Randomized, Phase II Study Comparing Interferon Combined with Hepatic Arterial Infusion of Fluorouracil plus Cisplatin and Fluorouracil Alone in Patients with Advanced Hepatocellular Carcinoma

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Clinical study

Title: Randomized, phase II study comparing interferon combined with the hepatic arterial infusion of fluorouracil plus cisplatin and fluorouracil alone in patients with advanced hepatocellular carcinoma

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Short title: Hepatic arterial infusion chemotherapy for HCC

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Abstract

Objective: This randomized phase II trial compared the response rates to treatment with interferon combined with hepatic arterial infusion of fluorouracil plus cisplatin and fluorouracil alone in patients with advanced hepatocellular carcinoma. **Methods:** A total of 114 patients with measurable advanced hepatocellular carcinoma were enrolled and randomized into 2 groups. Fluorouracil (300 mg/m^2 days 1 – 5, days 8 – 12) with or without cisplatin (20 mg/m^2 , day 1, day 8) were administered via the hepatic artery. Interferon alfa-2b was administered 3 times per week for 4 weeks. **Results:** The response rates were 45.6% for the interferon/fluorouracil + cisplatin group and 24.6% for the interferon/fluorouracil group. The response rate was significantly higher in the interferon/fluorouracil + cisplatin group ($p = 0.030$). The median overall survival period was 17.6 months in the interferon/fluorouracil + cisplatin group vs. 10.5 months in the interferon/fluorouracil group ($p = 0.522$). The median progression-free survival period was 6.5 months in the interferon/fluorouracil + cisplatin group vs. 3.3 months in the interferon/fluorouracil group ($p = 0.0048$). Hematological toxicity was common, but no toxicity-related deaths were observed. **Conclusion:** These results show the clinical efficacy of adding cisplatin to the hepatic arterial infusion of fluorouracil in combined chemotherapy regimens with interferon.

Introduction

Hepatocellular carcinoma (HCC) is the 6th most frequent type of cancer in the world and ranks third among various causes of cancer death. In recent years, the incidence of HCC has been increasing in Western and Asian countries [1-3].

Clinical practice guidelines for HCC are currently available in Japan, and the number of early cases with an early single tumor with a major diameter of 2cm or less detected by regular screening is generally increasing[4]. The treatment of early cases, including hepatectomy and local therapy such as radiofrequency ablation and percutaneous ethanol injection therapy, has progressed markedly, achieving a 5-year survival rate of 60-70%[5]. Most patients with HCC often experience the repeated recurrence of tumors after treatment and the disease may eventually reach an advanced stage. Furthermore, it is still not uncommon to find patients with symptomatic advanced HCC who have not participated in regular screening.

The efficacy of hepatectomy, local ablation therapy, and transarterial chemoembolization (TACE) is limited for advanced HCC and the prognosis of such cases is poor. Under these circumstances, systemic therapy with the molecular targeting drug sorafenib has shown a statistically significant survival benefit, compared with placebo treatment, in two large-scale phase-III clinical trials [6, 7]. Based on these findings, this drug is now recommended as a standard treatment for advanced HCC. These trials did not compare sorafenib with other conventional treatments of advanced HCC, but with best supportive care as the placebo treatment. Although a significant difference in the survival time was noted, the response rate was as low as 2 – 3.3%, with no significant difference from the results in the placebo arm (1 – 1.3%)[6, 7].

As another optional treatment for advanced HCC, hepatic arterial infusion

chemotherapy (HAIC) has been employed mainly in Japan and other Asian countries. HAIC has been used for not only unresectable HCC accompanied by vascular invasion, but also uncontrollable cases of repeated recurrences within a short period of time despite a number of sessions of TACE.

In recent years, fluorouracil (FU) and cisplatin (CDDP) have been reported as the most commonly used anticancer drugs used for HAIC [8-17]. Favorable results with an HAIC protocol using low-dose CDDP and FU have also been reported [8, 14, 16, 17]. Similarly, combination of interferon (IFN) with FU has demonstrated relatively good results in HAIC [11, 13, 18].

With this background in mind and with the aim of establishing the most effective HAIC protocol for advanced HCC, we planned a phase-II randomized clinical comparative study to examine whether or not IFN combined with HAIC consisting of FU and CDDP might be associated with a higher response rate. Patients with advanced HCC were randomly allocated to two treatment arms, i.e., IFN combined with hepatic arterial infusion of FU with CDDP or IFN combined with hepatic arterial infusion of FU alone without CDDP. The results were then compared with regard to the efficacy, safety, and prognosis.

Materials and Methods

Patients

Patients who had histologically or clinically diagnosed HCC were included in this study. A clinical diagnosis of HCC was made based on underlying chronic liver disease, radiologic findings and elevation of tumor markers.

As for the tumor stage, the following patients were included: patients who had (1) severe vascular invasion (i.e., vascular invasion found in the main trunk to the secondary branches of the portal vein; or invasion in the right, middle, or left hepatic vein); (2) intrahepatic multiple lesions (i.e., 5 or more nodules in the left and/or right lobes as confirmed by radiology).

Patients were eligible when they were 20 years old or older, had an eastern clinical oncology group (ECOG) performance status of 2 or less, and had appropriate bone marrow, liver, kidney and cardiac functions as determined in terms of the following measurements obtained within 1 week before enrollment (hemoglobin, 8.0 g/dL or more; white blood cell count (WBC), 2,000/mm³ or more; platelet count, 30,000/mm³ or more; blood urea nitrogen (BUN), 30 mg/dL or less; serum creatinine, 2.0 mg/dL or less, percentage of prothrombin time, 30% or more; total bilirubin, 5 mg/dL or less (excluding elevations caused by biliary tract obstruction as a result of HCC).

Assignment

The present study was an open randomized single center study composed of a two-group comparison. All the patients who satisfied the inclusion criteria were randomized to either of the two treatments. The treatment protocol was approved by the ethical committee of Kanazawa University (approval number 5169). Patients were given full information

regarding the details of the clinical study and provided their written consent prior to participation in the study. This clinical study adhered to the Declaration of Helsinki and good clinical practice.

Treatment schedule

A reservoir for hepatic arterial infusion was implanted prior to HAIC. A catheter with a side hole was inserted from the right femoral artery using an image-guided procedure, and the tip of the catheter was placed in the gastroduodenal artery or splenic artery. When more than one hepatic artery was present, the hepatic arteries were unified to the original proper hepatic artery alone. When blood flow into the gastrointestinal tract was confirmed by catheter angiography, the route was embolized to prevent complications. The reservoir was placed beneath the skin in the lower right abdomen. Medication was started at least 3 days after implantation.

In the IFN/FU treatment group, patients underwent the continuous hepatic arterial infusion of fluorouracil (5-FU[®]; Kyowa Hakko, Tokyo, Japan) at a dose of 300 mg/m²/day for 5 days in the 1st and 2nd weeks (for 120 h) using an infuser pump (Baxter Infusor SV1[®]; Tokyo, Japan) in the same manner as in previous reports[18]. The maximum amount of FU infused over 5 days was 2500 mg. IFN α -2b (Intron A[®]; Schering-Plough, Osaka, Japan) at a dose of 3,000,000 units was intramuscularly injected 3 times a week for 4 weeks. In the IFN/FU + CDDP treatment group, cisplatin (Randa[®]; Nippon Kayaku, Tokyo, Japan) at a dose of 20 mg/m² was given by hepatic arterial infusion over 1.5 h on day 1 and day 8 prior to the administration of FU and after appropriate hydration and antiemetic medication. A treatment cycle comprised 4 weeks of drug administration including interferon administration and a subsequent 2-week rest period (Figure 1).

Sample size

Based on previous reports in the literature[9, 19] and the results of our studies concerning HAIC for the treatment of HCC using single-drug regimens, the response rate in the IFN/FU treatment group was assumed to be 20% and that in the IFN/FU + CDDP treatment group was assumed to be 50%. Based on the assumption that the ratio of the numbers of patients was 1:1, the α error was 0.05, the β error was 0.1, and 52 patients were necessary for each treatment group. Therefore, the number of patients to be included was 114, allowing a 10% dropout rate that would result in a total of 104 patients for the two groups.

Response Assessment

The primary endpoint was the response rate (RR), as determined using dynamic computed tomography (CT) or magnetic resonance imaging (MRI) performed at the end of each treatment cycle according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0[20].

Secondary endpoints were the overall survival time, progression-free survival time, and adverse events. The overall survival time was defined as the period from the time of randomization until death, and the progression-free survival time was defined as the period from the beginning of treatment until confirmation of progression or death. Adverse events were evaluated according to the Common Toxicity Criteria for Adverse reactions (CTC-AE) version 3.0.

Statistical analyses

The two treatment groups were compared using the Fisher direct method and the Wilcoxon

rank sum test. Response factors were analyzed using logistic regression analysis. The cumulative survival and prognostic factors were analyzed using the Kaplan-Meier method, log-rank test and the Cox proposal hazard regression model.

Results

Patients

A total of 155 patients with advanced HCC were treated at our hospital between October 2003 and September 2007. Eventually, 114 patients were allocated to the IFN/FU + CDDP treatment group or the IFN/FU treatment group. Three patients in the IFN/FU + CDDP group and two in the IFN/FU group dropped out before the end of the first cycle; therefore, a total of 109 patients, comprising 54 patients from the former group and 55 from the latter, were included in the efficacy evaluation (Figure 2).

The baseline clinical features of 114 patients are shown in Table 1. No significant differences in the clinical features and test results were observed between the two groups, with the exception of a slightly higher bilirubin level in the IFN/FU group. The patients classified into Barcelona Clinic Liver Cancer (BCLC) stage B had five or more nodules in the left and/or right lobes and were considered to be difficult to control by TACE after repeated TACE (68%) or multiple lesions inadequate to TACE.

Response to Treatment

Among the 57 patients in the IFN/FU + CDDP treatment group, the best study response was complete response (CR) in 1 (1.7%); partial response (PR) was observed in 25 (43.9%) patients, stable disease (SD) was observed in 15 (26.3%), and progressive disease (PD) was observed in 13 (22.8%). Among the 57 patients in the IFN/FU treatment group, the response was CR in 3 (5.3%), PR in 11 (19.3%), SD in 19 (33.3%), and PD in 22 (38.6%). The response rate (RR; CR + PR) was 45.6% in the IFN/FU + CDDP group and 24.6% in the IFN/FU group; the figure was significantly higher in the former group ($p = 0.030$) (Table 2).

Factor improved the response to treatment as indicated by a multivariate analysis was

only the addition of CDDP to the treatment (odds ratio [OR] 2.5 [95%CI: 1.1-6.0] (Table 3).

Safety

Table 4 shows the major adverse events. Grade 3 or 4 adverse events were found in 75 (65.8%) of the 114 patients. Bone marrow suppression of any grade was found in 65-90% of the patients. Leucopenia and neutropenia were noted in about 70% of the patients, and no significant difference was found between the IFN/FU + CDDP group and the IFN/FU group. An overall reduction in hemoglobin was observed more frequently in the IFN/FU + CDDP group than in the IFN/FU group (91.2% vs. 75.4%, $p = 0.021$), although the difference was not significant for hemoglobin reductions of grade 3 or 4. No significant difference in the all grade thrombocytopenia was observed between the two groups, but thrombocytopenia of grade 3 or 4 were significantly more frequently in the IFN/FU + CDDP group (45.6% vs. 22.8%, $p = 0.017$). However, no serious complications secondary to a reduction in platelets occurred.

Non-hematologic toxicities including general malaise, nausea, vomiting, stomatitis, and an elevation in serum creatinine were significantly more common in the IFN/FU + CDDP group, but no intergroup difference was found for grade 3 or grade 4 toxicities.

Peptic ulcer arising from the leakage of arterially infused anticancer drugs into the gastrointestinal tract, a complication characteristic of HAIC, was found in 6 (10.5%) patients in the IFN/FU + CDDP group and 1 (1.8%) patient in the IFN/FU group; the incidence was higher, but not significantly, in the IFN/FU + CDDP group ($p = 0.06$), and no grade 3 or grade 4 cases occurred.

Survival

The median overall survival period of the 114 patients who underwent HAIC was 12.0 months (95% confidence interval [CI]: 11.6 – 12.4 months). In the IFN/FU + CDDP group, the median survival time (MST) was 17.6 months (95% CI: 9.9 – 25.3 months). On the other hand, in the IFN/FU group, the median survival time was 10.5 months (95%CI: 5.6 – 15.4 months). Although the survival period tended to be higher in the group given combined CDDP, no statistically significant differences were observed between the two groups ($p = 0.522$, log-rank test, hazard ratio [HR] 0.88 [95%CI: 0.60-1.30]) (Figure 3A).

In the subgroup with presence of major vascular invasion, the MST was 5.8 months (95% CI: 3.3 – 8.3 months) in the IFN/FU + CDDP group, 4.7 months (95% CI: -7.6 – 31.6 months) in the IFN/FU group. On the other hand, in the subgroup with absence of major vascular invasion, the MST was 20.0 months (95% CI: 13.6 – 26.6 months) in the IFN/FU + CDDP group, 12.0 months (95% CI: 4.4 – 19.6 months) in the IFN/FU group. Subanalysis according to presence or absence of major vascular invasion showed no significant difference among the two treatment groups ($p = 0.571$, in presence of major vascular invasion, $p = 0.399$ in absence). In the subgroup with tumor stage, the MST was 22.6 months (95% CI: 0.4 – 44.7 months) in the IFN/FU + CDDP group, 12.0 months (95% CI: 5.5 – 18.5 months) in the IFN/FU group. On the other hand, in the subgroup with stage IVA and stage IVB, the MST was 7.5 months (95% CI: 5.7 – 9.3 months) in the IFN/FU + CDDP group, 7.5 months (95% CI: 0.4 – 14.5 months) in the IFN/FU group. Subanalysis according to tumor stage (stage II and III or stage IVA and IVB) also showed no difference among two treatment groups ($p = 0.625$ in stage II and III, $p = 0.906$ in stage IVA and IVB).

Similarly, the median overall progression-free survival period of the 114 patients was 4.5 months (95%CI: 3.5 – 5.5 months). In the IFN/FU + CDDP group, the median progression-free survival time was 6.5 months (95%CI: 2.6 – 10.4 months). On the other hand,

in the IFN/FU group, the median progression-free survival time was 3.3 months (95%CI: -0.6 – 7.2 months). The progression-free survival period was significantly longer in the IFN/FU + CDDP group than in the IFN/FU group ($p = 0.0048$, long-rank test, HR 0.57 [95%CI: 0.38-0.85]) (Figure 3B).

As predictors for survival, a multivariate analysis showed that positivity for hepatitis C virus antibody (HCV-Ab), an albumin level of 3.5 g/dL or more, and an aspartate amino transferase (AST) value of lower than 80 IU/L were improved survival (Table 5).

Discussion

The present study showed that the addition of CDDP to IFN combined with HAIC using FU significantly enhanced the antitumor effect from 24.6% to 45.6%. The response rates, obtained in previous studies of HAIC involving at least 30 patients, varied from 14 to 71% [8-17]. Regarding the use of IFN combined with HAIC using FU, Obi et al. used this treatment in patients with advanced HCC and a tumor embolus in the main trunk or the first branch of the portal vein and achieved a response rate of 52.6% [13]. Ota et al. also used IFN combined with HAIC using FU for similar cases of advanced HCC and reported a response rate of 43.6% [18]. We have previously reported a response rate of 45% in 34 patients who underwent multidrug HAIC using FU and CDDP in combination with IFN treatment [11]. Uka et al. used IFN in combination with HAIC using FU in 55 patients who had a tumor embolus of the portal vein and reported a response rate of 29% [21]. The response rates obtained in the present study were similar to that obtained in the report by Uka et al. and lower than those obtained in the two other reports. This discrepancy may be explained by the different criteria used to evaluate antitumor efficacy, as Uka et al. suggested in their discussion. Obi et al. and Ota et al. used the ECOG criteria, whereas Uka et al. and the present study used the RECIST criteria.

The combined use of FU and IFN is reportedly beneficial because IFN serves as a modulator to enhance the antitumor effect of FU. More specifically, IFN induces p53, which enhances apoptosis by FU, and influences the cell cycle via p27^{Kip1} or apoptosis via Bcl-xL [22, 23]. From a clinical aspect, Takaki-Hamade et al and Eun et al concluded that combined IFN treatment did not have an incremental effect [24, 25]. Thus, the benefit of adding IFN to HAIC with FU has not been proven clinically. However, experimental data suggest that IFN should enhance the antitumor effect of FU [22] [26], and this supports the current use of

IFN-combined HAIC in clinical practice.

On the other hand, regarding the effect of CDDP combined with FU in a clinical setting, Ando et al. used HAIC with FU combined with low-dose CDDP for the treatment of patients with advanced HCC and a portal tumor embolus and reported a response rate of 48%[8]. After their report, several other reports on HAIC with FU combined with low-dose CDDP were made, with reported response rates ranging from 38.5 – 71%[14, 16, 17, 27]. Experimental studies have shown that low-dose CDDP blocks methionine transport into the cell causing a decrease in intracellular methionine and an increase in reduced folic acid, thus serving as a modulator of FU to enhance its antitumor efficacy [28]. It has also been reported that low-dose CDDP is involved in the inhibition of p53-mediated apoptosis and drug resistance [29]. The present study used two agents, IFN and CDDP, in combination with FU. Although IFN and CDDP seem to enhance the antitumor effect of FU through these pathways, a large amount of basic experimental research on FU combined with these two agents remains to be performed.

Our present study showed that the antitumor effect was significantly higher and the progression-free survival time was significantly longer in the IFN/FU + CDDP group. However, there was no statistically significant difference in the overall survival time. Subgroup analysis also did not show survival benefit in IFN + CDDP group. Since there were no limitations as to treatment after the end of the protocol treatment, 88 (77.2%) of the 114 patients underwent some treatment subsequently, and 34 (59.6%) patients in the IFN/FU group received HAIC (mainly IFN/FU + CDDP) eventually. This might have had some effects on the results concerning overall survival.

The factors that improved survival in this study included positivity for HCV-Ab, an albumin level of 3.5 g/dL or more, and an AST value of lower than 80 IU/L. Previous reports

have documented the presence of response to chemotherapy, the Cancer of the Liver Italian Program (CLIP) score, the Okuda stage, the Child-Pugh score, and alpha-fetoprotein (AFP) as prognostic factors of HAIC for advanced HCC [30, 31]. Obi et al. also reported that positivity for HCV-Ab was a predictor of the complete response to IFN combined with HAIC using FU[13]. Uka et al. reported that positivity for HCV-Ab was a factor involved in the early antitumor effect, progression-free time, and overall survival after IFN combined with HAIC [21]. Thus, positivity for HCV-Ab was determined as a prognosis improved factor. A possible explanation for this discrepancy may be that viral differences between HBV and HCV may be involved in the heterogeneity or anticancer drug sensitivity of HCC, or differences in the cytokine patterns of HBV and HCV infections may influence the effect of IFN [32-35]. However, the true explanation remains unclear. In connection with an AST value of lower than 80 IU/L, Cheong et al. also reported that low levels of AST and alkaline phosphatase (ALP) were associated with long-term survival exceeding 8 months in a study examining chemotherapy including HAIC for the treatment of patients with advanced HCC[36]. The basis of their argument requires further investigation.

Most patients with HCC have concomitant hepatic cirrhosis and thus have pancytopenia. Therefore, regarding the adverse events, we expected to see enhanced blood toxicity when IFN and CDDP were added to FU. As a result, this study showed a significantly higher frequency of cytopenia in the IFN/FU + CDDP group. However, as far as severe hematologic toxicities of grade 3 or 4 were concerned, thrombocytopenia alone was significantly more frequent in the IFN/FU + CDDP group, but no complications secondary to thrombocytopenia occurred. Although some of non-hematologic toxicities were significantly more frequent in the IFN/FU + CDDP group, these adverse events were controllable. Thus, IFN combined with HAIC using FU and CDDP seems to be tolerable with regard to the

occurrence of adverse events. The frequency of grade 3 or 4 toxicity of IFN-combined HAIC in our study was higher than sorafenib therapy reported previously [6, 7]. We enrolled 45 patients (39.5%) in Child-Pugh class B and pretreatment blood cell count in patients of Child-Pugh class B was generally lower than that in Child-Pugh class A. In addition, IFN has effect to decrease the blood cell count especially neutrophil and platelet. However these toxicity were controllable and there was no toxicity-related death.

In conclusion, the results of this phase II randomized clinical study on the effect of adding CDDP to IFN in combination with HAIC using FU for the treatment of advanced HCC show that the combined use of CDDP significantly increases the antitumor effect of the treatment and induces a significant improvement in the progression-free survival time. Although there was no significant difference in the overall survival time of the two treatment groups, the survival benefit of IFN combined with HAIC using CDDP should be examined in comparison with systemic therapy using sorafenib, the current standard treatment for advanced HCC. In this connection, a multicenter study of hepatic arterial infusion of FU versus sorafenib therapy is now underway in Japan, and the results are awaited.

References

- 1 Parkin DM, Bray F, Ferlay J, Pisani P: Global cancer statistics, 2002. *CA Cancer J Clin* 2005;55:74-108.
- 2 Bosch FX, Ribes J, Diaz M, Cleries R: Primary liver cancer: Worldwide incidence and trends. *Gastroenterology* 2004;127:S5-S16.
- 3 Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB: Hepatitis c infection and the increasing incidence of hepatocellular carcinoma: A population-based study. *Gastroenterology* 2004;127:1372-1380.
- 4 Arai S, Sata M, Sakamoto M, Shimada M, Kumada T, Shiina S, Yamashita T, Kokudo N, Tanaka M, Takayama T, Kudo M: Management of hepatocellular carcinoma: Report of consensus meeting in the 45th annual meeting of the japan society of hepatology (2009). *Hepatol Res* 2010;40:667-685.
- 5 Ikai I, Arai S, Okazaki M, Okita K, Omata M, Kojiro M, Takayasu K, Nakanuma Y, Makuuchi M, Matsuyama Y, Monden M, Kudo M: Report of the 17th nationwide follow-up survey of primary liver cancer in japan. *Hepatol Res* 2007;37:676-691.
- 6 Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, Xu J, Sun Y, Liang H, Liu J, Wang J, Tak WY, Pan H, Burock K, Zou J, Voliotis D, Guan Z: Efficacy and safety of sorafenib in patients in the asia-pacific region with advanced hepatocellular carcinoma: A phase iii randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009;10:25-34.
- 7 Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Haussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J: Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med*

2008;359:378-390.

- 8 Ando E, Tanaka M, Yamashita F, Kuromatsu R, Yutani S, Fukumori K, Sumie S, Yano Y, Okuda K, Sata M: Hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma with portal vein tumor thrombosis: Analysis of 48 cases. *Cancer* 2002;95:588-595.
- 9 Chung YH, Song IH, Song BC, Lee GC, Koh MS, Yoon HK, Lee YS, Sung KB, Suh DJ: Combined therapy consisting of intraarterial cisplatin infusion and systemic interferon-alpha for hepatocellular carcinoma patients with major portal vein thrombosis or distant metastasis. *Cancer* 2000;88:1986-1991.
- 10 Court WS, Order SE, Siegel JA, Johnson E, DeNittis AS, Principato R, Martz K, Zeiger LS: Remission and survival following monthly intraarterial cisplatin in nonresectable hepatoma. *Cancer Invest* 2002;20:613-625.
- 11 Kaneko S, Urabe T, Kobayashi K: Combination chemotherapy for advanced hepatocellular carcinoma complicated by major portal vein thrombosis. *Oncology* 2002;62 Suppl 1:69-73.
- 12 Lin CP, Yu HC, Cheng JS, Lai KH, Lo GH, Hsu PI, Lin CK, Chen HH, Lo CC, Liang HL, Tseng HH: Clinical effects of intra-arterial infusion chemotherapy with cisplatin, mitomycin c, leucovorin and 5-flourouracil for unresectable advanced hepatocellular carcinoma. *J Chin Med Assoc* 2004;67:602-610.
- 13 Obi S, Yoshida H, Toune R, Unuma T, Kanda M, Sato S, Tateishi R, Teratani T, Shiina S, Omata M: Combination therapy of intraarterial 5-fluorouracil and systemic interferon-alpha for advanced hepatocellular carcinoma with portal venous invasion. *Cancer* 2006;106:1990-1997.
- 14 Okuda K, Tanaka M, Shibata J, Ando E, Ogata T, Kinoshita H, Eriguchi N, Aoyagi S,

- Tanikawa K: Hepatic arterial infusion chemotherapy with continuous low dose administration of cisplatin and 5-fluorouracil for multiple recurrence of hepatocellular carcinoma after surgical treatment. *Oncol Rep* 1999;6:587-591.
- 15 Park JY, Ahn SH, Yoon YJ, Kim JK, Lee HW, Lee do Y, Chon CY, Moon YM, Han KH: Repetitive short-course hepatic arterial infusion chemotherapy with high-dose 5-fluorouracil and cisplatin in patients with advanced hepatocellular carcinoma. *Cancer* 2007;110:129-137.
 - 16 Sumie S, Yamashita F, Ando E, Tanaka M, Yano Y, Fukumori K, Sata M: Interventional radiology for advanced hepatocellular carcinoma: Comparison of hepatic artery infusion chemotherapy and transcatheter arterial lipiodol chemoembolization. *AJR Am J Roentgenol* 2003;181:1327-1334.
 - 17 Tanioka H, Tsuji A, Morita S, Horimi T, Takamatsu M, Shirasaka T, Mizushima T, Ochi K, Kiura K, Tanimoto M: Combination chemotherapy with continuous 5-fluorouracil and low-dose cisplatin infusion for advanced hepatocellular carcinoma. *Anticancer Res* 2003;23:1891-1897.
 - 18 Ota H, Nagano H, Sakon M, Eguchi H, Kondo M, Yamamoto T, Nakamura M, Damdinsuren B, Wada H, Marubashi S, Miyamoto A, Dono K, Umeshita K, Nakamori S, Wakasa K, Monden M: Treatment of hepatocellular carcinoma with major portal vein thrombosis by combined therapy with subcutaneous interferon-alpha and intra-arterial 5-fluorouracil; role of type 1 interferon receptor expression. *Br J Cancer* 2005;93:557-564.
 - 19 Tzoracoleftherakis EE, Spiliotis JD, Kyriakopoulou T, Kakkos SK: Intra-arterial versus systemic chemotherapy for non-operable hepatocellular carcinoma. *Hepatogastroenterology* 1999;46:1122-1125.

- 20 Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG: New guidelines to evaluate the response to treatment in solid tumors. European organization for research and treatment of cancer, national cancer institute of the united states, national cancer institute of canada. *J Natl Cancer Inst* 2000;92:205-216.
- 21 Uka K, Aikata H, Takaki S, Kawaoka T, Saneto H, Miki D, Takahashi S, Toyota N, Ito K, Chayama K: Systemic gemcitabine combined with intra-arterial low-dose cisplatin and 5-fluorouracil for advanced hepatocellular carcinoma: Seven cases. *World J Gastroenterol* 2008;14:2602-2608.
- 22 Takaoka A, Hayakawa S, Yanai H, Stoiber D, Negishi H, Kikuchi H, Sasaki S, Imai K, Shibue T, Honda K, Taniguchi T: Integration of interferon-alpha/beta signalling to p53 responses in tumour suppression and antiviral defence. *Nature* 2003;424:516-523.
- 23 Eguchi H, Nagano H, Yamamoto H, Miyamoto A, Kondo M, Dono K, Nakamori S, Umeshita K, Sakon M, Monden M: Augmentation of antitumor activity of 5-fluorouracil by interferon alpha is associated with up-regulation of p27kip1 in human hepatocellular carcinoma cells. *Clin Cancer Res* 2000;6:2881-2890.
- 24 Takaki-Hamabe S, Yamasaki T, Saeki I, Harima Y, Okita K, Terai S, Sakaida I: Hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma: Is the addition of subcutaneous interferon-alpha-2b beneficial? *Hepatol Res* 2009;39:223-230.
- 25 Eun JR, Lee HJ, Moon HJ, Kim TN, Kim JW, Chang JC: Hepatic arterial infusion chemotherapy using high-dose 5-fluorouracil and cisplatin with or without interferon-alpha for the treatment of advanced hepatocellular carcinoma with portal

- vein tumor thrombosis. *Scand J Gastroenterol* 2009;44:1477-1486.
- 26 Moriyama M, Hoshida Y, Kato N, Otsuka M, Yoshida H, Kawabe T, Omata M: Genes associated with human hepatocellular carcinoma cell chemosensitivity to 5-fluorouracil plus interferon-alpha combination chemotherapy. *Int J Oncol* 2004;25:1279-1287.
- 27 Ueshima K, Kudo M, Takita M, Nagai T, Tatsumi C, Ueda T, Kitai S, Ishikawa E, Yada N, Inoue T, Hagiwara S, Minami Y, Chung H: Hepatic arterial infusion chemotherapy using low-dose 5-fluorouracil and cisplatin for advanced hepatocellular carcinoma. *Oncology* 2010;78 Suppl 1:148-153.
- 28 Nishiyama M, Yamamoto W, Park JS, Okamoto R, Hanaoka H, Takano H, Saito N, Matsukawa M, Shirasaka T, Kurihara M: Low-dose cisplatin and 5-fluorouracil in combination can repress increased gene expression of cellular resistance determinants to themselves. *Clin Cancer Res* 1999;5:2620-2628.
- 29 Matsushashi N, Saio M, Matsuo A, Sugiyama Y, Saji S: Expression of p53 protein as a predictor of the response to 5-fluorouracil and cisplatin chemotherapy in human gastrointestinal cancer cell lines evaluated with apoptosis by use of thin layer collagen gel. *Int J Oncol* 2004;24:807-813.
- 30 Hamada A, Yamakado K, Nakatsuka A, Takaki H, Akeboshi M, Takeda K: Hepatic arterial infusion chemotherapy with use of an implanted port system in patients with advanced hepatocellular carcinoma: Prognostic factors. *J Vasc Interv Radiol* 2004;15:835-841.
- 31 Yamasaki T, Kimura T, Kurokawa F, Aoyama K, Ishikawa T, Tajima K, Yokoyama Y, Takami T, Omori K, Kawaguchi K, Tsuchiya M, Terai S, Sakaida I, Okita K: Prognostic factors in patients with advanced hepatocellular carcinoma receiving

- hepatic arterial infusion chemotherapy. *J Gastroenterol* 2005;40:70-78.
- 32 Buendia MA: Hepatitis b viruses and cancerogenesis. *Biomed Pharmacother* 1998;52:34-43.
- 33 Falasca K, Ucciferri C, Dalessandro M, Zingariello P, Mancino P, Petrarca C, Pizzigallo E, Conti P, Vecchiet J: Cytokine patterns correlate with liver damage in patients with chronic hepatitis b and c. *Ann Clin Lab Sci* 2006;36:144-150.
- 34 Robinson WS: Molecular events in the pathogenesis of hepadnavirus-associated hepatocellular carcinoma. *Annu Rev Med* 1994;45:297-323.
- 35 Someya T, Ikeda K, Saitoh S, Kobayashi M, Hosaka T, Sezaki H, Akuta N, Suzuki F, Suzuki Y, Arase Y, Kumada H: Interferon lowers tumor recurrence rate after surgical resection or ablation of hepatocellular carcinoma: A pilot study of patients with hepatitis b virus-related cirrhosis. *J Gastroenterol* 2006;41:1206-1213.
- 36 Cheong JY, Lee KM, Cho SW, Won JH, Kim JK, Wang HJ, Hahm KB, Kim JH: Survival benefits of intra-arterial infusion chemotherapy in patients with advanced hepatocellular carcinoma with portal vein tumor thrombosis. *Hepatol Res* 2005;32:127-133.

Tables

Table 1. Patient demographics and baseline characteristics

	IFN/FU+CDDP (n=57)	IFN/FU (n=57)	<i>p</i> -value
Gender (male/female)	49/8	46/11	0.62 [*]
Age (median, range, years)	65 (40-82)	68 (40-82)	0.27 [†]
PS (ECOG) (0/1/2)	36/19/2	34/21/2	0.92 [†]
Primary or recurrence	20/37	23/34	0.70 [*]
Prior TACE (+/-)	32/25	33/24	1.00 [*]
Prior Chemotherapy (+/-)	4/53	3/54	1.00 [*]
HCV-Ab (positive/negative)	32/25	35/22	0.70 [*]
HBsAg (positive/negative)	16/41	18/39	0.83 [*]
Liver cirrhosis (+/-)	46/11	47/10	1.00 [*]
Child-Pugh class (A/B/C)	33/23/1	32/22/3	0.74 [†]
LCSGJ TNM Stage (II/III/IVA/IVB)	7/26/17/7	7/20/25/5	0.53 [†]
UICC TNM Stage (II/III/IV)	6/43/8	12/38/7	0.30 [†]
BCLC Stage (B/C/D)	33/23/1	23/31/3	0.13 [†]
Diameter of tumor (median, range, mm)	37 (10-250)	40 (11-200)	0.71 [†]
Major portal vein invasion (+/-)	12/45	19/38	0.21 [*]
Lymph node metastasis (+/-)	2/55	4/53	0.68 [*]
Distant metastasis (+/-)	7/50	5/52	0.76 [*]
Treatment cycles [‡]	3.2 ± 2.6	2.9 ± 2.4	0.37 [†]
Albumin (g/dL) [‡]	3.36 ± 0.6	3.49 ± 0.5	0.22 [†]
Total bilirubin (mg/dL) [‡]	1.10 ± 0.7	1.44 ± 0.88	0.07 [†]
Active prothrombin (%) [‡]	78.6 ± 18.9	74.9 ± 13.8	0.22 [†]
Platelet count (x104/μL) [‡]	12.3 ± 6.4	11.0 ± 5.4	0.26 [†]
AST (IU/L) [‡]	83.1 ± 74.4	82.5 ± 51.8	0.47 [†]
ALT (IU/L) [‡]	64.2 ± 53.6	68.5 ± 88.6	1.00 [†]
DCP (<100/100 ≤ mAU/mL)	33/24	37/20	0.56 [*]
AFP (<400/400 ≤ ng/mL)	24/33	28/29	0.57 [*]
AFP-L3 (<30/30 ≤ %)	22/35	27/30	0.45 [*]

PS; performance status; ECOG: Eastern Cooperative Oncology Group;
TACE: transarterial chemoembolization; HCV-Ab: hepatitis C virus antibody
HBsAg: hepatitis B surface antigen; LCSGJ: liver cancer study group of Japan;
UICC: Unio Internationalis Contra Cancrum; BCLC: Barcelona Clinic Liver Cancer
AST: aspartate aminotransferase; ALT: alanine aminotransferase;
DCP: des-gamma-carboxy prothrombin

AFP: alpha-fetoprotein

^{*} Fisher's exact test

[†] Wilcoxon rank sum test

[‡] Mean ± standard deviation

Table 2. Comparison of best study response between treatment arms

Best study response	IFN/FU+CDDP (n=57)	IFN/FU (n=57)	<i>p</i>-value[*]
CR, n (%)	1 (1.7)	3 (5.3)	
PR, n (%)	25 (43.9)	11 (19.3)	
SD, n (%)	15 (26.3)	19 (33.3)	
PD, n (%)	13 (22.8)	22 (38.6)	
NE, n (%)	3 (5.3)	2 (3.5)	
RR (CR+PR), n (%)	26 (45.6)	14 (24.6)	0.030
TCR (CR+PR+SD), n (%)	41 (71.9)	33 (57.9)	0.169

Abbreviations: CR, complete response; PR, partial response; SD, stable response; PD, progressive disease; NE, not evaluable; RR, response rate; TCR, tumor control rate.

^{*} The between-group *p*-value was determined with the Fisher's exact test.

Table 3. Factorial analysis of predictors for response

	response rate (%)	univariate analysis <i>p</i> -value*	multivariate analysis <i>p</i> -value [†] odds ratio (95%CI)	
IFN/FU+CDDP / IFN/FU	45.6 / 25.6	0.0302	0.0268	2.5 (1.1-6.0)
Gender (male / female)	35.8 / 31.6	0.7979		
Age (<65/65≤years)	31.1 / 39.6	0.4318		
Primary / recurrence	32.6 / 36.6	0.6909		
Prior TACE (+/-)	35.4 / 36.7	1.00		
Prior Chemotherapy (+/-)	57.1 / 33.6	0.2382		
HCV-Ab (positive/negative)	40.3 / 27.7	0.2315		
HBsAg (positive/negative)	38.2 / 33.7	0.6722		
Liver cirrhosis (+/-)	37.6 / 23.8	0.3134		
Child-Pugh class (A / B, C)	41.5 / 26.5	0.115		
LCSGJ TNM Stage (II, III / IVA, IVB)	46.7 / 22.2	0.0102	0.2877	1.3 (0.4-4.0)
Diameter of tumor (<50/50≤ mm)	44.0 / 17.9	0.008	0.1817	2.2 (0.7-7.0)
Major portal vein invasion (+/-)	16.1 / 42.2	0.0143	0.1266	1.8 (0.5-6.8)
Lymph node metastasis (+/-)	33.3 / 35.2	1.00		
Distant metastasis (+/-)	16.7 / 37.3	0.2098		
Albumin (<3.5/3.5≤ g/dL)	27.6 / 42.9	0.1165		
Total bilirubin (<1.5/1.5≤ mg/dL)	39.2 / 25.7	0.2038		
Active prothrombin (<70/70≤ %)	26.8 / 39.7	0.2203		
Platelet (<10x104/10x104≤ μL)	33.3 / 36.7	0.8444		
AST (<80/80≤ IU/L)	40.8 / 25.6	0.1096		
ALT (<80/80≤ IU/L)	33.7 / 40.0	0.6372		
DCP (<100/100≤ mAU/mL)	42.5 / 57.5	0.5511		
AFP (<400/400≤ ng/mL)	38.7 / 30.8	0.4334		
AFP-L3 (<30/30≤ %)	46.6 / 22.4	0.0094	0.2898	1.7 (0.7-4.4)

TACE: transarterial chemoembolization; HCV-Ab: hepatitis C virus antibody

HBsAg: hepatitis B surface antigen; LCSGJ: liver cancer study group of Japan

Major portal vein invasion: tumor invasion in main trunk or 1st branches of portal vein

AST: aspartate aminotransferase; ALT: alanine aminotransferase;

DCP: des-gamma-carboxy prothrombin; AFP: alpha-fetoprotein; CI: confident interval

* Fisher's exact test

[†] Logistic Procedure Model

Table 4. Most common adverse events

Adverse events	IFN/FU+CDDP (n=57) n (%)		IFN/FU (n=57) n (%)	
	Any grade	CTC Grade 3-4	Any grade	CTC Grade 3-4
Neutrophils	44 (77.2)	17 (29.8)	37 (64.9)	19 (33.3)
Leukocytes	43 (75.4)	12 (21.1)	38 (66.7)	18 (31.6)
Hemoglobin	52 (91.2)*	4 (7.0)	43 (75.4)*	2 (3.5)
Platelets	50 (89.5)	26 (45.6) [†]	48 (84.2)	13 (22.8) [†]
Prothrombin time	30 (52.6)	3 (5.3)	32 (56.1)	1 (1.8)
Asthenia	34 (59.6)*	1 (1.8)	21 (36.8)*	3 (5.3)
Fever	41 (71.9)	1 (1.8)	37 (64.9)	0 (0.0)
Nausea	32 (56.1)*	10 (17.5)	22 (38.6)*	3 (5.3)
Vomiting	15 (26.3)*	4 (7.0)	4 (7.0)*	1 (1.8)
Mucositis	22 (38.6)*	3 (5.3)	9 (15.8)*	1 (1.8)
Liver function	42 (73.7)	4 (7.0)	43 (75.4)	10 (17.5)
Creatinine	10 (17.5)*	0 (0.0)	2 (3.5)*	0 (0.0)
Peptic ulcer	6 (10.5)	0 (0.0)	1 (1.8)	0 (0.0)

CTC: common toxicity criteria

* p<0.05, Wilcoxon rank sum test

[†] p<0.05, Fisher exact test

Table 5. Factorial analysis of predictors for survival

	median survival time (months)	univariate analysis <i>p</i> -value*	multivariate analysis <i>p</i> -value [†]	hazard ratio (95%CI)
IFN/FU+CDDP / IFN/FU	17.6 / 10.5	0.522		
Gender (male / female)	12.0 / 12.0	0.236		
Age (<65/65≤years)	9.9 / 19.5	0.115		
Primary / recurrence	7.7 / 16.5	0.394		
Prior TACE (+/-)	14.4 / 12.0	0.491		
Prior Chemotherapy (+/-)	18.6 / 12.0	0.936		
HCV-Ab (positive/negative)	19.5 / 7.6	0.0049	0.0219	0.60 (0.39-0.93)
HBsAg (positive/negative)	7.6 / 15.4	0.1145		
Liver cirrhosis (+/-)	13.7 / 9.0	0.5063		
Child-Pugh class (A / B,C)	18.6 / 9.2	0.0636		
LCSGJ TNM Stage (II, III / IVA, IVB)	19.4 / 7.5	0.0019	0.6326	0.87 (0.49-1.54)
Diameter of tumor (<50/50≤ mm)	19.4 / 5.8	0.0014	0.1068	0.64 (0.37-1.10)
Major portal vein invasion (+/-)	5.1 / 18.6	0.0005	0.3203	0.73 (0.40-1.35)
Lymph node metastasis (+/-)	4.5 / 12.0	0.0789		
Distant metastasis (+/-)	4.5 / 14.0	0.0037	0.1806	0.60 (0.29-1.27)
Albumin (<3.5/3.5≤ g/dL)	9.3 / 16.5	0.0200	0.0017	0.50 (0.32-0.77)
Total bilirubin (<1.5/1.5≤ mg/dL)	15.4 / 9.5	0.2774		
Active prothrombin (<70/70≤ %)	9.3 / 14.5	0.9470		
Platelet (<10x104/10x104≤μL)	16.5 / 10.5	0.6273		
AST (<80/80≤ IU/L)	19.4 / 7.4	0.0056	0.0356	0.62 (0.39-0.97)
ALT (<80/80≤ IU/L)	13.7 / 9.5	0.8973		
DCP (<100/100≤ mAU/mL)	20.0 / 9.4	0.2294		
AFP (<400/400≤ ng/mL)	21.5 / 6.6	0.0002	0.1588	0.69 (0.41-1.16)
AFP-L3 (<30/30≤ %)	20.8 / 7.5	0.0002	0.0730	0.61 (0.35-1.05)

TACE: transarterial chemoembolization; HCV-Ab: hepatitis C virus antibody;

HBsAg: hepatitis B surface antigen

LCSGJ: liver cancer study group of Japan

Major portal vein invasion: tumor invasion in main trunk or 1st branches of portal vein

AST: aspartate aminotransferase; ALT: alanine aminotransferase;

DCP: des-gamma-carboxy prothrombin

AFP: alpha-fetoprotein

* Log-rank test

[†]Cox Proportional Hazard Model

Figure legends

Figure 1. Treatment protocol

Figure 2. CONSORT flow diagram

Figure 3. Kaplan-Meier analysis of overall survival (A) and progression-free survival (B) according to chemotherapeutic regimens

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