Adjuvant Adenovirus-Mediated Delivery of Herpes Simplex Virus Thymidine Kinase Administration Improves Outcome of Liver Transplantation in Patients with Advanced Hepatocellular Carcinoma

Ning Li,[1,2] Jianfeng Zhou,[3] Danhui Weng,[3] Chenghua Zhang,[4] Lixin Li,[2] Beibei Wang,[3] Yang Song,[2] Qiang He,[2] Dongdong Lin,[1] Dazhi Chen,[2] Gang Chen,[3] Qinglei Gao,[3] Shixuan Wang,[3] Gang Xu,[3] Li Meng,[3] YunPing Lu,[3] and Ding Ma[3]

Abstract

Purpose: Previous poor results of liver transplantation (LT) have been confirmed in patients with advanced hepatocellular carcinoma (HCC). Adenovirus-mediated delivery of herpes simplex virus thymidine kinase (ADV-TK) therapy is an established adjuvant treatment in cancer, and we evaluated its potential as an adjuvant treatment for HCC patients who underwent LT.

Experimental Design: Forty-five HCC patients with tumors >5 cm in diameter participated in the study over a follow-up period of 50 months. Among these patients, 22 received LT only, and 23 received LT combined with ADV-TK therapy. All HCC patients enrolled in this study had tumors >5 cm in diameter and no metastasis in lungs or bones was detected by computed tomography or magnetic resonance imaging scans.

Results: The recurrence-free survival and the overall survival in the LT plus ADV-TK therapy group were 43.5% and 69.6%, respectively, at 3 years; both values were significantly higher than those in the LT-only group (9.1% and 19.9%, respectively). In the nonvascular invasion subgroup, overall survival was 100% and recurrence-free survival was 83.3% in the patients receiving LT plus ADV-TK, significantly higher than the patients receiving LT only.

Conclusions: HCC patients with no vascular invasion could be selected for LT followed by adjuvant ADV-TK therapy, regardless of intrahepatic huge or diffuse tumor. We propose that the current criteria for LT based on tumor size may be expanded if accompanied by ADV-TK therapy due to improved prognosis.

Hepatocellular carcinoma (HCC) is currently the fifth most common neoplasm in the world (1, 2). The majority of HCC (80-90%) are associated with underlying liver diseases related to post-hepatitis cirrhosis, hemochromatosis, or alcohol abuse, and the incidence of HCC continues to increase steadily (3). The population of HCC patients in China is the largest around the world and ~230, 000 people die of HCC yearly, counting 53% of the total number of the global death population (4). In the past 30 years, liver transplantation (LT) has become an important technique in HCC treatment because of the triple advantage of removing the tumor, preventing formation of metachronous lesions on underlying cirrhosis, and restoring normal liver function (5). However, high recurrence rates (32-54%) and poor outcomes (5-year survival rate of <40%) were recorded from the first series of transplanted patients. These poor results were mostly related to unrestrictive selection criteria and inclusion of patients with macroscopic vascular invasion, lymph node involvement, and extrahepatic spread (6, 7). The landmark study of Mazzaferro et al. (8) in 1996 established LT as a viable option to treat HCC, based on the poor outcome of advanced HCC patients receiving LT and the limited source of donor organs. Their criteria have come to be known as the Milan criteria (unifocal ≤5 cm in diameter or ≤3 foci ≤3 cm in diameter) and have been widely applied throughout the world in the selection of patients with small HCC for transplantation (9–11). However, as a result of using these criteria for selecting patients, a large proportion of patients were excluded from LT and the chance of cure is unavailable for them (12). To date, it remains controversial whether the Milan criteria should be expanded or not, although

Authors' Affiliations: [1]Beijing YouAn Hospital, Capital Medical University; [2]Beijing ChaoYang Hospital, Capital Medical University, Beijing, P.R. China; [3]Cancer Biology Research Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, P.R. China; and [4]The No. 180 Hospital of People's Liberation Army, Quanzhou, Fujian, P.R. China

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N. Li and J. Zhou contributed equally to this work.

Requests for reprints: Ding Ma, Cancer Biology Research Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095 Jiefang Avenue, Wuhan, Hubei 430030, P.R. China. Phone: 86-27-83663351; Fax: 86-27-83662681; E-mail: dma@tjh.tjmu.edu.cn or dingma424@yahoo.com.

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several centers have tried to expand the criteria (13–15). The concern is that liberalizing the criteria will result in increased recurrence (16).

Gene therapy for malignant neoplasm has currently been receiving much attention in the field. Extensive experience related to gene therapy, such as toxicity, pharmacology, and clinical indications, has been gained and reported (17–26). Adenovirus-mediated delivery of herpes simplex virus thymidine kinase (ADV-TK) into tumor cells is one of the well-studied approaches to facilitate the eradication of tumors. Herpes simplex virus thymidine kinase (HSV-TK) converts a benign substance (prodrug) ganciclovir into toxic nucleotide analogues, which are incorporated into DNA during cell division, terminate DNA replication, and lead to cancer cell death. Moreover, TK transduction effectively eradicates cancer cells via a bystander effect of gene therapy. Recent phase I/II clinical trials reported promising results using ADV-TK alone or in combination with chemotherapeutic agents or irradiation in treatment of recurrent prostate cancer (27). A routine chemotherapy scheme is administered to HCC patients after LT for the purpose of controlling metastasis or relapse. The advantages of ADV-TK highlight its potential as adjuvant treatment for HCC patients after LT, especially because of its chemotherapeutic efficacy-promoting ability and its hepatotropic characteristics.

Improving the prognosis of patients with advanced HCC is a great challenge. It is not advisable to blindly expand the criteria of LT without available renovation in adjuvant treatment that have been shown to improve outcome. In this study, we report the result of LT plus adjuvant ADV-TK therapy followed by ganciclovir i.v. administration in patients with advanced HCC. Careful assessment was made for the clinical effect of this combined treatment, and the possibility of expanding the transplantation selection criteria was also explored.

Patients and Methods

The study was conducted in accordance with the Declaration of Helsinki. All patients provided written, informed consent. The study was approved by the local ethics committee.

Eligibility criteria. Forty-five patients in the Beijing Transplantation Center, ChaoYang Hospital (Beijing, P.R.China) and in The No. 180 Hospital of People's Liberation Army (Quanzhou, Fujian, P.R. China) participated in the study during the period between September 2000 and October 2006. All patients participating in the study were evaluated with computed tomography (CT) or magnetic resonance imaging (MRI) and a complete liver function panel (Table 1). All patients who had unresectable HCC >5 cm, with no metastasis in lungs and bones, were eligible to participate in the study. Patients with distant metastasis detected by CT or MRI scan or at the time of surgery were excluded. Tumor involvement of intrahepatic portal branches was not considered contraindications for acceptance into the study. Vascular invasion was evaluated by ultrasonography or pathologic examination.

Study design and treatment. All patients participating in the study were randomized into a LT-only group or a LT plus ADV-TK therapy group. The comparison of patients' baseline data from the two groups was shown in Table 1; the baseline characteristics were compatible between the two groups. The primary aim of the study was to evaluate and compare the recurrence-free survival and the overall survival rates in each group. The safety of ADV-TK therapy was also assessed.

Table 1. Baseline characteristics of 45 HCC patients undergoing LT

Various	Transplantation only	Transplantation plus ADV-TK therapy	*P*
Demography		32-61	9
Age (y)			
Average	43.9	44.3	NS
Range	26-65	32-61	
Sex			
Male	20	23	NS
Female	2	0	
Hepatic function related			
Child-Pugh stage			
B	15	9	NS
C	7	14	
Tumor related			
No. tumors			
1	4	7	NS
≥2	18	16	
Diameter of tumor (cm)			
5~8	1	5	NS
>8	21	18	
TNM stage			
T_3	8	3	NS
T_4	14	20	
Vascular invasion			
Absent	12	12	NS
Present	10	11	
Serum AFP (ng/mL)			
Preoperation	2,630.1 ± 14,602.5	1,036.6 ± 12,034.1	NS
Post-operation	105.8 ± 12,253.6*	15.8 ± 1,674.4†	0.022

Abbreviations: TNM, tumor-node-metastasis; NS, not significant.
*P = 0.050 (versus preoperation).
†P < 0.0001 (versus preoperation).

Orthotopic LT was done in all patients, followed by triple regimen immunosuppression consisting of cyclosporin A (Novartis AG) and mycophenolate mofetil (Shanghai Roche Pharmaceuticals Ltd.) in combination with lifelong administration of corticosteroids. Rejection episodes were treated with basiliximab (Novartis) and Zenapax (Hoffman-La Roche, Inc.). All patients received routinely systemic postoperative epirubicin (60 mg/d) for 3 days at months 1, 2, 3, 6, and 12 after LT. Of a total of 45 patients, 23 patients received ADV-TK (Tian Dakang Co.) therapy during the operation. Based on the toxicity results from the phase I dose escalation trial at Sun Yat-sen University Cancer Center (Guangzhou, Guangdong, P.R.China), a total of 5.0×10^{11} viral particles ADV-TK were determined for current clinical trial. A single-dose intratumoral injection of 5.0×10^{11} viral particles ADV-TK caused an objective response with no significant toxicity (phase I test, Supplementary Data 3). To ensure uniform dosing, a total 5.0×10^{11} viral particles of ADV-TK in 60 mL of 0.9% saline were injected into peritoneum tissues around the liver, including the lesser curvature of stomach, abdominal aorta side, head of the pancreas surface of the right kidney, and the area under the right diaphragm at a dose of 1.0×10^{11} viral particles for each. Most of the viral dose was administrated at the middle of the peritoneum tissues contacting with the liver. The first dose (5 mg/kg) of ganciclovir (Hoffman-La Roche) was administered i.v. 24 h after LT and twice daily for 10 days.

The follow-up for these patients always included CT scans or MRI, done monthly up to the third postoperative month, then once every 3 months up to the third postoperative year, and then at least once every 6 months. Postoperative α-fetoprotein (AFP) was tested monthly up to the third postoperative month and then every 3 months up to the third postoperative year. A positron emission tomography (PET) examination, if possible, was done for detecting micrometastatic foci or early recurrence. Adverse effects were evaluated based on the symptoms reported by the patients or observed by clinicians.

ADV-TK handling and processing. The replication-deficient adenovirus mutant ADV-TK is a chimeric human group C adenovirus (ADV5) that expresses HSV-TK. The transgene of *HSV-TK* gene under control of a Rous sarcoma virus long terminal repeat promoter is inserted in the region of the excised E1 adenoviral genes. ADV-TK was constructed in this laboratory (Chinese patent issued number ZL 98 1 24960.4) and had been approved for clinical trials by State Food and Drug Administration, P.R. China (28). Clinical grade of ADV-TK was produced and purified as described elsewhere (28) and provided as a sterile viral solution containing 1 mL virus solution/5.0×10^{11} viral particles of ADV-TK. The viral solution was then further diluted to a final volume of 60 mL for injection.

Detection of ADV-TK viral DNA in blood. The persistence of ADV-TK viral DNA in patient blood was detected using a real-time fluorescent quantitative PCR assay. Blood samples were collected from patients receiving LT plus ADV-TK therapy at the time of 0 h, 4 h, 12 h, 24 h, 48 h, 7 days, 14 days, and 21 days after administration of ADV-TK. Plasma samples were stored at -80°C up to assay time. ADV-TK viral DNA was purified using QIAamp DNA Blood Mini kit (Qiagen). A standard curve was generated by spiking 10^{11} viral particles of CsCl gradient-purified ADV-TK into 1 mL of heparinized human blood and preparing serial 10-fold dilutions. To amplify ADV-TK viral genome, 5′-end sense primer (5′-CATTGGTGTGCACCTCCAAG-3′), 5′-end antisense primer (5′-CGCAGACGCGTGCTGAT-3′), and fluorescence-labeled probe (5′-FAM-AGCTCGGATCTTGGTGGCGTGAAACT-TAMRA-3′) for *HSV-TK* gene were used. Twenty microliters of each standard dilution and patent samples were added to a 25-μL PCR.

Statistical analysis. Continuous variables were tested for normal distribution with the use of the Kolmogorov-Smirnov test. Means between two groups were compared using a two-tailed, unpaired Student's *t* test. The Mann-Whitney test was used to test the medians of two groups if continuous variables tested did not exhibit a normal distribution. The χ^2 test or, where appropriate, Fisher's exact test was used for categorical variables.

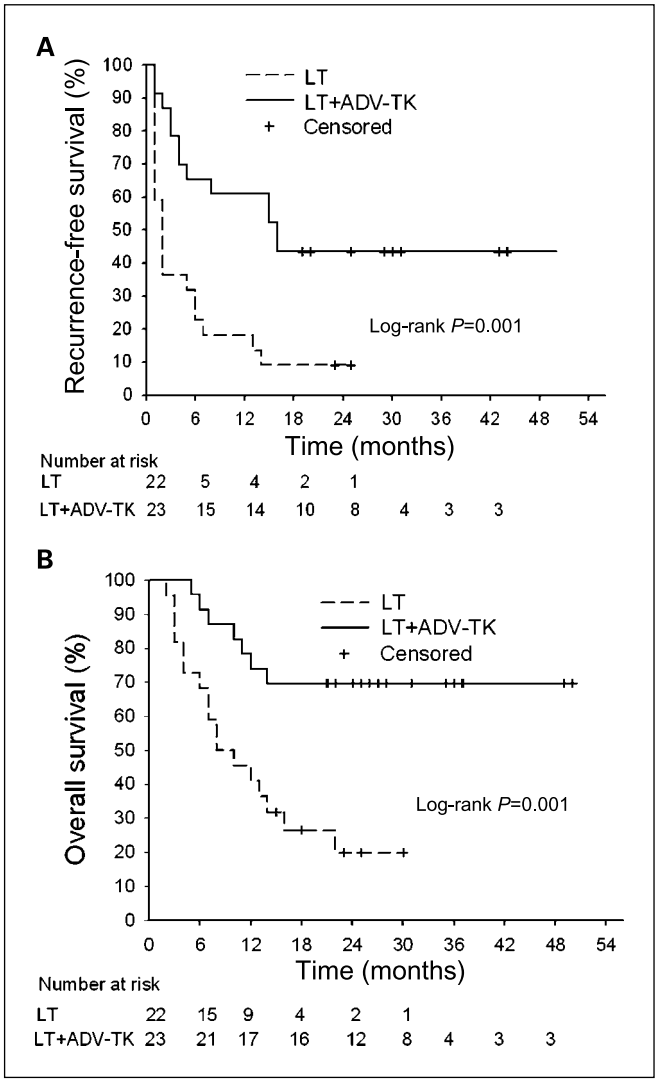

Fig. 1. Recurrence-free survival rate and overall survival rate of 45 patients based on different therapy protocols. *A,* recurrence-free survival rate in 23 patients receiving LT plus ADV-TK gene therapy (*solid line*) was significantly higher than that in 22 patients receiving LT only (*dashed line*; $P = 0.001$, log-rank test; $P = 0.001$, Breslow test). *B,* overall survival rate in 23 patients receiving LT plus ADV-TK gene therapy (*solid line*) was significantly higher than that in 22 patients receiving LT only (*dashed line*; $P = 0.001$, log-rank test; $P = 0.002$, Breslow test).

Recurrence-free and overall survivals were presented as Kaplan-Meier estimates and analyzed by log-rank tests and Breslow tests for comparisons. The Cox proportional hazards model [adjusted for age, tumor-node-metastasis stage, Child-Pugh classification, number of tumors, diameter of tumor, and vascular invasion] was used to estimate recurrence-free and overall survival. Hypothesis testing was two sided. A P value of <0.05 was considered to indicate statistical significance. Hazard ratios and 95% confidence intervals were obtained from the Cox proportional hazards model. Subgroup analyses of recurrence-free and overall survival were also prospectively planned for level of significance. Data were analyzed with SPSS statistical software (version 12.0 for Windows, SPSS).

Results

Characteristics of the patients. As of October 31, 2006, 128 advanced HCC patients in the Beijing Transplantation Center, ChaoYang Hospital (Beijing, P.R. China) and in The No. 180

Table 2. Multivariate analysis of tumor characteristics as predictors for mortality after LT

Predictors	Hazard ratio (95% CI)	*P*
Age	0.981 (0.934-1. 031)	0.456
Child-Pugh stage at transplantation	1.435 (0.555-3.715)	0.456
No. tumors	1.034 (0.289-3.696)	0.959
Diameter of tumor	0.553 (0.099-3.101)	0.501
TNM stage	0.885 (0.270-2.908)	0.841
Vascular invasion	4.323 (1.692-11.047)	0.002

Abbreviation: 95% CI, 95% confidence interval.

Hospital of People's Liberation Army (Quanzhou, Fujian, P.R. China) were registered, and 47 of them were enrolled in the study. There were 2 discarded from 47 patients, 1 died during operation and 1 patient died from multiple organ system failure due to infection at 16 days after operation (less than 1 month). The median follow-up time for the 45 patients who participated in the study was 26 months (range, 2-50 months). Baseline characteristics were compatible in the transplantation-only group and transplantation plus ADV-TK therapy group (Table 1) with no significant between-group differences in variables assessed. Subgroup analyses were also based on the homogeneity of the groups.

Overall recurrence. Among the 22 patients who received LT alone, 18 relapsed at 1 year and 2 relapsed at 2 years, making the 1-year recurrence-free survival rate 18.2% and 2-year overall recurrence-free rate 9.1%, respectively; a 9.1% recurrence-free survival rate was maintained until the end of study. Among 23 patients who received LT plus ADV-TK therapy, 9 relapsed at 1 year and 6 relapsed at 2 years, making the 1-year overall recurrence rate 60.9% and the 2-year overall recurrence-free rate 43.5%, respectively; a 43.5% overall recurrence-free rate was maintained until the end of study. The actuarial probability of recurrence-free survival rates was significantly different between two groups ($P = 0.001$, log-rank test; $P = 0.001$, Breslow test; Fig. 1A).

Overall survival. Among the 22 patients who received LT alone, 13 of 22 patients died at 1 year and 17 of 22 patients died at 2 years, making the 1-year overall survival rate 40.9% (accumulative survival rate) and the 2-year overall survival rate at 2 years 19.9%, respectively; a 19.9% overall survival rate was maintained until the end of study (Fig. 1B). The median follow-up time was 16 months (range, 2-30 months). Among the 23 patients who received LT plus ADV-TK therapy, 6 of 23 patients died at 1 year and 7 of 23 patients died at 2 years, making the 1-year overall survival rate 73.9% and the 2-year overall survival rate 69.6%, respectively; a 69.6% survival rate was maintained until the end of study (Fig. 1B). The median follow-up time of this group was 27.5 months (range, 5-50 months). The difference in the overall survival rates between these two groups was significant ($P = 0.001$, log-rank test; $P = 0.002$, Breslow test; Fig. 1B).

Multivariable analyses. The multivariate analyses of potentially relevant factors of patients in this study (Tables 2 and 3) showed that only vascular invasion was an independent prognostic factor significantly affecting both survival and recurrence ($P = 0.002$ and $P < 0.0001$).

Subgroup analyses. When the analysis was stratified according to the vascular invasion condition, more details about survival and recurrence were revealed. In the subgroup with vascular invasion, 10 patients received LT only and 11 patients received LT plus ADV-TK therapy. All 10 patients in the transplantation-only group experienced recurrence within 2 months after the operation. The recurrence occurred in the liver ($n = 4$), lung ($n = 5$), and bone ($n = 1$). Alternatively, all the patients who received LT plus ADV-TK therapy experienced relapse between 1 to 16 months. The recurrence site was in the liver ($n = 3$), lung ($n = 6$), and both liver and lung ($n = 2$). Adjuvant ADV-TK therapy seemed to have postponed recurrence time after LT. The significant difference in the recurrence-free survival rate between the two groups was identified by log-rank test and Breslow test ($P = 0.001$, log-rank test; $P = 0.003$, Breslow test; Fig. 2A).

Kaplan-Meier survival analysis showed that, in the LT-only group, 5 of 10 patients died at 6 months, 9 of 10 patients died at 12 months, and only 1 patient had been living for 30 months; in the LT plus ADV-TK therapy group, 2 of 11 patients died at 6 months, 7 of 11 patients died at 14 months, and 4 patients had been living for 28 months. The difference in survival rates between these two groups was examined by log-rank test and Breslow test ($P = 0.042$, log-rank test; $P = 0.026$, Breslow test; Fig. 2B).

In the nonvascular invasion subgroup, 12 patients received LT only and 12 patients received LT plus ADV-TK therapy. Among the 12 patients in the LT-only group, 10 patients relapsed and the recurrence time was between 1 to 14 months; 4 of 12 had died at 1 year and 8 of 12 had died at 2 years. The 2-year recurrence-free survival rate and the overall survival rate were 16.7% and 26.7% at 2 years, respectively. Among the 12 patients who received LT plus ADV-TK therapy, 2 patients relapsed between 15 to 16 months. No patients died until the end of study. The recurrence-free survival rates and the overall survival rates were 83.3% and 100%, respectively. The significant differences of the recurrence-free survival rate ($P < 0.001$, log-rank test; $P < 0.001$, Breslow test; Fig. 2C) and overall survival rates ($P < 0.001$, log-rank test; $P = 0.001$, Breslow test; Fig. 2D) between the two groups were analyzed by the log-rank test and Breslow test. Likewise, the recurrence-free survival rate and overall survival rate in the group without vascular invasion who received LT plus ADV-TK were significantly higher than that in other two groups, including group with vascular invasion who received LT plus ADV-TK ($P < 0.001$) and group with vascular invasion who received LT only ($P < 0.001$). One patient in the LT plus ADV-TK therapy group was diagnosed pathologically with primary colon cancer

Table 3. Multivariate analysis of tumor characteristics as predictors for recurrence after LT

Predictors	Hazard ratio (95% CI)	*P*
Age	0.973 (0.931-1.017)	0.229
Child-Pugh class at transplantation	0.503 (0.197-1.284)	0.151
No. tumors	1.850 (0.501-6.828)	0.356
Diameter of tumor	1.011 (0.224-4.564)	0.989
TNM stage	0.931 (0.325-2.669)	0.894
Vascular invasion	6.781 (2.625-17.519)	0.000

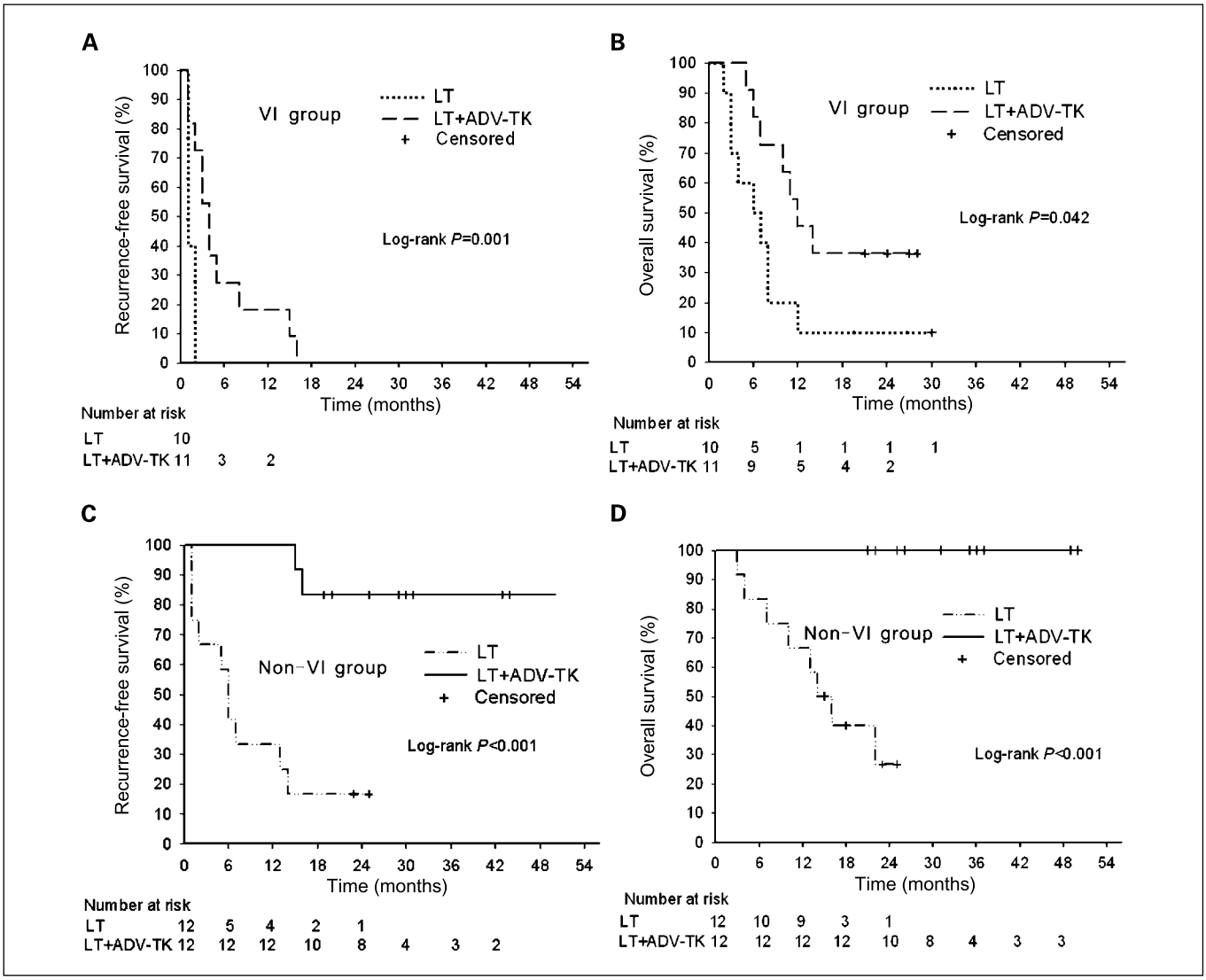

Fig. 2. Recurrence-free survival rate and overall survival rate of patients according to vascular invasion (*VI*) stations. In vascular invasion group, 10 patients received LT only (*dotted line*) and 11 patients received LT plus ADV-TK (*dashed line*). *A,* the recurrence-free survival rate in the patients who received LT plus ADV-TK was significantly higher than that in the patients who received LT only ($P = 0.001$, log-rank test; $P = 0.003$, Breslow test). *B,* the overall survival rate in the group who received LT plus ADV-TK was higher than that in the group who received LT only ($P = 0.042$, log-rank test; $P = 0.026$, Breslow test). In nonvascular invasion group, 12 patients received LT only (*dashed line*/*dotted line*) and 12 patients received LT plus ADV-TK (*solid line*). *C,* the recurrence-free survival rate in the patients who received LT plus ADV-TK was significantly higher than that in the patients who received LT only ($P < 0.001$, log-rank test; $P < 0.001$, Breslow test). *D,* the overall survival rate in the group who received LT plus ADV-TK was significantly high than that in the group who received LT only ($P < 0.001$, log-rank test; $P = 0.001$, Breslow test).

18 months after LT. After the colon cancer was removed, the patient recovered and remains alive at the time of this manuscript writing.

Changes in AFP before and after LT. AFP levels were tested before and after LT in all patients. Before LT, there was no significant difference between the LT-only group and the LT plus ADV-TK therapy group. After LT, AFP levels in both groups decreased ($P = 0.05$ in the LT-only group; $P < 0.0001$ in the LT plus ADV-TK group; Table 1); the decreasing tendency of AFP in the LT plus ADV-TK group was more noticeable compared with that of the LT-only group ($P = 0.022$; Table 1). Whether the ADV-TK therapy would prompt the decrease of AFP level still needs to be confirmed.

Safety. ADV-TK treatment was well tolerated and no significant toxicity was evident. Mild catarrhal symptoms were reported in 10 of 23 patients who received ADV-TK therapy. Slight fever with no chills was also observed after injection of ADV-TK in the first 3 days in the same patients. The temperatures ranged from 37.3°C to 38.3°C. The same 10 patients also suffered from light headache. All these symptoms subsided in 5 days. There was no evidence of liver or kidney dysfunction caused by ADV-TK in our study. Despite the abnormality of liver and renal function were observed in patients who received LT, especially in the first 2 weeks after operation, no added liver and renal toxicities were observed after injection of ADV-TK (Supplementary Data 3; Supplementary Table S3). There were no significant differences of liver and renal function tests between LT-only group and LT plus ADV-TK group (Supplementary Data 3; Supplementary Table S3). Liver function tests assayed included alanine aminotransferase, aspartate

aminotransferase, total bilirubin, and direct bilirubin. Renal function tests assayed included blood urea nitrogen and creatinine.

The pharmacokinetics of viral genome in the serum was measured in 12 randomized chosen patients from 23 patients who received LT plus ADV-TK treatment. The ADV-TK viral DNA in serum was detected at 4 h, peaked at 12 h, and disappeared at 7 days after ADV-TK. administration.

A representative case report. A 41-year-old man suffering from hepatitis B virus–related cirrhosis and portal hypertension presented with intermittent abdominal distention. Laboratory results showed markedly impaired hepatic function and an AFP level of 77 ng/mL (normal, ≤20 ng/mL). MRI of the liver showed a single tumor mass, 7.0 × 7.5 cm in diameter, located in right lobe of the liver (Fig. 3A, *white arrows*). Esophageal varices and fundus gastricus varicosities were also apparent. A diagnostic of HCC was confirmed by pathologic examination after operation, and no metastasis in the portal lymph node or tumor thrombosis of the extrahepatic portal vein was found. The patient was followed up for 45 months

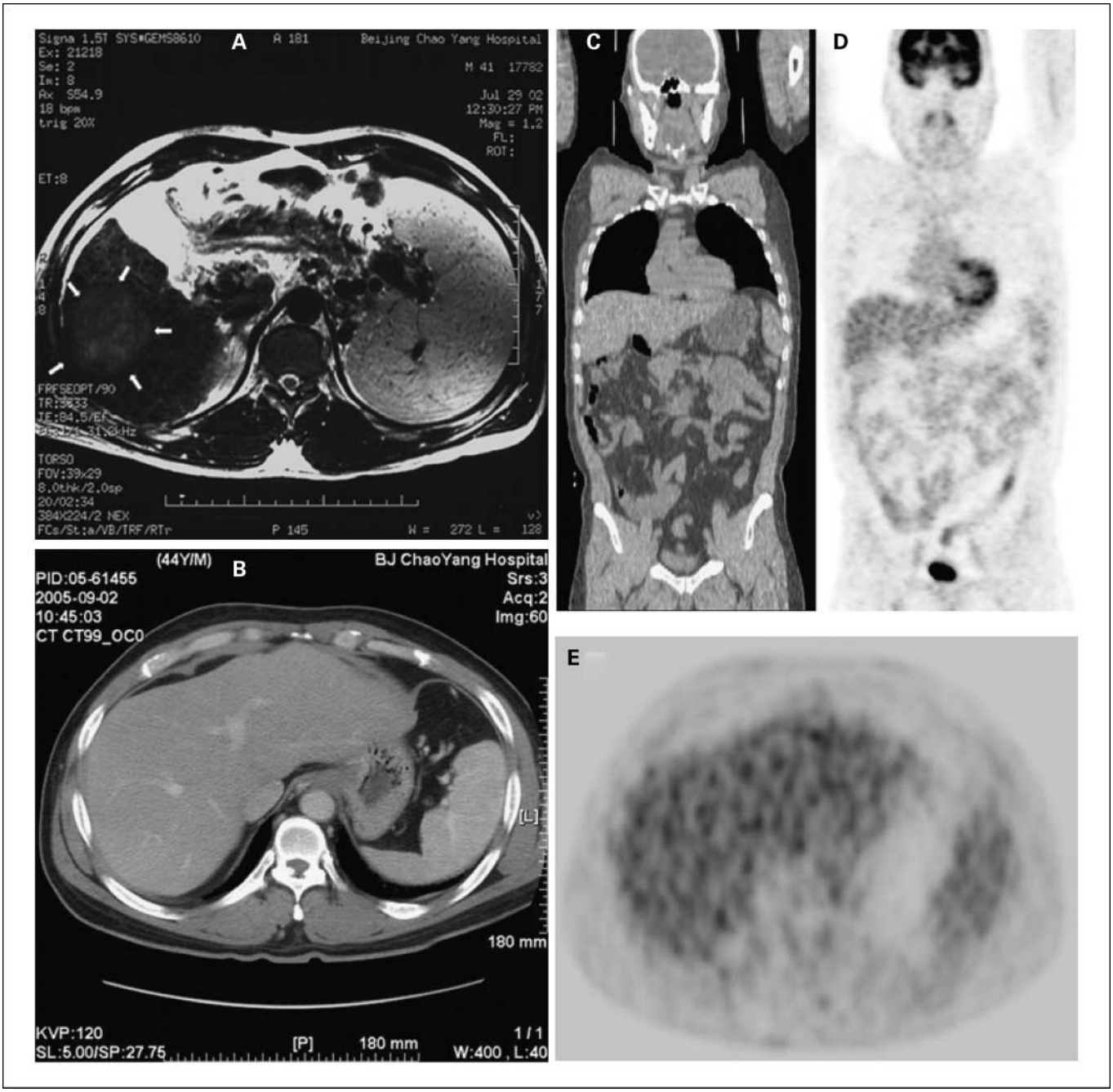

Fig. 3. A typical case with nonvascular invasion receiving LT plus ADV-TK treatment. *A,* a 7 × 7.5–cm single tumor in diameter located in the right lobe of the liver, by MRI. *B,* no tumor recurrence found by CT in transplanted liver 38 mo after LT. *C* and *D,* no tumor recurrence or metastasis found by CT or PET, respectively. *E,* no microrecurrent tumor in liver by PET.

after receiving LT plus ADV-TK treatment. CT and PET were done at 38 and 40 months after the operation, and no metastasis or recurrence signs were revealed (Fig. 3B–E; more details in Supplementary Data 1).

Discussion

The advantage of transplantation versus other types of treatments is that transplantation not only eliminates the tumor but also provides potential oncogenic cures for the underlying liver disease (10). It is not advisable to blindly expand the criteria of LT without appropriate adjuvant treatment. Many transplant center started to give patients post-LT chemotherapy to reduce the recurrence rate because recurrence of tumor is the most important factor affecting mortality (10, 20–26, 29–32). The safety and efficacy of ADV-TK has been assessed in several clinical studies (23–26, 33). In our study, ADV-TK therapy was combined with systemic chemotherapy after LT. Encouraging outcomes were achieved with this combined approach.

Several studies have clearly shown that vascular invasion is associated with an up to 15-fold increased risk of HCC recurrence following LT (32, 34–36). Adjuvant ADV-TK therapy seemed to postpone the recurrence time after LT in the advanced HCC patients with vascular invasion but did not affect the survival rate. It is noteworthy that the patients without vascular invasion, receiving ADV-TK therapy, achieved 100% overall survival rate over a 50-month follow-up period, and only 2 had recurrence within 3 years. A representative case was presented in Supplementary Data 1.

The efficacy of ADV-TK has been assessed in several clinical studies (23–25, 33), and the outcome in our study is more successful than expected. We tentatively attribute this excellent outcome to two factors: the hepatotropic tendency of ADV-TK and possibly the immune evasion of ADV-TK reagent in host patients. It is possible that adenovirus can aggregate into liver cells to improve gene transfer efficiency, resulting in stronger transgene expression in the target tissue (37, 38). In addition, numerous studies have suggested that adenovirus-mediated gene delivery induces systemic cellular and humoral immune responses that inhibit the effectiveness of repeated transfection of therapeutic genes (33, 39, 40). Research has shown that the transplant immunosuppression regimen enhances and prolongs adenovirus transgene expression (40), which we confirmed in our study (Supplementary Data 2). Post-transplant immunosuppression provides the means to attenuate the severe immune response to adenoviral-mediated gene transfection and thereby increase and prolong transgene expression rather than to promote tumor recurrence.

If our results are further confirmed by other studies, the criteria for selection of patients for LT may be modified and expanded. Patients who have a single HCC >5 cm in diameter, or even those with diffused type, without cancer vascular invasion, could be selected as candidates for LT combined with effective ADV-TK adjuvant therapy. Vascular invasion is still a strict restriction against LT.

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