

Adoptive immunotherapy to lower postsurgical recurrence rates of hepatocellular carcinoma: a randomised trial

Tadatoshi Takayama, Teruaki Sekine, Masatoshi Makuuchi, Susumu Yamasaki, Tomoo Kosuge, Junji Yamamoto, Kazuaki Shimada, Michiie Sakamoto, Setsuo Hirohashi, Yasuo Ohashi, Tadao Kakizoe

Summary

Background Postsurgical recurrence of hepatocellular carcinoma (HCC) is frequent and fatal. Adoptive immunotherapy is active against HCC. We assessed whether postoperative immunotherapy could lower the frequency of recurrence.

Methods Between 1992 and 1995, we did a randomised trial in which 150 patients who had undergone curative resection for HCC were assigned adoptive immunotherapy (n=76) or no adjuvant treatment (n=74). Autologous lymphocytes activated *in vitro* with recombinant interleukin-2 and antibody to CD3 were infused five times during the first 6 months. Primary endpoints were time to first recurrence and recurrence-free survival and analyses were by intention to treat.

Findings 76 patients received 370 (97%) of 380 scheduled lymphocyte infusions (mean cell number per patient 7.1×10^{10} [SD 2.1]; CD3 and HLA-DR cells 78% [16]), and none had grade 3 or 4 adverse events. After a median follow-up of 4.4 years (range 0.2–6.7), adoptive immunotherapy decreased the frequency of recurrence by 18% compared with controls (45% [59] vs 57% [77]) and reduced the risk of recurrence by 41% (95% CI 12–60, $p=0.01$). Time to first recurrence in the immunotherapy group was significantly longer than that in the control group (48% [37–59] vs 33% [22–43] at 3 years, 38% [22–54] vs 22% [11–34] at 5 years; $p=0.008$). The immunotherapy group had significantly longer recurrence-free survival ($p=0.01$) and disease-specific survival ($p=0.04$) than the control group. Overall survival did not differ significantly between groups ($p=0.09$).

Interpretation Adoptive immunotherapy is a safe, feasible treatment that can lower recurrence and improve recurrence-free outcomes after surgery for HCC.

Lancet 2000; **356**: 802–07

Department of Surgery (T Takayama MD, S Yamasaki MD, T Kosuge MD, J Yamamoto MD, K Shimada MD), **National Cancer Centre Hospital** (T Kakizoe MD); **Divisions of Experimental Medicine** (T Sekine PhD) and **Pathology** (M Sakamoto MD, S Hirohashi MD), **National Cancer Centre Research Institute; Department of Biostatistics, School of Health Sciences and Nursing** (Prof Y Ohashi PhD); and **Department of Surgery, Faculty of Medicine** (Prof M Makuuchi MD), **University of Tokyo, Tokyo, Japan**

Correspondence to: Dr Tadatoshi Takayama, Division of Hepato-Biliary-Pancreatic and Transplantation Surgery, Department of Surgery, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan (e-mail: takayamat-2su@h.u-tokyo.ac.jp)

Introduction

The rising incidence of hepatocellular carcinoma (HCC) in at-risk patients with chronic hepatitis B or C is an important issue in more-developed countries.¹ Although early diagnosis and treatment improve survival,² HCC is rarely cured and recurs frequently after regional therapy or transplantation.^{3–8} Hepatic resection can improve 5-year recurrence-free survival by up to 25%,^{3–7} and median survival after recurrence is about 7–28 months.^{2,5,6} Micrometastases of HCC have been detected by molecular techniques in 88% of patients at the time of surgery,⁹ and probably cause postoperative recurrence.

Adoptive immunotherapy is no longer thought to be useful because clinical success has been confined to melanoma, renal cancer, and lymphoma.^{10,11} However, phase 2 studies in patients with HCC showed objective response rates of around 20%.^{12–14} In animal models, hepatic micrometastases were suitable targets.¹⁵ We thus postulated that immunotherapy would be most beneficial when used as a postsurgical adjuvant, since any residual tumour would probably be minimal. We developed a 2-week culture system that ensures about 1000-fold T-cell expansion from a sample of peripheral blood (with no need for leucapheresis).¹⁶ We did a randomised controlled trial to assess whether adoptive immunotherapy could lower the frequency of recurrence and prolong time to first recurrence and recurrence-free survival in patients who had undergone curative resection for HCC.

Methods

Patients

Patients treated at the National Cancer Centre in Tokyo were eligible if they had histologically confirmed HCC; UICC tumour-node-metastasis clinical grouping of stage I, II, IIIA, or IVA; hepatic function of Child-Pugh class A or B; had undergone curative hepatic resection; had adequate bone-marrow and renal reserve (white cell count $>3 \times 10^9/L$, platelets $>5 \times 10^{10}/L$, and creatinine $<88.4 \mu\text{mol/L}$); and were aged between 18 and 80 years. Exclusion criteria were clinically confirmed extrahepatic metastasis (stage IIIB or IVB); previous or simultaneous other malignant disorders; previous cancer treatment; or postoperative dysfunction of any organ.

Study design

We randomly assigned eligible patients adoptive immunotherapy or no adjuvant treatment. Randomisation was done by permuted block is without stratification, on receipt of the pathological confirmation

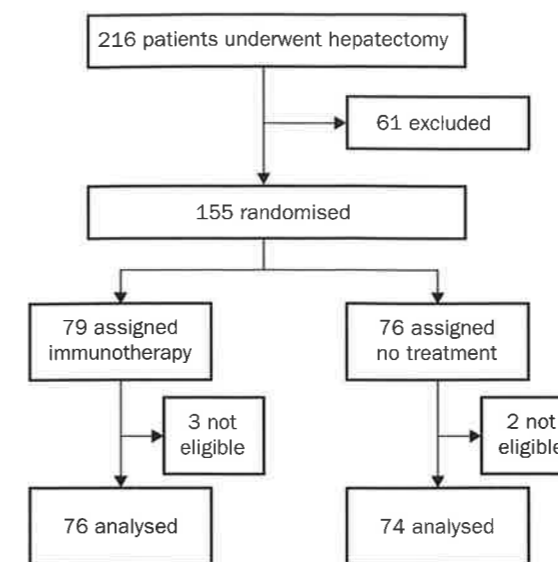


Figure 1: Trial profile

of T category, no later than 1 week after surgery. We obtained approval from the institutional ethics committee, and written consent from each patient.

Hepatectomy was indicated according to criteria based on tumour extension and hepatic functional reserve.¹⁷ Surgery was defined as curative when all gross lesions were removed with a histologically proven tumour-free margin, and as palliative when any gross lesion was left in the remnant liver or when tumour tissue remained at any resection margin. Microscopic examination of surgical samples included assessments of tumour-cell differentiation according to Edmondson's system, the status of cancer spread (defined as histologically proven vascular invasion or intrahepatic metastasis in samples),² and the presence of dysplastic lesions (adenomatous hyperplasia or dysplastic foci) representing precursors of HCC.¹⁸

We took (50 mL) peripheral blood from all eligible patients on the day before surgery. Mononuclear cells were separated and cultured for 2 weeks with recombinant interleukin-2 (700 IU/mL; Shionogi Pharmaceutical, Osaka, Japan) and immobilised monoclonal antibody to CD3 (5 $\mu\text{g}/\text{mL}$; Janssen-Kyowa, Tokyo, Japan).¹⁶ Cell culture was continued for patients assigned to immunotherapy, preserving (about 2×10^9) cells as the source for the second to fifth infusions, and was discontinued for patients assigned no adjuvant treatment. Lymphocytes before and after *in-vitro* culture were phenotyped with the appropriate monoclonal antibodies. The final cell products were assessed for viability by the dye-exclusion test and checked twice for possible contamination by bacteria, fungi, and endotoxins.

Before the start of the trial, we confirmed that the activated T-cells we cultured killed autologous HCC cells *in vitro*,¹⁹ localised at tumour sites *in vivo*,¹³ and had no major toxic effects after repeated transfer.²⁰ Patients in the immunotherapy group received autologous lymphocytes intravenously at weeks 2, 3, 4, 12, and 24 after surgery (the last two or three infusions as outpatients). This schedule was designed to transfer sufficient cells ($>3 \times 10^{10}$) to produce a tumour response, as confirmed in phase 2 studies.²¹ All adverse events were

Characteristic	Immunotherapy (n=76)	Control (n=74)
Age (years)		
<60	26 (34%)	32 (43%)
≥60	50 (66%)	42 (57%)
Cause of liver injury		
Hepatitis B	15 (20%)	14 (19%)
Hepatitis C	50 (66%)	49 (66%)
Unknown	11 (14%)	11 (15%)
Child-Pugh class		
A	54 (71%)	50 (68%)
B	22 (29%)	24 (32%)
Alanine aminotransferase (IU/L)		
<100	69 (91%)	69 (93%)
≥100	7 (9%)	5 (7%)
Indocyanine clearance (%)		
<20	49 (64%)	45 (61%)
≥20	27 (36%)	29 (39%)
α-fetoprotein (ng/mL)		
<400	58 (76%)	57 (77%)
≥400	18 (24%)	17 (23%)
Tumour size (mm)		
<30	38 (50%)	32 (43%)
≥30	38 (50%)	42 (57%)
Tumour number		
Single	51 (67%)	53 (72%)
Multiple	25 (33%)	21 (28%)
Hepatectomy*		
Minor	48 (63%)	50 (68%)
Major	28 (37%)	24 (32%)
TNM stage†		
I or II	40 (53%)	41 (55%)
IIIa or IVa	36 (47%)	33 (45%)
Edmondson's grade		
1 or 2	62 (82%)	61 (82%)
3	14 (18%)	13 (18%)
Cancer spread‡		
Negative	42 (55%)	42 (57%)
Positive	34 (45%)	32 (43%)
Dysplastic lesions		
Absent	60 (79%)	56 (76%)
Present	16 (21%)	18 (24%)
Background liver		
Chronic hepatitis	41 (54%)	36 (49%)
Cirrhosis	35 (46%)	38 (51%)

*Minor hepatectomy included limited resection or subsegmentectomy; major hepatectomy included segmentectomy or lobectomy. †Workups for staging consisted of preoperative imaging methods (hepatic ultrasonography, dynamic computed tomography, angiography, lipiodol computed tomography, and chest radiography), and surgical exploration with operative ultrasonography. ‡Positive status was defined as histologically proven vascular invasion or metastasis within resected liver sample, and negative status as absence of these two findings.

Table 1: Baseline characteristics

recorded, and we graded severity according to the WHO toxicity scale. Treatment was stopped if extrahepatic metastasis was detected, grade 3 or 4 toxic effects developed, or the patient withdrew from the study. We defined adherence to treatment as transfer of the processed lymphocytes within 1 week of the assigned dates.¹¹

The primary endpoints were time to first recurrence and recurrence-free survival, and the secondary endpoints were disease-specific survival and overall survival. After surgery we screened all patients in the two groups by hepatic ultrasonography and α -fetoprotein measurement every 2 months, dynamic computed tomography every 4 months, and chest radiography every 6 months. Hepatic angiography followed by lipiodol, chest computed tomography, or bone scintigraphy was done if recurrence was suspected. By the time of recurrence, no other cancer treatment was being given to all patients.

Event	Immunotherapy (n=76)*	Cellular transfer (n=370)
Fever	36 (47%)	50 (14%)
Headache	3 (4%)	5 (1%)
Nausea	3 (4%)	4 (1%)
Dizziness	1 (1%)	1 (0.3%)
Itching	1 (1%)	1 (0.3%)
Tachycardia	1 (1%)	1 (0.3%)
Total	45 (59%)	62 (17%)

*Five patients had more than one type of event attributed to immunotherapy.

Table 2: Adverse events

Type	Immunotherapy (n=76)	Control (n=74)	Relative risk (95% CI)	p*
Overall recurrence	45 (59%)	57 (77%)	0.59 (0.40-0.88)	0.01
Early recurrence	25 (33%)	40 (54%)	0.49 (0.30-0.80)	0.005
Local recurrence	2 (3%)	5 (7%)	0.31 (0.06-1.62)	0.17
Extensive recurrence	11 (14%)	19 (26%)	0.44 (0.21-0.93)	0.03
Distant metastasis	4 (5%)	6 (8%)	0.49 (0.14-1.75)	0.27
Multicentric tumour	3 (4%)	7 (9%)	0.37 (0.09-1.46)	0.15

*Likelihood-ratio test.

Table 3: Postsurgical recurrence

We recorded first recurrences and deaths from any cause. We defined recurrence as the appearance of new lesions with radiological features typical of HCC, seen by at least two imaging methods,^{2,6} and was separated into six classifications: overall recurrence (tumours at any time or at any site); early recurrence (any recurrence type during the first 2 years); local recurrence (tumours within 2 cm of the surgical margin); extensive recurrence (more than five tumours, or tumours with vascular invasion); distant metastasis (tumours at extrahepatic sites as the first sign of recurrence); or multicentric tumour (tumours in the different segments after 3 or more years, if the initial tumour had had no cancer spread). The first detected recurrence was documented by two independent radiologists unaware of the study group; any recurrence arising after the first event was not recorded. According to our criteria,² patients with recurrence underwent a second hepatic resection, ethanol injection, hepatic arterial embolisation, or systemic chemotherapy. Study group was not taken into account when assigning secondary treatment.

Statistical analysis

We calculated that we would need a sample size of at least 143 patients (86 events) to detect a 20% difference in 3-year recurrence-free survival (from 30 to 50%)⁴⁻⁶ at 5% type-I error and 80% power for a one-tailed log-rank test. All analyses were done by intention-to-treat.

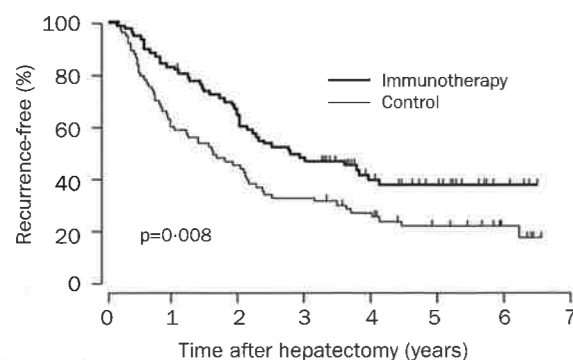


Figure 2: Time to first recurrence

Variable	Univariate analysis p*	Multivariate analysis	
		Relative risk (95% CI)	p†
Adoptive immunotherapy (yes vs no)	0.01	0.60 (0.41-0.89)	0.01
Cancer spread (negative vs positive)	0.01	0.52 (0.35-0.77)	0.001
Indocyanine clearance (<20% vs ≥20%)	0.02	0.63 (0.42-0.95)	0.03

*Log-rank test. †Likelihood-ratio test.

Table 4: Analyses on recurrence

Kaplan-Meier curves were generated for time to first recurrence, recurrence-free survival, disease-specific survival, and overall survival. We compared curves for the two groups with the log-rank test. All time estimates were done with the date of hepatectomy as the baseline. All patients were followed up for at least 3 years as of December, 1998, or until death.

We used Cox's proportional-hazards model to estimate risk reduction in each of six recurrence types for competing risks (ie, when assessing risk of early recurrence, patients who did not have recurrence by the end of 2 years were not included; when assessing risks of four types of recurrence, the other three types of recurrence were treated as censored at the date of diagnosis). The prognostic relevance of immunotherapy and the 14 baseline variables (table 1). recurrence was analysed univariately by log-rank tests. All variables that had p values less than 0.05 were included in the Cox's model. We assessed the interaction between the treatment effect on recurrence and cancer spread, a strong prognostic factor for recurrence,^{1-3,6,7} by the Cox's model with the interaction term. The effect of recurrence status on overall survival was assessed by the Cox's model, which included recurrence status as a time-dependent dummy covariate. All p values were two-tailed, and significance was set at p<0.05.

Results

From May, 1992, to September, 1995, we undertook hepatic resection in 216 patients. 61 patients were not eligible for inclusion in the study because of palliative resection (46 patients), non-HCC tumours (six), stage IVB status (four), or other reasons (five). 155 patients were included, but five were found to be not eligible after randomisation because of tumour-positive surgical margin (three), cholangiocarcinoma (one), and peritoneal metastases (one; figure 1). Baseline characteristics in the two groups were similar (table 1).

Autologous lymphocytes were successfully cultured in all 76 patients of the immunotherapy group. After 2 weeks of culture, the mean number of cells increased 1400-fold from 1.0×10^7 (SD 0.2) to 1.4×10^{10} (0.4), and most were positive T-cell markers (CD3 87% [10], CD3 and HLA-DR 78% [16], CD4 25% [13], CD8 72% [8]). The final cell products in all patients were highly viable (97% [2]) and uncontaminated.

72 patients received all five lymphocyte infusions, two received four infusions because of detection of extrahepatic metastases, and two received only one infusion because they subsequently refused treatment. Of 380 scheduled infusions, 370 (97%) were completed. The mean number of transferred cells was 1.5×10^{10} (SD 0.4) per session and 7.1×10^{10} (2.1) per patient. 62 adverse events developed in 45 patients, all of which were grade 1 or 2 and self-limiting (table 2). No patient had pulmonary or renal symptoms or any sign of

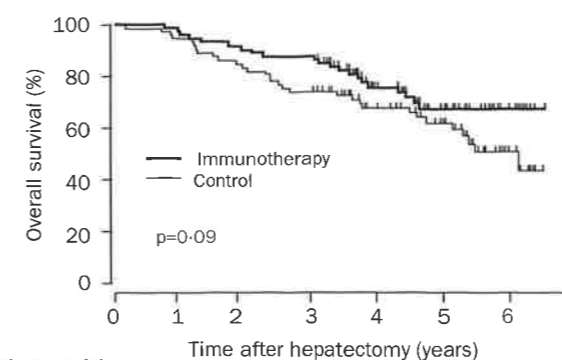


Figure 3: Overall survival

infection, hepatic functional deterioration, or autoimmune disorder.

No patients were lost to follow-up, and the median length of follow-up was 4.4 years (range 0.2-6.7). HCC recurred in 45 (59%) immunotherapy patients compared with 57 (77%) controls (table 3). The time to first recurrence in the immunotherapy group was significantly longer than that in the control group (p=0.008). Estimated rates for years 3 and 5 were 48% (95% CI 37-59) compared with 33% (22-43), and 38% (22-54) compared with 22% (11-34), respectively (figure 2). The median time to first recurrence was 1.6 years (range 0.2-6.7) for the control group and 2.8 years (0.2-6.6) for the immunotherapy group. Recurrence-free survival was also significantly higher in the immunotherapy group than in the control group (28 [37%] vs 16 [22%] patients; p=0.01). Inclusion of five non-eligible patients randomised in these analyses gave similar results (p=0.01 for time to first recurrence, p=0.02 for recurrence-free survival).

Compared with no adjuvant treatment, adoptive immunotherapy significantly lowered the risks of overall recurrence by 41% (p=0.01), early recurrence by 51% (p=0.005), and extensive recurrence by 56% (p=0.03; table 3). Of 15 variables assessed, three were significantly related to recurrence in univariate analysis: immunotherapy, cancer spread, and indocyanine clearance (table 4). In multivariate analysis, the risk of overall recurrence in the immunotherapy group, compared with the control group remained unchanged. The interaction between the treatment effect on recurrence and cancer spread was significant (p=0.02), but the effect was clinically important in the two groups: risk reduction of recurrence in the stratum with spread was 0.31 (95% CI -0.20 to 0.60), and that in the stratum without spread was 0.50 (0.13-0.71). The initial treatment for recurrence was similar in the immunotherapy and the control groups: arterial embolisation 24 patients (53%) compared with 34 (60%), second surgery or ethanol injection 19 patients (42%) compared with 19 (33%), and systemic chemotherapy 2 patients (5%) compared with 4 (7%).

21 patients (28%) in the immunotherapy group and 31 (42%) in the control group died. The causes of death were recurrent HCC in 48 patients, and other diseases with no evidence of recurrence in four (three treated patients died of ruptured oesophageal varices, hepatic failure, or myocardial infarction, and one control died of

pulmonary squamous-cell carcinoma). Recurrence status significantly affected overall survival (relative risk of death 21.8 [95% CI 7.2-65.8], p<0.0001). Disease-specific survival was significantly higher in the immunotherapy group than in the control group (p=0.04). The difference in overall survival was not significant (p=0.09); the estimated rates for years 3 and 5 were 88% (95% CI 81-95) compared with 74% (64-85), and 68% (53-83) compared with 62% (47-77), respectively (figure 3). The relative risk of death attributable to HCC for treated patients compared with non-treated patients was 0.56 (95% CI 0.31-1.01, p=0.05), and that of death from all causes was 0.64 (0.37-1.11, p=0.11).

Discussion

We showed that adoptive immunotherapy could lower frequency of recurrence and improve recurrence-free outcomes after surgery for HCC. Recurrence of HCC after treatment is frequent.³ Acyclic retinoid and intra-arterial 131-iodine-labelled lipiodol have been reported to diminish recurrence, but their potential usefulness remains to be accepted because of a limited effect on overall recurrence²² or on hepatitis-C-related HCC.²³ Adoptive immunotherapy is perhaps thought of as out-of-date, since the efficacy on cancer may be lower than was initially understood,¹⁰ and new T-cell-based treatments have been introduced.²⁴ A preliminary trial of postoperative immunotherapy in 45 patients with renal cancer suggested a significant time delay to recurrence;²⁵ such adjuvant use has, however, been precluded by the complexity and toxicity of treatment. We reliably obtained an average 1400-fold expansion of autologous T-cells and a safe cell product. Our double-stimulation technique^{16,20} resulted in a higher yield of cells than standard methods involving leucapheresis,^{10,11} and is less invasive and more economical than other techniques. Concomitant infusion of interleukin-2 was avoided to keep grade 3 and 4 toxic effects to a minimum (reported in 30% of patients),¹⁰ although this might have increased activity. In our study, treatment adherence was satisfactory (97%), all adverse events were grade 1 or 2, and patients could be treated as outpatients. We believe that our treatment is justified by its technical feasibility and minimum toxicity.

Patients with HCC probably have microscopic lesions that cannot be removed by surgery. We confirmed just after surgical interventions that our patients were free from macroscopic evidence of tumour by intraoperative ultrasonography. Clinically occult metastases of HCC present at the time of surgery, probably became detectable during follow-up. Recurrence was defined as the development of new lesions meeting predefined criteria,^{2,6} which have a diagnostic accuracy rate of 96%.² The two groups were well balanced for known factors for recurrence, such as HCC status (size, number, vascular invasion, and tumour-node-metastasis stage), α -fetoprotein, and liver dysfunction.^{1-3,6,7} Recurrence data in the control group were similar to those reported previously.⁶ Therefore, the significant risk reduction in recurrence is attributable to adoptive immunotherapy, which suggests that the treatment's antitumour activity is against residual HCC. The substantial suppression of early recurrence is consistent with our hypothesis that

immunotherapy would be most beneficial to patients with minimum residual tumours. The substantial inhibition of extensive recurrence might indicate that immunotherapy decreased emergence of intrahepatic metastases, possibly via the portal venous system.¹⁷ This treatment might, therefore, have the greatest impact on intrahepatic tumour residues during the first 2 years after surgery.

Case-control studies reported that risk of recurrence was 2.2-fold higher in patients who had cancer spread at the time of surgery than in those who did not.^{2,6,7} Although interaction between treatment effect and cancer spread was significant, risk reduction was clinically important in the two groups, with a possibly greater degree of reduction in the stratum without spread than in that with spread. Taken together, patients with no cancer spread might have had a lesser burden of micrometastases, and consequently benefited more from immunotherapy than those with the spread.

Even if residual tumour is eliminated, our patients remained at risk of multicentre HCC.²⁶ We defined multicentricity by more stringent criteria than those proposed in other studies,²² since clonal discrimination is not done in clinical practice. Similar baseline incidence of precursor coexistence in the two groups may indicate no striking difference in such a risk.¹⁸ Immunotherapy had no apparent effect on this event, which was not unexpected because of the short duration of treatment. Nevertheless, use of more intensive treatment is promising, because a recurrence-free state can last for many years in other patients.²⁰

Reduced recurrence is expected to improve survival. This theory seems reasonable because recurrent HCC was responsible for 48 (92%) of 52 deaths in our study and recurrence status significantly affected overall survival. The possibility that salvage treatment for recurrence had an effect on overall survival is unlikely, since choice of treatment was not dependent on the study group and the proportions of patients receiving each type of treatment did not differ between groups. Immunotherapy gave a significantly higher disease-specific survival, but did not do so for overall survival, which could become significant with further follow-up.

Transferred T-cell-based cytotoxicity seems to be the most probable mechanism for our results.^{13-15,19,21} Micrometastatic HCC cells are plausible targets, since they frequently upregulate class I MHC antigens.²⁷ Use of peripheral blood as the source of effectors is supported by the fact that tumour-specific cytotoxic T-cells can be isolated from the peripheral repertoire (eg, frequency in melanoma patients is as much as 2% of CD8 T-cells).²⁸ Our culture system would increase the total number of potential effectors, because antibodies to CD3 can mimic antigen and stimulate tumour-sensitized T-cells.²¹ The extent to which specific T-cell responses contribute to clinical outcome needs to be analysed.

Adoptive immunotherapy can be recommended as a new adjuvant in patients with HCC. Treatment refinements, such as defining the best schedule, finding the optimum use of known immunomodulators, and developing more potent effectors, could improve clinical benefits.

Contributors

T Takayama, T Sekine, and M Makuuchi contributed to planning, execution, and analysis of the study. S Yamasaki, T Kosuge, J Yamamoto, and K Shimada participated in the clinical execution of the study. M Sakamoto and S Hirohashi were responsible for the pathological assessments. Y Ohashi contributed to statistical analysis and T Kakizoe was the overall coordinator. All investigators contributed to writing of the paper.

Acknowledgments

We thank: Masao Omata (Department of Gastroenterology, University of Tokyo) for his critical review of the manuscript; Kouichi Ishikawa and Masashi Fujii (Department of Pharmacology and Third Department of Surgery, Nihon University) for their review of the study design; Chikuma Hamada (Department of Pharmacoepidemiology, University of Tokyo) for his statistical help; Nobuaki Higashi (Laboratory of Cancer Biology and Molecular Immunology, University of Tokyo) for his valuable suggestions; and Hidetaka Kawabata, Kyoji Moriya, Masami Minagawa, Kazuto Inoue, and Guido Torzilli (Department of Surgery and Department of Gastroenterology, University of Tokyo) for their helpful discussions about the data. This work was supported in part by a grant-in-aid for Cancer Research and a grant-in-aid for the Comprehensive 10-year Strategy of Cancer Control from the Ministry of Health and Welfare, Japan.

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