm, *arrow*); (C-3) partial response (4 × 3.2 cm, *arrow*) 1 month after the second administration. ANT = anterior; POST = posterior; LLAT = left lateral; RLAT = right lateral.

Iodine-131 is widely used clinically, especially in RIT, as a therapeutic radionuclide for its convenient source, satisfying labeling effect, and low cost. Because it is a high-energy β-particles emitter and can kill tumor cells not only by its direct effect but also by the cross-fire effect that expands its killing range, ^{131}I is believed to have good clinical efficacy for the therapy of solid tumors with high-density antigen (20).

In this study, we performed RIT of HCC by iodine (^{131}I) metuximab injection, the ^{131}I-labeled metuximab, and achieved better clinical efficacy. After two cycles of therapy, the clinical effect of iodine (^{131}I) metuximab injection was 27.40%, and the 21-month survival rate reached 44.54%.

Our hypotheses regarding the mechanism responsible for the clinical results are based on data from *in vitro*, *in vivo,* and clinical trials. First, the specificity and affinity of metuximab and the high expression of target antigen on HCC cells make it possible for the conjugated ^{131}I to con-

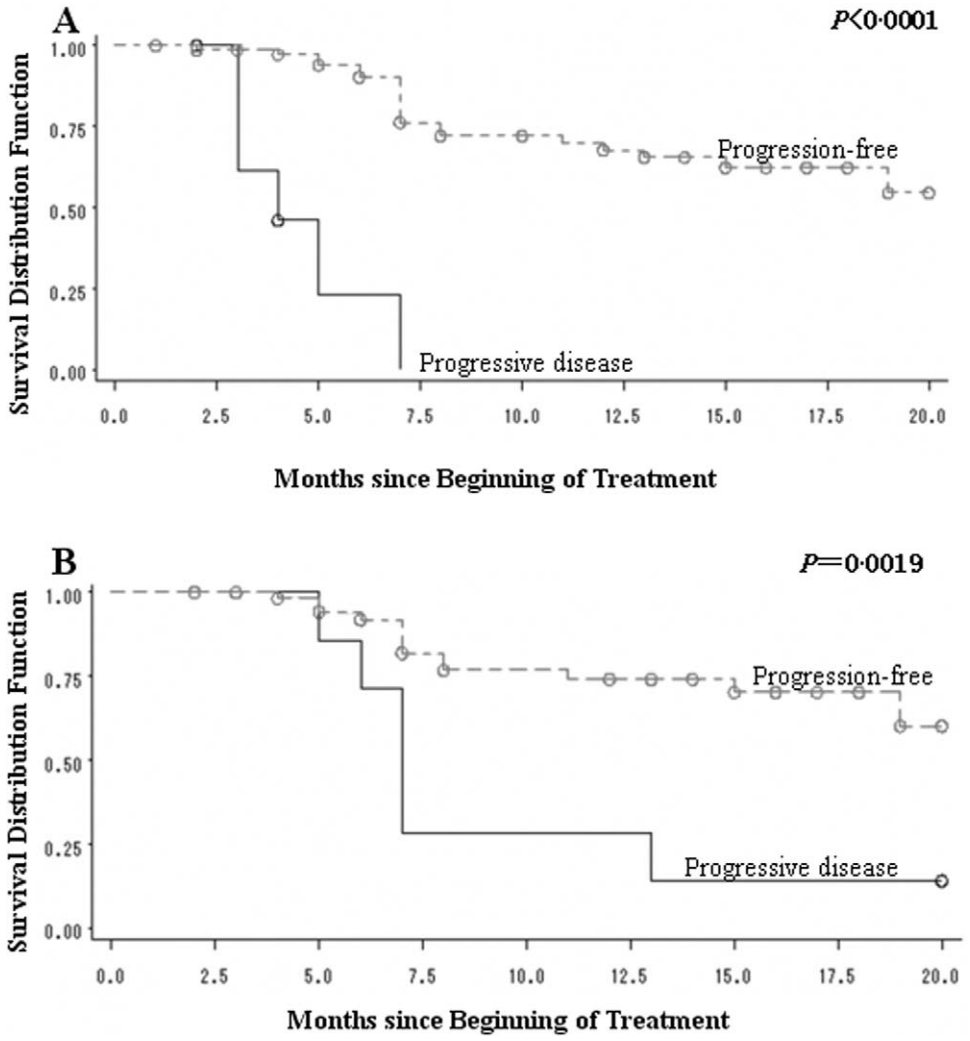

Fig. 3. Survival curves for patients after (A) one and (B) two cycles with iodine (^{131}I) metuximab injection.

centrate in HCC tissues and kill tumor cells directly and indirectly by its cross-fire effect. As a result, iodine (^{131}I) metuximab injection can effectively kill tumor cells without the internalization of mAb, which makes it a better therapy than that with mAb-conjugated chemicals and toxins. Second, the target antigen, HAb18G/CD147, is a cell adhesion molecule with multiple functions and closely relates to tumor metastasis (21, 22). It is involved in the adhesion and motion of tumor cells, angiogenesis, and signal transduction and can induce fibroblast to produce matrix metalloproteinases (MMPs), including MMP-1, MMP-2, and MMP-9 (23–26). These MMPs can degrade the extracellular matrix and promote the metastasis of HCC cells. Iodine (^{131}I) metuximab injection was found both *in vitro* and *in vivo* (HCC xenografts) to have obvious inhibitory effects on oncogenesis and metastasis in liver and other organs (unpublished data). From the above findings, we believe that iodine (^{131}I) metuximab injection has the potential ability to block and destroy HAb18G/CD147 target site, thus inhibiting the metastasis of HCC. This might partly explain the fact that nearly half of our patients in trials survived over 20 months.

In our study we did not use the placebo for comparison because of ethical considerations and because HCC is characterized by rapid progression: patients usually have only a 3.2-month natural survival time. We also did not use any other drugs for comparison because no appropriate congener drug was available. Using unlabeled antibody metuximab as a control was not approved by the Medical Ethics Committee because metuximab obviously showed no satisfying responses in the preclinical studies. However, we compared our present work with studies on TACE reported previously, in which the trials were conducted in HCC patients with similar clinical status. It was reported that the tumor response rate ranged from 12% to 57.9%, that the median survival time ranged from 7 to 19 months (13.63 ± 6.10 months), and that 1- and 2-year survival rates were 42%–72% (58.30% ± 10.14%) and 0–55% (28.74% ± 15.88%), respectively (27–35). In our study, iodine (^{131}I) metuximab injection exhibited comparable clinical efficacy to TACE and more encouraging survival considering the possible difference of the enrolled patients' status and race; it was also well-tolerated, with no

obvious related toxicity after two courses of treatment. Compared with our preliminary clinical trial, that no CR was observed in the present study might be due to the larger tumor size (mean 7.85 ± 3.82 cm × 6.44 ± 2.97 cm) and the fewer treatment courses (one to two courses). In the preliminary clinical study, a patient finally showed CR after four courses of treatment by iodine (^{131}I) metuximab injection. The fact that the patients with tumor response in the present study could have a better response after a second treatment also suggested a possible better efficacy of long-term treatment by iodine (^{131}I) metuximab injection. The combination of iodine (^{131}I) metuximab injection with other treatment, such as TACE and chemical drugs, might also be a promising direction in unresectable HCC therapy. Thus, iodine (^{131}I) metuximab injection might be used as one of the first-choice candidate drugs for unresectable HCC. Our single-agent trials serve primarily as proof of principle and the basis for further investigation in this area, while providing some evidence for the clinical efficacy of iodine (^{131}I) metuximab injection.

REFERENCES

1. Tang ZY. Review. In: Tang ZY, editor. Tang ZY's clinical hepatocellular carcinoma. 1st ed. Shanghai, China: Shanghai Scientific and Technological Education Publishing House; 2001. p. 7–35.
2. Chang X. Review on treatment of primary liver cancer. In: Chang X, Chen JS, Qu QJ, *et al.* editors. Treatment of primary liver cancer. 1st ed. Zhengzhou, China: Henan Medical University Press; 1999. p. 3.
3. Chen ZN, Liu YF. Monoclonal antibody HAb18 to human hepatoma. *Monoclonal Antibodies* 1990;8:11.
4. Liu YF, Chen ZN, Ji YY. Localization of hepatocellular carcinoma with monoclonal antibodies. *Zhonghua Yi Xue Za Zhi* 1991;71:362–365.
5. Chen ZN, Xing JL, Zhang SH, inventors; NTD Patent & Trademark Agency, Beijing office, assignee. Anti-human hepatoma monoclonal antibody HAb18 light/heavy chain variable region gene, and use thereof. Chinese patent ZL02114471.0, 2002 March 15; PCT international patent WO03/078469. 2003 March 17.
6. Toole BP. Emmprin (CD147), a cell surface regulator of matrix metalloproteinase production and function. *Curr Top Dev Biol* 2003;54:371–389.
7. Chen ZN, Shang P, Li Y, Qian AR, Zhu P, Xing JL, inventors; NTD Patent & Trademark Agency, Beijing office, assignee. HAb18G/CD147, its agonist and application. Chinese patent ZL01131735.3, 2001 Sep 28; PCT international patent WO02/094875. 2002 May 27.
8. Yu JY, Sui YF, Chen ZN. Pharmacokinetics of monoclonal antibody HAb18 and its F(ab')$_2$ and Fab fragments in mice. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* 1996;12:16–21.
9. Chen ZN, Liu YF, Jiang MD, *et al.* Radiolocalization of human hepatoma with anti-human hepatoma monoclonal antibodies and its F(ab')$_2$ in tumor-bearing nude mice. *Zhonghua Yi Xue Za Zhi* 1989;69:566–568.
10. Xu SY. Toxicity test. In: Xu SY, Bian RL, Chen X, editors. The methodology of pharmacological experiment. 1st ed. Beijing, China: People's Medical Publishing House; 1982. p. 412.
11. Mi L, Chen ZN, Feng Q, Yu XL, inventors; Xi'an Tongda Patent & Trademark Agency, assignee. Preparation of monoclonal antibody bivalent fragment and Fab fragment. Chinese patent ZL99115730.3. 1999 Mar 12.
12. Mi L, Li L, Feng Q, Yu XL, inventors; Intellectual Property Services Center of Shaanxi Province, assignee. Technical methods of continuous perfusion culture hybridoma cells and CHO cells for production of monoclonal antibody. Chinese patent ZL01131736.1. 2001 Sep 29.
13. Yamada R, Kishi K, Terada M. Transcatheter arterial chemoembolization for unresectable hepatocellular carcinoma. In: Tobe T, Okudaira KH, editors. Primary liver cancer in Japan. Tokyo: Springer Verlag; 1992. p. 259–271.
14. Bronowicki JP, Vetter D, Dumas F, *et al.* Transcatheter oily chemoembolization for hepatocellular carcinoma. A 4-year study of 127 French patients. *Cancer* 1994;74:16–24.
15. Wiseman GA, White CA, Stabin M, *et al.* Phase I/II ^{90}Y Zevalin (90 yttrium ibritumomab tiuxetan, IDEC-Y2B8) radioimmunotherapy dosimetry results in relapsed or refractory non-Hodgkin's lymphoma. *Eur J Nucl Med* 2000;27:766–777.
16. Wiseman GA, White CA, Sparks RB, *et al.* Biodistribution and dosimetry results from a phase III prospectively randomized controlled trial of Zevalin radioimmunotherapy for low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma. *Crit Rev Oncol Hematol* 2001;39:181–194.
17. Khawli LA, Alauddin MM, Hu P, *et al.* Tumor targeting properties of indium-111 labeled genetically engineered Fab' and F(ab')$_2$ constructs of chimeric tumor necrosis treatment (chTNT)-3 antibody. *Cancer Biother Radiopharm* 2003;18:931–940.
18. Chen ZN, Liu YF, Sui YF, *et al.* Significance and application of anti-malignant hepatoma MAb HAb18 in radioimmunal diagnosis of human hepatocellular carcinoma. *Chin J Oncol* 1992;14:9–12.
19. Lu W, Li X, Wang C, *et al.* Biological distribution of ^{131}I-HAb18F(ab')$_2$ in patients with hepatocellular carcinoma. *J Biomed Eng* 2003;20:689–691.
20. Mattes MJ. Radionuclide-antibody conjugates for single-cell cytotoxicity. *Cancer* 2002;94:1215–1223.
21. Chen ZN, Yang Z, Mi L, *et al.* The structural and functional analysis of the hapetoma related factor, HAb18G. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* 1999;15:34.
22. Li Y, Shang P, Qian AR, *et al.* Inhibitory effects of antisense RNA of HAb18G/CD147 on invasion of hepatocellular carcinoma cells in vitro. *World J Gastroenterol* 2003;9:2174–2177.
23. Jiang JL, Zhou Q, Yu MK, *et al.* The involvement of HAb18G/CD147 in regulation of store-operated calcium entry and metastasis of human hepatoma cells. *J Biol Chem* 2001;267:46870–46877.
24. Jiang JL, Yu MK, Chen ZN, *et al.* cGMP-regulated store-operated calcium entry in human hepatoma cells. *Cell Biol Int* 2001;25:993–995.
25. Huang BC, Shang P, Qian AR, *et al.* Bio-panning of antagonistic peptides against HAb18G/CD147 and their function of anti-hepatoma invasion. *Chin J Oncol* 2003;25:111–114.
26. Jiang JL, Chan HC, Zhou Q. HAb18G/CD147-mediated calcium mobilization and hepatoma metastasis require both C-terminal and N-terminal domains. *Cell Mol Life Sci* 2004;61:2083–2091.
27. Yamada K, Izaki K, Sugimoto K, *et al.* Prospective trial of combined transcatheter arterial chemoembolization and three-dimensional conformal radiotherapy for portal vein tumor thrombus in patients with unresectable hepatocellular carcinoma. *Int J Radiat Oncol Biol Phys* 2003;57:113–119.
28. Ueno K, Miyazono N, Inoue H, *et al.* Transcatheter arterial

chemoembolization therapy using iodized oil for patients with unresectable hepatocellular carcinoma: Evaluation of three kinds of regimens and analysis of prognostic factors. *Cancer* 2000;88:1574–1581.
29. Lo CM, Ngan H, Tso WK, *et al.* Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. *Hepatology* 2002;35: 1164–1171.
30. Yamada R, Kishi K, Sonomura T, *et al.* Transcatheter arterial embolization in unresectable hepatocellular carcinoma. *Cardiovasc Intervent Radiol* 1990;13:135–139.
31. Nakamura H, Hashimoto T, Oi H, *et al.* Treatment of hepatocellular carcinoma by segmental hepatic artery injection of adriamycinin oil emulsion with overflow to segmental portal veins. *Acta Radiol* 1990;31:347–349.
32. Chen MS, Li JQ, Zhang YQ, *et al.* High-dose iodized oil transcatheter arterial chemoembolization for patients with large hepatocellular carcinoma. *World J Gastroenterol* 2002;8:74–78.
33. Wang MQ. Transcatheter arterial chemoembolization for 850 patients with primary hepatocellular carcinoma. *Chin J Med Imaging Technol* 1996;12:23.
34. Pellentier G, Ducreux M, Gay F, *et al.* Treatment of unresectable hepatocellular carcinoma with lipiodol chemoembolization:a multicenter randomized trial. *J Hepatol* 1998;29: 129–134.
35. Raoul J, Guyader D, Bretagne JF, *et al.* Prospective randomized trial of chemoembolization versus intra-arterial injection of ^{131}I-labeled-iodized oil in the treatment of hepatocellular carcinoma. *Hepatology* 1997;26:1156–1161.