40. Torimura T, Ueno T, Kin M, *et al*: Overexpression of angiopoietin-1 and angiopoietin-2 in hepatocellular carcinoma. J Hepatol 40: 799-807, 2004.
41. Mitsuhashi N, Shimizu H, Ohtsuka M, *et al*: Angiopoietins and Tie-2 expression in angiogenesis and proliferation of human hepatocellular carcinoma. Hepatology 37: 1105-1113, 2003.
42. Maisonpierre PC, Suri C, Jones PF, *et al*: Angiopoietin-2, a natural antagonist for Tie2 that disrupts *in vivo* angiogenesis. Science 277: 55-60, 1997.
43. Davis S, Aldrich TH, Jones PF, *et al*: Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. Cell 87: 1161-1169, 1996.
44. Suri C, Jones PF, Patan S, *et al*: Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. Cell 87: 1171-1180, 1996.
45. Papapetropoulos A, Garcia-Cardena G, Dengler TJ, Maisonpierre PC, Yancopoulos GD and Sessa WC: Direct actions of angiopoietin-1 on human endothelium: evidence for network stabilization, cell survival and interaction with other angiogenic growth factors. Lab Invest 79: 213-223, 1999.
46. Asahara T, Chen D, Takahashi T, *et al*: Tie2 receptor ligands, angiopoietin-1 and angiopoietin-2, modulate VEGF-induced postnatal neovascularization. Circ Res 83: 233-240, 1998.
47. Harada H, Kitagawa M, Tanaka N, *et al*: Anti-oncogenic and oncogenic potentials of interferon regulatory factors-1 and -2. Science 259: 971-974, 1993.
48. Zhang L, Yang N, Park JW, *et al*: Tumor-derived vascular endothelial growth factor up-regulates angiopoietin-2 in host endothelium and destabilizes host vasculature, supporting angiogenesis in ovarian cancer. Cancer Res 63: 3403-3412, 2003.
49. Potente M, Urbich C, Sasaki K, *et al*: Involvement of Foxo transcription factors in angiogenesis and postnatal neovascularization. J Clin Invest 115: 2382-2392, 2005.
50. Tran H, Brunet A, Grenier JM, *et al*: DNA repair pathway stimulated by the forkhead transcription factor FOXO3a through the Gadd45 protein. Science 296: 530-534, 2002.
51. Kops GJ, Dansen TB, Polderman PE, *et al*: Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. Nature 419: 316-321, 2002.

Hepatology Research 2007; 37 (Suppl. 2): S238–S250 doi: 10.1111/j.1872-034X.2007.00191.x

Combined intra-arterial 5-fluorouracil and subcutaneous interferon-alpha therapy for highly advanced hepatocellular carcinoma

Bazarragchaa Damdinsuren, Hiroaki Nagano and Morito Monden

Department of Surgery, Graduate School of Medicine, Osaka University, Osaka, Japan

Because of the difficulties of low sensitivity for anticancer agents and giving sufficient dose because of poor liver function, chemotherapy may not play a central role for treatment of hepatocellular carcinoma (HCC) patients, especially those with liver cirrhosis. However, chemotherapy must be one of the important possibilities of multimodal treatment for advanced HCC, for which hepatic resection, percutaneous ablation, transcatheter arterial embolization and other general therapies would not be effective or even possible. Also, intra-arterial perfusion chemotherapy is a common therapy for HCC and it is not difficult to maintain; but the effective rate is not sufficient. Recently, the combination therapy of s.c. interferon (IFN)-α and intra-arterial 5-fluorouracil (5-FU) showed an outstandingly effective rate for intractable HCC (with portal vein thrombosis). In addition, recent preclinical and clinical studies have revealed that the mechanism of combination therapy may concern direct antitumor effects (through cell-cycle arrest and induction of apoptosis) and indirect actions (through immunocompetent cells and anti-angiogenic effect). For the further advance of HCC treatment and prognosis, this therapy might be a promising treatment modality and is expected to develop. In this review, we summarize recent clinical and preclinical data regarding IFN-α and 5-FU combination therapy and discuss the further prospects of this therapy.

Key words: 5-fluorouracil, antitumor effect, chemotherapy, hepatocellular carcinoma, interferon-α

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most common malignancies worldwide, with an estimated number of more than half a million new cases per year, most of which occur in Asia and Africa.[1] Recently, a trend of increasing rates of HCC has been reported from several developed countries in North America, Europe and Asia, adding to the increasing incidence in Japan over the past 40 years.

Many investigators have reported a putative link between the development of HCC and chronic viral infection and/or liver cirrhosis. Hepatic cirrhosis is observed in 80% of patients with HCC, and the major risk factor for HCC is infection with hepatitis B virus (HBV) or C virus (HCV), 20–70% or 10–70%, respectively, depending on geographic location.[1] In studies on the Japanese population, HBV-related or anti-HCV-positive HCC accounted for 14% and 81%, respectively, of cases in 2003,[2] showing that HCC in Japan is mainly related to HCV infection.

The prognosis of HCC is generally poor. In 2001, the death rates from primary liver cancer were 27.3 in men (third leading cause of death from malignant neoplasms) and 8.8 in women (fifth leading cause) per 100 000 cancer deaths in Japan.[2] Curative therapies such as hepatic resection, liver transplantation, transcatheter arterial embolization or percutaneous ablation have led to improvement in the survival of patients with HCC. However, the majority of patients are still diagnosed at an inoperable advanced stages and/or have recurrence or metastasis after therapy, and their prognoses remain extremely poor.[1,3] Almost all patients with unresectable tumors, especially those with tumor thrombi in the major branches of the portal vein (Vp3–4), die within several months with poor quality of life (QOL) due to liver failure, intractable ascites or esophageal bleeding. Also, tumor cells may spread out through the portal tract, resulting in extensive intrahepatic metastases.

For such highly advanced HCC, surgical resection, use of transcatheter chemoembolization and systematic chemotherapy have been reported, but the results were

Correspondence: Professor Morito Monden, Department of Surgery, Graduate School of Medicine, Osaka University, E2, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan. Email: monden@surg2.med.osaka-u.ac.jp

Table 1 Clinical studies of 5-fluorouracil (5-FU) and interferon (IFN)-α alone for hepatocellular carcinoma (HCC)

Author	Regimen	Response CR + PR/total (%)	Survival over-all (responders)	Patient characteristics
Ansfield (1971)[11]	5-FU i.a.	3/11 (27%)		
Ramming (1976)[12]	5-FU i.a. 10 mg/kg/day	1/7 (14%)		Unresectable
Link *et al.* (1977)[13]	5-FU i.v. p.o.	0/21 (0%)		
Lin (1988)[14]	5-FU	2/21 (9.5%)		Unresectable
Doci *et al.* (1988)[4]	5-FU i.a.	2/9 (22%)		Unresectable, stage II
Stehlin *et al.* (1988)[5]	5-FU i.a.	5/30 (16%)		Advanced
Ueno (2002)[15]	5-FU i.v. continuous 300 mg/m^2	0/20 (0%)	12 weeks	Advanced
Sachs (1985)[16]	IFN-α (12, 50 MU/m^2, i.m., 3 times/week)	0/30 (0%)		
Lai *et al.* (1989)[17]	IFN-α (9–18 MU/m^2 i.m. daily or 25–50 MU/m^2, i.m., 3 times/week)	red 25–50% in 12% and >50% in 10% (22%)	8.3 weeks	Inoperable
GTSG (1990)[18]	IFN-α (?MU/m^2, i.m., 3 times/week)	2/28 (7%)	22 weeks	
Lai *et al.* (1993)[19]	IFN-α (50 MU/m^2, i.m., 3 times/week)	11/35 (31%)	14.5 weeks	Inoperable
Llovet *et al.* (2000)[20]	IFN-α (3 MU, 3 times/week)	2/30 (7%)	Around 13 m	Advanced
Yuen *et al.* (2003)[21]	IFN-α (10–50 MU/m^2, i.a. embolization)	11/18 (61%)	15.9 months	Inoperable

CR, complete response; PR, partial response.

unsatisfactory.[4–7] In addition, conventional therapies such as percutaneous ethanol injection, microwave coagulation therapy and transcatheter arterial embolization are not generally indicated due to lack of efficacy and possible complications.[8]

CLINICAL PERSPECTIVE OF COMBINED INTRA-ARTERIAL 5-FLUOROURACIL AND S.C. INTERFERON-α THERAPY

Summary of clinical trails of single chemotherapeutic agents and/or in combinations for advanced HCC

ALTHOUGH VARIOUS CHEMOTHERAPIES have been used for the treatment of advanced HCC, it could not play a central role for HCC patients, especially those with liver cirrhosis, because of low sensitivity to the anticancer agents and difficulty in giving a sufficient dose due to poor liver function.[8,9] However, chemotherapy must be one of the important possibilities of multimodal treatment for advanced HCC, for which hepatic resection and other general therapies would not be effective.

Several randomized controlled trials (RCT) have assessed the role of systemic or intra-arterial chemotherapy using different anti-neoplastic agents (doxorubicin [DOX], cisplatin [CDDP], mitomycin, 5-fluorouracil [5-FU] and others) either alone or in combination on tumor progression and survival. These trials described an overall partial response (PR) rate less than 20%, and complete response (CR) rates were negligible. Therefore, most investigators advise against using chemotherapy as a single therapy.[10]

The pyrimidine anti-metabolite FU was the first reported chemotherapeutic agent tested in the treatment of HCC (Table 1, upper part).[11,12,14] Ueno *et al.* conducted a phase I clinical study to evaluate the maximum tolerated dose of 5-FU administered by 5-day continuous infusion every 4 weeks in patients with HCC.[15] The maximum tolerated dose for this continuous infusion of 5-FU in HCC patients was 500 mg/m^2/day. As summarized in Table 1, the treatment schedules, dosage and durations have varied among reports studying 5-FU monotherapy. Response rates (RR) of the monotherapy ranged 0–27%, with a median survival of only <13.8 months. Also, systemic administration of 5-FU showed no response at all for HCC patients.[13,15]

The antitumor effects of interferon (IFN)-α therapy in HCC remain controversial (Table 1, lower part).[16,18,21] Two RCT from Hong Kong showed that high doses of IFN-α were better than no treatment or DOX administration with RR in 22–31% and average survival for 8.3–14.5 weeks in patients with inoperable HCC.[17,19] However, an RCT of Western patients failed to show benefit of an average dose of IFN-α (RR 7%) and the treatment was associated with high rates of severe side-effects leading to treatment discontinuation.[20]

Taken together, the data from published studies show that treatment with either IFN-α or 5-FU as a single agent is of little, if any, benefit in patients with advanced

HCC. However, it has to be kept in mind that a small proportion of patients may present a PR to the treatments and, thus, it could be appropriate to test the usefulness of this agent in a multidrug approach. In that sense, recent reports have described encouraging results when combining IFN-α with the administration of 5-FU alone and with CDDP or DOX. However, because of the rate of severe side-effects, the cost of 5-FU with DOX is possibly higher than in 5-FU with CDDP.[22]

Other combination chemotherapies with or without IFN-α

Systemic combination therapy with IFN-α and DOX was found to be ineffective with an RR ranging 3–17%.[23,24] Using intra-arterial chemotherapy with methotrexate, 5-FU, CDDP and s.c. IFN-α administration, Urabe *et al.* reported an RR of 47% (7/15) in patients with Vp3.[25] Chung *et al.* presented a PR in 33% (6/18) of patients with major portal veinous tumor thrombus (PVTT) or distant metastases, who received systemic combination therapy with IFN-α and CDDP.[26] Recent clinical studies reported that the RR in combination therapy with IFN-α and multiple anticancer agents including CDDP, 5-FU and DOX were 26% (13/50),[27] 15.4% (4/26)[28] and 20.9% (19/91)[29] for advanced HCC. However, treatment was unlikely to be tolerated by patients with HCC and cirrhosis. Moreover, the other studies discussed hepatic arterial infusion chemotherapy using low-dose CDDP (3–7 mg/m^2 or 10 mg per day) and 5-FU, which may be a useful alternative for the treatment of patients with complicated PVT or recurrence of HCC with RR in 29% (9/31),[30] 48% (23/48),[31] 47% (18/37)[32] and 33% (6/18).[33]

It is difficult to accurately compare the effectiveness of various therapeutic regimens among different studies because of patient selection bias in liver function or extent of tumor progression and differences in the evaluation methods of the clinical effect.

Clinical trails of combination therapy with 5-FU and IFN-α

The use of a combination of 5-FU and IFN-α had controversial results in patients with gastrointestinal malignancies. Although Wadler *et al.* suggested that the addition of IFN-α to 5-FU improved the activity of the latter in colorectal cancer patients,[34] phase III trials of the combination in that malignancy,[35] and phase II trials in advanced carcinoid and pancreatic cancer, were negative.[36,37] Despite these results, the use of the combination for HCC seemed warranted in view of the association of HCC with HBV or HCV, by virtue of the concurrent anti-neoplastic and antiviral effects of IFN-α and the potential synergism between IFN-α and 5-FU.

Recent studies have indicated the beneficial effects of combined intra-arterial 5-FU and s.c. IFN-α therapy (we abbreviated this treatment as FAIT) for advanced HCC. For the further advance of HCC treatment and prognosis, this therapy might be a promising treatment modality and is expected to develop. Up to date, four clinical studies have been published using intra-arterial 5-FU, and two studies using systemic 5-FU, combined with s.c. IFN-α therapy for advanced HCC (Table 2). First, Patt *et al.* reported that IFN-α in combination with systemic 5-FU injection showed 21% RR in patients with unresectable advanced HCC and low α-fetoprotein (AFP) levels.[38] In the phase II study conducted by the same group, the antitumor response of this combination was assessed as a PR in four patients (14.3%) and minor response (tumor regression of 25–49%) in two (7.1%) of 28 patients with HCC.[39] The median survival duration of all patients was 15.5 months.

In 1997, we experienced a patient with advanced HCC and lung metastasis, who was successfully treated with a combination therapy of IFN-α and tegafur-uracil (UFT), an oral anti-neoplastic drug consisting of uracil and tegafur (a prodrug of 5-FU).[40] Thereafter we studied the effect of FAIT for patients with advanced HCC with PVT in major branches.[22] We reported for the first time that FAIT showed CR (two cases) or PR (three cases) in five patients from the subsequent eight patients (objective RR, 63%) treated with this therapy.[23]

In our second prospective clinical trail we enrolled 55 patients with advanced HCC with major PVTT.[41] The treatment was done on an outpatient basis. An intra-arterial catheter was inserted through the subclavian or femoral artery with a s.c. implanted drug-delivery system. Each patient was treated with natural IFN-α (OIF, Otsuka Pharmaceutical, Tokyo, Japan) and intra-arterial infusion of 5-FU (Kyowa Hakko, Tokyo, Japan) as shown in Figure 1. Of the 55 patients, eight (14.5%) were evaluated as CR, 16 (29.1%) as PR, four (7.3%) as no change (NC) and 27 (49.1%) as progressive disease (PD), with an objective response in 43.6% of the patients. The median progression-free and survival periods of the patients were 5.2 and 11.8 months, respectively. The median survival periods of CR/PR cases (responders) was 24.4 months, while that of NC/PD cases (non-responders) was 5.4 months. The 1- and 3-year survival rates of responders were 82.9% and 30.9%, respectively, and those of non-responders were 13.1% and 0%, respectively, with a significant difference between the groups ($P = 0.0001$).

Table 2 Clinical studies of 5-FU and IFN-α in combinations for HCC

Author	Regimen	Response CR + PR/total (%)	Survival over-all (responders)	Patient characteristics
Patt *et al.* (1993)[38]	IFN-α (5 MU, i.m., 3 times/ week) 5-FU (750 mg/m², continuous i.v., 5 days)	6/28 (21%)		
Sakon *et al.* (2002)[22]	IFN-α (5 MU, i.m., 3 times/ week) 5-FU (450–500 mg/ day, continuous i.a., 2 weeks)	5/8 (63%)	n/a	Vp3 multiple
Patt *et al.* (2003)[39]	IFN-α (4 MU/m², s.c., 3 times/ week) 5-FU (200 mg/m²/day, continuous i.v., 21 days)	4/28 (14%)	15.5 months	tumor node metastasis (TNM) stage >III
Ota *et al.* (2005)[41]	IFN-α (5 MU, i.m., 3 times/ week) 5-FU (300 mg/m²/day, continuous i.a., 2 weeks)	24/55 (44%)	11.8 months (24.4)	Vp3 or 4, IM3
Enjoji *et al.* (2005)[42]	IFN-α (3 MU, s.c., 3 times/ week) 5-FU (500 mg/day, i.a., 5 days)	6/28 (21.5%)		Advanced HCC with IM or portal vein thrombosis
Obi *et al.* (2006)[43]	IFN-α (5 MU, i.m., 3 times/ week) 5-FU (500 mg/day, continuous i.a., 2 weeks)	61/116 (53%)	6.9 months?	Portal venous invasion
Nagano *et al.* (2006)[45]	IFN-α (5 MU, i.m., 3 times/ week) 5-FU (300 mg/m²/day, continuous i.a., 2 weeks)			Resectable advanced HCC (Vp3) as a postoperative adjuvant

Enjoji *et al.* published that the overall RR was 21.5% (6/28) in patients with advanced HCC received FAIT.[36] The study by Obi *et al.* of 116 patients with advanced HCC with PVTT reported that 19 (16.4%) patients had CR, 42 (36.2%) had PR, two (1.7%) had SD, and 53 (45.7%) had PD, resulting in 52.6% RR.[37] The average duration of complete and partial responses was 13.6 and 4.8 months, respectively.

The RR of i.v. 5-FU and IFN-α therapy was lower than that of FAIT (Table 2). Perhaps the major dissimilarities of the above studies were in the different administrations of 5-FU in different patient populations; there are no evidences of unlike responses to the therapies.

Taken together, the efficacy of FAIT for patients with highly advanced HCC ranged 21.5–63% (overall RR, 46.4%; 96/207), which was better than the previous reports with other combination chemotherapies for patients of similar stage (see above). Generally, the prognosis of such patients is extremely poor and survival is generally limited to a few months after diagnosis, despite multimodal therapies, even in cases suitable for surgical resection.[44] The FAIT markedly decreased tumor size and levels of tumor markers with an encouraging RR and prolonged survival time in the responders. Furthermore, the clinical response completely reflected the survival benefits.

FAIT as an adjuvant therapy after curative operation

From our clinical study on 30 patients treated with FAIT (n = 15) or without FAIT (m = 15) as adjuvant therapy after curative hepatectomy, disease-free survival was 11 of 15 patients during 5–55 months, and survival with recurrence in two patients for 13 and 48 months in the FAIT group.[45] In the group that did not receive adjuvant FAIT, almost all patients (11/15) died of recurrent cancer in the residual liver or had lung and lymph node metastasis within 2 years. The overall survival rates at 1 and 3 years were 100% and 74%, respectively, in patients with FAIT, and 41% and 22%, respectively, in the controls without FAIT ($P = 0.0031$).

With respect to postoperative adjuvant therapy for HCC, a recent review mentioned that systemic chemotherapy, hepatic-artery chemotherapy or TAE, as well as combinations of these therapies did not improve overall or disease-free survival after potentially curative surgery for localized HCC.[46] Compared with previous studies, the clinical outcome using FAIT as a postoperative adjuvant was excellent and highly satisfactory in terms of disease-free and overall survival rates.

The study suggested that FAIT is not only promising for treatment of highly advanced HCC, and also effec-

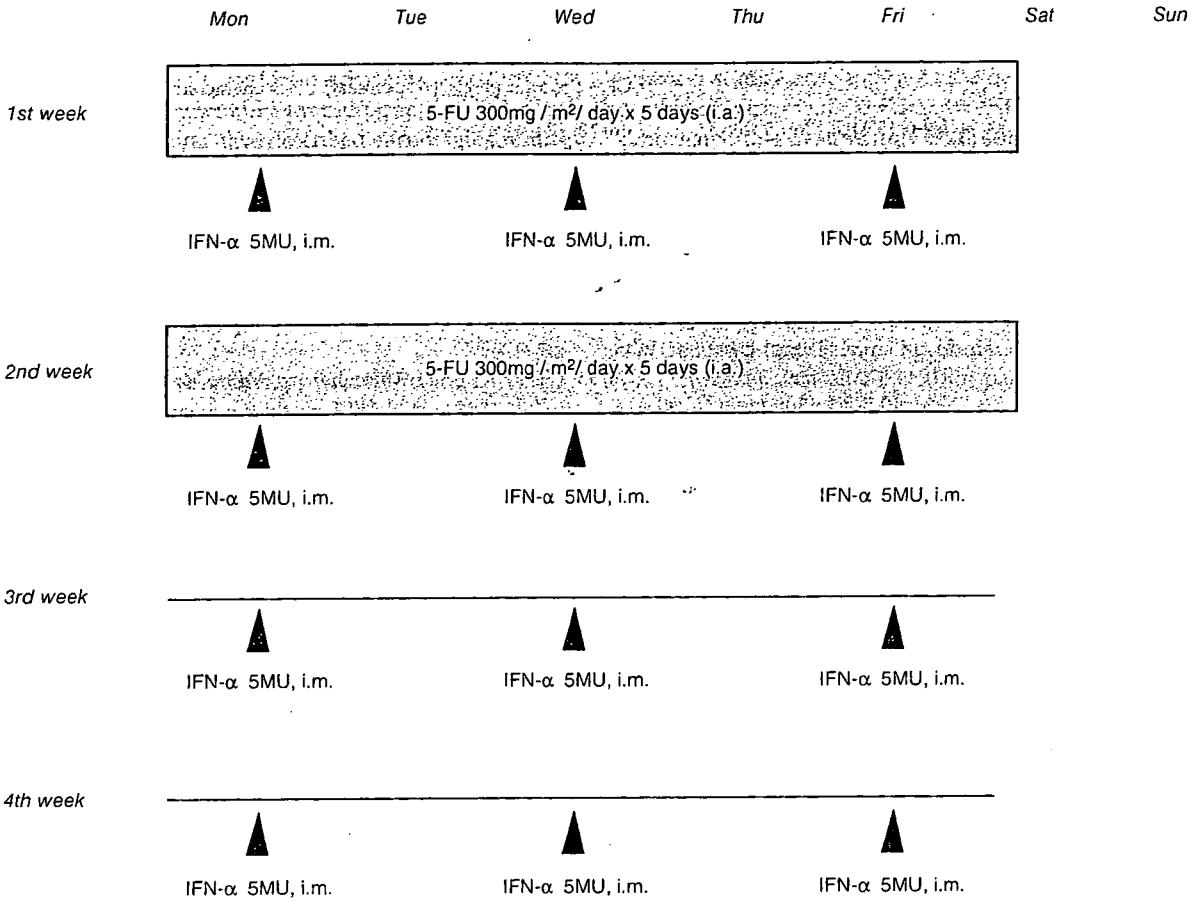

Figure 1 Combined intra-arterial 5-fluorouracil (5-FU) and s.c. interferon (IFN)-α therapy (FAIT) protocol; one cycle.

tive for prevention of recurrence in residual liver as a postoperative adjuvant treatment in resectable tumors.

Adverse effects of FAIT in HCC patients

Although 5-FU and IFN-α is known to result in multiple adverse effects, the occurrence of life-threatening side-effects among advanced HCC patients (even with cirrhosis) receiving FAIT was rare from the published data.[22,41,43,45] Non-hematological side-effects, such as fever, chills and flu-like syndrome, mostly in grades 1 and 2, were commonly (90–100% of the patients) observed but were easily controlled by non-steroidal anti-inflammatory drugs. In 20–50% of the patients, grade 1 generalized fatigue and nausea (5.5–50%) were observed. In a lesser percentage of patients (–5%), diarrhea, depression, gastric ulceration and skin reaction occurred. Only one case of grade 3 stomatitis and another of grade 3 depression, were noted and the latter patient discontinued the therapy due to the side-effects.[43] Myelosuppressive adverse effects were particularly important in patients. Decreased numbers of leukocytes or platelets were found in 30–80% of patients, and 5.5–9.1% of patients developed grade 3 leukopenia, thrombocytopenia or anemia (1.8%), but none resulted in termination of the therapy or required granulocyte-colony-stimulating factor administration, as reported. All were manageable. No complications resulting from the arterial catheter were reported. In addition, from our experience, the QOL of HCC patients was good. Moreover, they had no symptoms related to liver dysfunction. No hospital admission was necessary to receive FAIT. In studies with i.v. 5-FU and s.c. IFN-α reported by Patt *et al.*, grade 3 or 4 toxicity occurred in leukopenia (8.8%), anemia (5.9%), thrombocytopenia (11.8%) and mucositis (41.2%) of treated patients and were similar to those with FAIT.[38,39]

In our experience, only one case of the occurrence of interstitial pneumonia during FAIT was reported.[47] The patient with advanced HCC died 32 days after start of the therapy due to respiratory failure, suspected to have been caused by interstitial pneumonia, after steroid pulse therapy was started. The association of IFN-α with the development of interstitial pneumonia has been reported.[48] However, the prognosis of IFN-induced interstitial pneumonia has mostly been favorable when the medication was discontinued. It has been postulated that interstitial pneumonia induced by FAIT may be therapy-resistant. Thus, interstitial pneumonia in these patients should be carefully managed.

MECHANISM OF ANTITUMOR EFFECTS OF IFN-α AND 5-FU COMBINATION THERAPY

TO ADVANCE THE effect of FAIT and to increase the RR, it is necessary to understand the mechanism of action of IFN-α, 5-FU alone and in combination. Tumor cell resistance seems to be an important reason for failure of IFN-α and chemotherapy. It is therefore appropriate to consider what specific mechanisms might be involved in antitumor activity of those agents in HCC cells.

Action mechanism of 5-FU

5-Fluorouracil is an analog of uracil with a fluorine atom at the C-5 position in place of hydrogen. It rapidly enters the cell using the same facilitated transport mechanism as uracil. 5-FU is converted intracellularly to several active metabolites: fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP) and fluorouridine triphosphate (FUTP). These active metabolites incorporate into RNA disrupting its synthesis, and inhibit the nucleotide (DNA) synthetic enzyme thymidylate synthase (TS).[49] The rate-limiting enzyme in 5-FU catabolism is dihydropyrimidine dehydrogenase (DPD), which converts 5-FU to dihydrofluorouracil (DHFU). More than 80% of administered 5-FU is normally catabolized primarily in the liver, where DPD is abundantly expressed.[50] It is known that 5-FU induces dysfunction of RNA by FUTP or inhibition of DNA synthesis by FdUMP; however, it has not been demonstrated clearly that which mainly contributes to antitumor effects of clinically administrated 5-FU. Also, when 5-FU concentration is high (10–100 mM), it preferably induces dysfunction of RNA, while at a lower concentration (0.5–1.0 mM) DNA synthesis can be inhibited.[51] Based on pharmacological characteristics of 5-FU, various studies have been conducted to find the optimal way of 5-FU administration. As a result, experimental studies have revealed that 5-FU is a time-dependent chemotherapeutic agent.[52] That is, antitumor effects of 5-FU are poor even at high concentration when it contacts tumor cells for a short time, while enhanced antitumor effects can be obtained even at low concentration when it contacts tumor cells for a long time. In addition, many prospective randomized clinical studies have demonstrated that both lower hematological toxicities[53] and higher antitumor effects[54,55] can be achieved with continuous infusion of 5-FU as compared with single bolus injection, which has been confirmed in a recent meta-analysis study.[56] Altogether, maintenance of a certain concentration of 5-FU in liver tumor for a long time by continuous hepatic artery infusion for 5 days, in ours and other clinical studies, has been established as the optimal way of 5-FU administration.[21,41,43,45]

Action mechanism of IFN-α

The actions of type I IFN, which includes IFN-α, -β -ω, are mediated by their interaction with a multisubunit cell-surface receptor, IFN-α receptor (IFNAR)1 and IFNAR2 (long, short and soluble subunits).[57,58] After IFN bind to the receptors, IFNAR-associated tyrosine kinases (JAK), including JAK1 and Tyk2, are activated, followed by phosphorylation of signal transducer and activator of transcription factor (STAT)1, 2, 3 and 5. Phosphorylated STAT (pSTAT) form hetero- or homodimers with an IFN-stimulated gene factor 3 (ISGF3), and that transfer into the nucleus where they induce the transcription of numerous IFN-responsive genes, which ultimately results in the biological effects of the IFN-α treatment, including antiviral, growth inhibitory, apoptotic, anti-angiogenic and immunomodulatory effects.[58]

Theoretically, the patients may respond to IFN therapy for a variety of reasons. The IFN-α may have direct effects on the tumor cells, for example, it may be cytotoxic, affect the proliferation of the tumor cells or induce cellular differentiation. Alternatively, or in addition, the IFN-α may have indirect effects; for example, on host immune functions, the tumor stromal cells or the vascularization of the tumor.[58] Some of these possibilities have been investigated in HCC.

Hepatocellular carcinoma cells differ greatly in their sensitivity to growth-inhibitory effect of IFN-α.[59–62] In IFN-sensitive cells, the IFN-α treatment resulted in a time- and dose-dependent reduction of proliferation.

Interferon-α-induced events that lead to cell cycle arrest have also been studied in HCC cell lines.[59,60] IFN-α treatment inhibits growth of HCC cells by specifically mainly delaying S-phase progression, most likely

because of inhibition of cyclin A induction, resulting in decreased activity of the associated Cdk2 and Cdc2 kinases.[60]

Several studies have investigated the role of IFN-α in apoptotic events, and in some instances IFN-α have now been shown to exert limited proapoptotic activity that is unrelated to its cell growth inhibitory action.[62,63]

Interferons also have profound effects on a number of immunological functions such as natural killer (NK)-cell activity, T-cell cytotoxicity and macrophage function, and induction of class I major histocompatibility complex antigens.[58] Another possible mechanism is via its antiangiogenesis activity; IFN-α has been shown to inhibit HCC angiogenesis in various experimental settings.[64,65]

Interestingly, a negative regulator of the IFN signal transduction, SOCS-1, was found to be silenced by methylation in human HCC.[66] In addition, our unpublished results showed a clear relationship between sensitivity to IFN-α and IFNAR expression; the expression rate of IFNAR2 in HCC was higher than in esophageal, gastric, colorectal, cholangiocarcinoma and pancreatic cancer samples.[67] Therefore, the antitumor effect of IFN may be better than those in other gastrointestinal cancers. Also, the expressions of IFNAR with subsequent activation of STAT were important for antiproliferative effect of IFN-α in HCC cells.[68]

Cooperative effect of 5-FU and IFN-α in HCC cells

From clinical data, the combination of 5-FU and IFN-α seems to have some synergism. Also, synergistic cooperative effects were clearly observed in experiments on HCC cell lines.[61,69] Also, Kondo *et al.* and Moriyama *et al.* found that IFN-α markedly increases susceptibility to 5-FU, respectively, in three of four, and five of eight human HCC cell lines.[70,71] Besides the above, several experimental studies have demonstrated that IFN-α enhanced the cytotoxic effect of 5-FU in other cultured malignant cells.[34]

The mechanism underlying the ability of IFN-α to strengthen the anticancer effect of 5-FU has been studied previously. Possible mechanisms of the cooperative effect pathways are schematically summarized in Figure 2. IFN-α enhances the conversion of 5-FU to an active metabolite, FdUMP, through an increase of thymidine phosphorylase (TP)[72] and a suppression of DPD in HCC.[73] Increased levels of FdUMP inhibit TS activity, resulting in an increase in DNA double-strand breaks.[74] In addition, Braybrooke *et al.* have demonstrated that a single dose of IFN-α could upregulate TP in peripheral blood lymphocytes within few hours of the administration and that the effect could be sustained for at least several days.[75] However, it is not clear for HCC.

We have investigated the mechanism of the cooperative effect of the IFN-α and 5-FU on HCC cells, and published in serial in our previous papers. A study by Eguchi *et al.* showed that augmentation of antitumor activity of 5-FU by IFN-α was associated with upregulation of p27Kip1, by delaying the progression of G1 to S phase in IFNAR2 expressing the HCC cell line.[67] Also, a possible explanation for the synergistic or additive effects was suggested by up- or downregulation of the Bcl-2 protein family, especially Bcl-xL, which was correlated with the incidence of apoptosis.[71] In these direct actions, IFNAR2 on tumor cells has been shown to be important and working as a "gatekeeper" of the cooperative action in this combination.[41,68,71,76] We reported that the modulation of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptor-mediated cytotoxic pathway could partially contribute to the anti-HCC effect of IFN-α/5-FU combination therapy.[77] Recently, we also demonstrated contribution of the Fas/FasL pathway in this combination.[78] Another possible mechanism is via its antiangiogenesis activity; the combination has been shown to inhibit cooperatively tumor angiogenesis in HCC (unpubl. data). This antiangiogenesis activity may be clinically important because we observed reduced tumor blood flow demonstrated by dynamic computed tomography (CT) scan as an initial finding leading to clinical response. It was reported that IFN-α induces p53, which enhances apoptotic responses to 5-FU.[79]

Taken together, these *in vitro* findings provide supportive evidence for the beneficial effect of combination therapy with IFN-α and 5-FU on HCC. Also, using gene profiling, several genes showed distinct gene expression profiles in the responsive cells and others. Further investigation of these genes may elucidate underlying molecular mechanisms, enabling us to improve the efficacy of this combination therapy.

FUTURE DIRECTIONS TO IMPROVE THE EFFICACY OF FAIT

Prediction of response to the therapy

HOWEVER EFFECTIVE FAIT is for advanced HCC with significant prolongation of survival, it did not have any survival benefits for non-responders. Of the patients receiving FAIT, 37–78.5% (overall, 53.6%) did not respond and average survival time was only a few months, which is similar to patients symptomatically

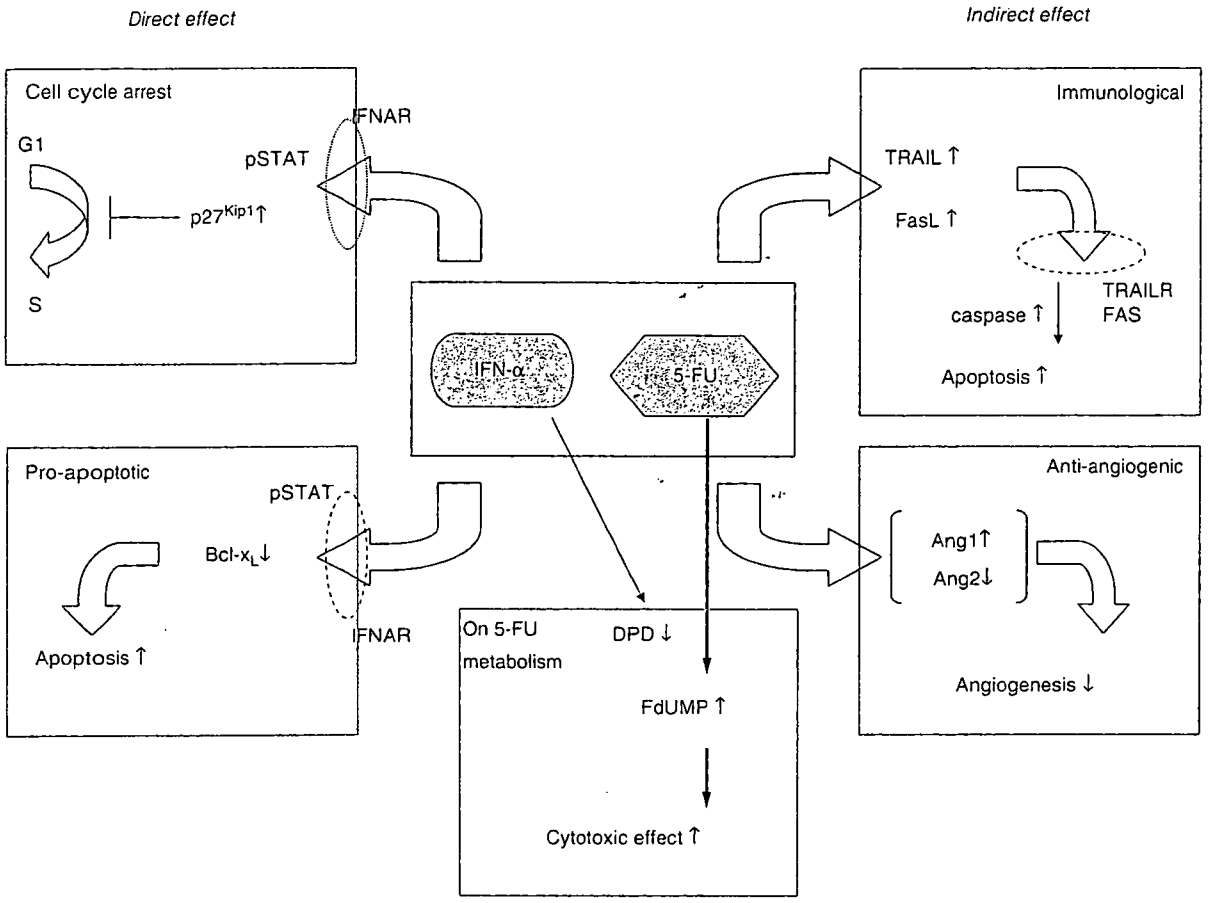

Figure 2 FAIT mechanism (version 1).

treated.[21,41,42,43] They lose crucial survival time by precluding their chance to undertake other treatment options. Moreover, FAIT induces side-effects (see Adverse effects). Therefore, it is vital that the patients be appropriately selected for FAIT and that sensitivity to this chemotherapy is predicted accurately.

Several studies have attempted to distinguish between responders and non-responders to FAIT. To investigate the role of clinical and pathological parameters in clinical effects of therapy, we compared some factors of responders ($n = 8$) with those of non-responders ($n = 5$).[41] The results showed that patient age, gender, serum AFP, protein induced by vitamin K absence or antagonist II (PIVKA-II), Child–Pugh, Okuda scores and Cancer of the Liver Italian Program (CLIP) scores did not correlate with the response to combination therapy. On the other hand, IFNAR2 expression in tumors correlated significantly with the response to the therapy ($P = 0.007$). Moreover, survival analysis showed the significant role of IFNAR2 expression on prognosis; IFNAR2-positive cases had better prognosis than negative cases.[41,76] Thus, the expression level of IFNAR2 was the sole factor that influenced the response to the combination therapy and that might be a potentially useful predictor of the response to FAIT.

The investigators from Kyoundo Hospital noted that the CR rate was higher among patients with HCV infection (22%) than among others (5%).[43] The final response to treatment was predictable, with the early response of tumor biomarker levels at the second week among patients whose markers were positive before treatment (AFP, L3 fraction of AFP, and des-γ-carboxy prothrombin; prediction with 90% sensitivity and 80% specificity). Otherwise, Yamamoto *et al.* reported that

among HCC patients who received FAIT, the expression of TRAIL mRNA in peripheral blood mononuclear cells was significantly higher in clinical responders than in non-responders.[75]

From the study by Patt *et al.*, all patients who achieved CR or PR to 5-FU (i.v.) and IFN-α had a corresponding significant decrease in serum AFP level (<50 ng/mL) as compared with the baseline value (median decrease, 82.5%), while most patients (11/14) with DP showed an increase in their follow-up serum AFP levels.[39]

The advent of DNA microarray technology could be used directly as a therapeutic tool. A novel prediction method using gene expression profiling has recently been reported for the treatment of breast cancer patients with the taxanes, docetaxel and paclitaxel.[80] The study by Kurokawa *et al.* identified subsets of 63 genes which, when analyzed simultaneously by gene expression profile analysis using adaptor-tagged competitive polymerase chain reaction (ATAC-PCR) technology, predict the response to FAIT in advanced HCC.[81] Further studies for prediction of the therapy are needed adopting modern methods, such as gene expression profiling technology.

From the available data, it will be helpful to examine the expression of IFNAR2 in HCC before therapy and whether only those with IFNAR2 must enroll in the therapy. We advocate that, in practice, responders and non-responders should be discriminated after the first cycle of the therapy by evaluating tumor size and the levels of tumor markers. For early non-responders, therapy should not proceed to the next cycle and instead a different therapeutic option should be explored.

Questions regarding non-responders to FAIT

Despite the prominent improvement in survival among complete responders, we must admit that the CR rate was not satisfactory, and that we need to enhance the response among partial responders by modifying the protocol. The studies show that approximately half of the patients with HCC will remain unsusceptible to the combination therapy.

We reported previously that FAIT had no effect in IFNAR2-negative cases.[35] Upregulation of IFNAR2 may be considered in order to induce a better response to the therapy in such cases. In our recent study involving *in vitro* experiments, we showed that IFNAR2 gene transfer is effective for augmenting the biological activity of IFN-α/5-FU combination therapy in HCC cells.[71] Thus, IFNAR2 gene transfection might enhance the response to FAIT in IFNAR2-negative patients. On the other hand, not all IFNAR2-positive cases benefited from FAIT. In such patients, increasing the doses or modifying t combination therapy (e.g. addition of other antitum agents) might increase the RR. Other parameters, ap from the expression of IFNAR2, might be important a necessary for the response to the therapy.

Further challenges

What can be done to make combination chemothera more effective? Increasing the doses or modifying t protocol of the combination therapy, monitoring ca fully the toxic effects, may be considered initially.

Higher concentrations of cancer chemotherapeu agents can be delivered directly to the HCC via t hepatic arterial route, considering that this is the maj vascular supply of these tumors. Yuen *et al.* show that, from 18 patients with inoperable HCC who we recruited to receive i.a. high doses of IFN-α (10 MU/n 30 MU/m², or 50 MU/m²), CR and PR were observ in 28.6% and 33.3% of patients, respectively.[21] T median survival was 15.9 months. No significant liv decompensation was observed. This pilot study show that transcatheter arterial IFN-α embolization was effective method for the treatment of patients with ino erable HCC without significant hepatic toxicity.

It is also a problem whether the coupling of 5-F and IFN-α is the best combination as chemothera for HCC. A better regimen for IFN-α/5-FU combin tion chemotherapy could be developed. In our pre ous *in vitro* study, we showed that the spectra of t antiproliferative activity and synergistic effect of IFN when combined with anticancer drugs (5-FU, DC and CDDP) were more potent than those of IFN-α.[61] Thus, combinations of IFN-β with other anticanc drugs may provide a better treatment of HCC wh FAIT is ineffective.

SUMMARY

CURATIVE THERAPIES SUCH as hepatic resectio liver transplantation, transcatheter arterial emb lization or percutaneous ablation have led to improv ment in survival of patients with HCC. Howev standard therapy for advanced, inoperable HCC was n established.

Both clinical and preclinical studies suggest effica of combination of 5-FU with IFN-α for such high advanced HCC. The efficacy of FAIT ranged 21.5–63 (overall RR, 46.4%), which was better than the previo reports with other combination chemotherapies f patients of a similar stage. Generally, the prognosis such patients is extremely poor and survival is general

limited to a few months after diagnosis. The FAIT markedly decreased tumor size and levels of tumor markers with an encouraging RR and prolonged survival time in the responders. Furthermore, the clinical response completely reflected the survival benefits.

It will be helpful to examine the expression of IFNAR2 in HCC before therapy and if only those with IFNAR2 should enroll. At present, we recommend starting the combination therapy with close monitoring of response, preferably that of tumor biomarkers, and treatment should be continued if there is a response after the first cycle of chemotherapy. For early non-responders, therapy should not proceed to the next cycle and instead a different therapeutic option should be explored.

Although the limitations in comparing the clinical response between some studies cannot be neglected, the marked effect and acceptable toxicity of the therapy for HCC patients with extremely poor prognosis suggests that FAIT is a potential, promising treatment regimen. To obtain conclusive evidence of the effect of this treatment, a large phase II trial and further investigation are essential.

ACKNOWLEDGMENTS

THIS STUDY WAS supported by a Grant-in-Aid for Scientific Research provided by the Ministry of Education, Culture, Sports, Science and Technology, Japan. B.D. is a postdoctoral fellow supported by the Japan Society for the Promotion of Science.

CONFLICT OF INTEREST

NO CONFLICT OF interest statement has been received from the authors.

REFERENCES

1 Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003; 362: 1907–17.
2 Kiyosawa K, Umemura T, Ichijo T *et al.* Hepatocellular carcinoma: recent trends in Japan. *Gastroenterology* 2004; 127: S17–26.
3 Befeler AS, Di Bisceglie AM. Hepatocellular carcinoma: diagnosis and treatment. *Gastroenterology* 2002; 122: 1609–19.
4 Doci R, Bignami P, Bozzetti F *et al.* Intrahepatic chemotherapy for unresectable hepatocellular carcinoma. *Cancer* 1988; 61: 1983–7.
5 Stehlin JS Jr, de Ipolyi PD, Greeff PJ, McGaff CJ Jr, Davis BR, McNary L. Treatment of cancer of the liver. Twenty years' experience with infusion and resection in 414 patients. *Ann Surg* 1988; 208: 23–35.
6 Iwamiya T, Sawada S, Ohta Y. Repeated arterial infusion chemotherapy for inoperable hepatocellular carcinoma using an implantable drug delivery system. *Cancer Chemother Pharmacol* 1994; 33 (Suppl): S134–8.
7 Furuse J, Iwasaki M, Yoshino M *et al.* Hepatocellular carcinoma with portal vein tumor thrombus: embolization of arterioportal shunts. *Radiology* 1997; 204: 787–90.
8 Mathurin P, Rixe O, Carbonell N *et al.* Review article: overview of medical treatments in unresectable hepatocellular carcinoma – an impossible meta-analysis? *Aliment Pharmacol Ther* 1998; 12: 111–26.
9 Johnson PJ. Hepatocellular carcinoma: is current therapy really altering outcome? *Gut* 2002; 51: 459–62.
10 Nerenstone SR, Ihde DC, Friedman MA. Clinical trials in primary hepatocellular carcinoma: current status and future directions. *Cancer Treat Rev* 1988; 15: 1–31.
11 Ansfield FJ, Ramirez G, Skibba JL *et al.* Intrahepatic arterial infusion with 5-fluorouracil. *Cancer* 1971; 28 (5): 1147–51.
12 Ramming KP, Sparks FC, Eilber FR *et al.* Hepatic artery ligation and 5-fluorouracil infusion for metastatic colon carcinoma and primary hepatoma. *Am J Surg* 1976; 132 (2): 236–42.
13 Link JS, Bateman JR, Paroly WS, Durkin WJ, Peters RL. 5-Flourouracil in hepatocellular carcinoma: report of twenty-one cases. *Cancer* 1977; 39: 1936–9.
14 Lin DY, Liaw YF, Lee TY, Lai CM. Hepatic arterial embolization in patients with unresectable hepatocellular carcinoma – a randomized controlled trial. *Gastroenterology* 1988; 94 (2): 453–6.
15 Ueno H, Okada S, Okusaka T, Ikeda M, Kuriyama H. Phase I and pharmacokinetic study of 5-fluorouracil administered by 5-day continuous infusion in patients with hepatocellular carcinoma. *Cancer Chemother Pharmacol* 2002; 49: 155–60.
16 Sachs E, Di Bisceglie AM, Dusheiko GM *et al.* Treatment of hepatocellular carcinoma with recombinant leucocyte interferon: a pilot study. *Br J Cancer* 1985; 52 (1): 105–9.
17 Lai CL, Wu PC, Lok AS *et al.* Recombinant alpha 2 interferon is superior to doxorubicin for inoperable hepatocellular carcinoma: a prospective randomised trial. *Br J Cancer* 1989; 60: 928–33.
18 The Gastrointestinal Tumor Study Group. A prospective trial of recombinant human interferon alpha 2B in previously untreated patients with hepatocellular carcinoma. *Cancer* 1990; 66: 135–9.
19 Lai CL, Lau JY, Wu PC *et al.* Recombinant interferon-alpha in inoperable hepatocellular carcinoma: a randomized controlled trial. *Hepatology* 1993; 17: 389–94.
20 Llovet JM, Sala M, Castells L *et al.* Randomized controlled trial of interferon treatment for advanced hepatocellular carcinoma. *Hepatology* 2000; 31: 54–8.

21 Yuen MF, Ooi CG, Hui CK *et al.* A pilot study of transcatheter arterial interferon embolization for patients with hepatocellular carcinoma. *Cancer* 2003; 97: 2776–82.
22 Sakon M, Nagano H, Dono K *et al.* Combined intraarterial 5-fluorouracil and subcutaneous interferon-alpha therapy for advanced hepatocellular carcinoma with tumor thrombi in the major portal branches. *Cancer* 2002; 94: 435–42.
23 Kardinal CG, Moertel CG, Wieand HS *et al.* Combined doxorubicin and alpha-interferon therapy of advanced hepatocellular carcinoma. *Cancer* 1993; 71: 2187–90.
24 Feun LG, Savaraj N, Hung S *et al.* A phase II trial of recombinant leukocyte interferon plus doxorubicin in patients with hepatocellular carcinoma. *Am J Clin Oncol* 1994; 17: 393–5.
25 Urabe T, Kaneko S, Matsushita E, Unoura M, Kobayashi K. Clinical pilot study of intrahepatic arterial chemotherapy with methotrexate, 5-fluorouracil, cisplatin and subcutaneous interferon-alpha-2b for patients with locally advanced hepatocellular carcinoma. *Oncology* 1998; 55: 39–47.
26 Chung YH, Song IH, Song BC *et al.* 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: 1986–91.
27 Leung TW, Patt YZ, Lau WY *et al.* Complete pathological remission is possible with systemic combination chemotherapy for inoperable hepatocellular carcinoma. *Clin Cancer Res* 1999; 5: 1676–81.
28 Yin XY, Lu MD, Liang LJ, Lai JM, Li DM, Kuang M. Systemic chemo-immunotherapy for advanced-stage hepatocellular carcinoma. *World J Gastroenterol* 2005; 11: 2526–9.
29 Yeo W, Mok TS, Zee B *et al.* A randomized phase III study of doxorubicin versus cisplatin/interferon alpha-2b/doxorubicin/fluorouracil (PIAF) combination chemotherapy for unresectable hepatocellular carcinoma. *J Natl Cancer Inst* 2005; 97: 1532–8.
30 Okuda K, Tanaka M, Shibata J *et al.* 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: 587–91.
31 Ando E, Tanaka M, Yamashita F *et al.* Hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma with portal vein tumor thrombosis: analysis of 48 cases. *Cancer* 2002; 95: 588–95.
32 Tanioka H, Tsuji A, Morita S *et al.* Combination chemotherapy with continuous 5-fluorouracil and low-dose cisplatin infusion for advanced hepatocellular carcinoma. *Anticancer Res* 2003; 23: 1891–7.
33 Lai YC, Shih CY, Jeng CM *et al.* Hepatic arterial infusion chemotherapy for hepatocellular carcinoma with portal vein tumor thrombosis. *World J Gastroenterol* 2003; 9: 2666–70.
34 Wadler S, Schwartz EL, Goldman M *et al.* Fluorouracil and recombinant alfa-2a-interferon: an active regimen against advanced colorectal carcinoma. *J Clin Oncol* 1989; 7: 1769–75.
35 Greco FA, Figlin R, York M *et al.* Phase III randomized study to compare interferon alfa-2a in combination with fluorouracil versus fluorouracil alone in patients with advanced colorectal cancer. *J Clin Oncol* 1996; 14: 2674–81.
36 Saltz L, Kemeny N, Schwartz G, Kelsen D. A phase II trial of alpha-interferon and 5-fluorouracil in patients with advanced carcinoid and islet cell tumors. *Cancer* 1994; 74: 958–61.
37 Hughes MJ, Kerr DJ, Cassidy J *et al.* A pilot study of combination therapy with interferon-alpha-2a and 5-fluorouracil in metastatic carcinoid and malignant endocrine pancreatic tumours. *Ann Oncol* 1996; 7: 208–10.
38 Patt YZ, Yoffe B, Charnsangavej C *et al.* Low serum alpha-fetoprotein level in patients with hepatocellular carcinoma as a predictor of response to 5-FU and interferon-alpha-2b. *Cancer* 1993; 72: 2574–82.
39 Patt YZ, Hassan MM, Lozano RD *et al.* Phase II trial of systemic continuous fluorouracil and subcutaneous recombinant interferon Alfa-2b for treatment of hepatocellular carcinoma. *J Clin Oncol* 2003; 21: 421–7.
40 Miyamoto A, Umeshita K, Sakon M *et al.* Advanced hepatocellular carcinoma with distant metastases, successfully treated by a combination therapy of alpha-interferon and oral tegafur/uracil. *J Gastroenterol Hepatol* 2000; 15: 1447–51.
41 Ota H, Nagano H, Sakon M *et al.* 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: 557–64.
42 Enjoji M, Morizono S, Kotoh K *et al.* Re-evaluation of antitumor effects of combination chemotherapy with interferon-alpha and 5-fluorouracil for advanced hepatocellular carcinoma. *World J Gastroenterol* 2005; 11: 5685–7.
43 Obi S, Yoshida H, Toune R *et al.* Combination therapy of intraarterial 5-fluorouracil and systemic interferon-alpha for advanced hepatocellular carcinoma with portal venous invasion. *Cancer* 2006; 106: 1990–7.
44 Poon RT, Fan ST, Ng IO, Wong J. Prognosis after hepatic resection for stage IVA hepatocellular carcinoma: a need for reclassification. *Ann Surg* 2003; 237: 376–83.
45 Nagano H, Sakon M, Eguchi H *et al.* Hepatic resection followed by IFN-alpha and 5-FU for advanced hepatocellular carcinoma with tumor thrombus in the major portal branch. *Hepatogastroenterology* 2007; 54 (73): 172–9.
46 Schwartz JD, Schwartz M, Mandeli J, Sung M 2002 Neoadjuvant and adjuvant therapy for resectable hepatocellular carcinoma: review of the randomised clinical trials. *Lancet Oncol* 2006; 3: 593–603.
47 Yamamoto S, Tomita Y, Hoshida Y *et al.* Interstitial pneumonia induced by combined intraarterial 5-fluorouracil

and subcutaneous interferon-alpha therapy for advanced hepatocellular carcinoma. *J Gastroenterol* 2004; 39: 793–7.
48 Okanoue T, Sakamoto S, Itoh Y *et al.* Side effects of high-dose interferon therapy for chronic hepatitis C. *J Hepatol* 1996; 25: 283–91.
49 Longley DB, Harkin DP, Johnston PG. 5-Fluorouracil: mechanisms of action and clinical strategies. *Nat Rev Cancer* 2003; 3 (5): 330–8.
50 Diasio RB, Harris BE. Clinical pharmacology of 5-fluorouracil. *Clin Pharmacokinet* 1989; 16: 215–37.
51 Tanaka F, Fukuse T, Wada H, Fukushima M. The history, mechanism and clinical use of oral 5-fluorouracil derivative chemotherapeutic agents. *Curr Pharm Biotechnol* 2000; 1: 137–64.
52 Pinedo HM, Peters GF. Fluorouracil: biochemistry and pharmacology. *J Clin Oncol* 1988; 6: 1653–64.
53 Leichman CG, Fleming TR, Muggia FM *et al.* Phase II study of fluorouracil and its modulation in advanced colorectal cancer: a Southwest Oncology Group study. *J Clin Oncol* 1995; 13: 1303–11.
54 Lokich JJ, Ahlgren JD, Gullo JJ, Philips JA, Fryer JG. A prospective randomized comparison of continuous infusion fluorouracil with a conventional bolus schedule in metastatic colorectal carcinoma: a Mid-Atlantic Oncology Program Study. *J Clin Oncol* 1989; 7: 425–32.
55 Hansen RM, Ryan L, Anderson T *et al.* Phase III study of bolus versus infusion fluorouracil with or without cisplatin in advanced colorectal cancer. *J Natl Cancer Inst* 1996; 88: 668–74.
56 Cunningham D. Mature results from three large controlled studies with raltitrexed ("Tomudex"). *Br J Cancer* 1998; 77 Suppl 2: 15–21.
57 Darnell JE Jr, Kerr IM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* 1994; 264: 1415–21.
58 Stark GR, Kerr IM, Williams BR, Silverman RH, Schreiber RD. How cells respond to interferons. *Annu Rev Biochem* 1998; 67: 227–64.
59 Yano H, Iemura A, Haramaki M *et al.* Interferon alfa receptor expression and growth inhibition by interferon alfa in human liver cancer cell lines. *Hepatology* 1999; 29: 1708–17.
60 Murphy D, Detjen KM, Welzel M, Wiedenmann B, Rosewicz S. Interferon-alpha delays S-phase progression in human hepatocellular carcinoma cells via inhibition of specific cyclin-dependent kinases. *Hepatology* 2001; 33: 346–56.
61 Damdinsuren B, Nagano H, Sakon M *et al.* Interferon-beta is more potent than interferon-alpha in inhibition of human hepatocellular carcinoma cell growth when used alone and in combination with anticancer drugs. *Ann Surg Oncol* 2003; 10: 1184–90.
62 Inamura K, Matsuzaki Y, Uematsu N, Honda A, Tanaka N, Uchida K. Rapid inhibition of MAPK signaling and anti-proliferation effect via JAK/STAT signaling by interferon-alpha in hepatocellular carcinoma cell lines. *Biochim Biophys Acta* 2005; 1745: 401–10.
63 Legrand A, Vadrot N, Lardeux B, Bringuier AF, Guillot R, Feldmann G. Study of the effects of interferon a on several human hepatoma cell lines: analysis of the signalling pathway of the cytokine and of its effects on apoptosis and cell proliferation. *Liver Int* 2004; 24: 149–60.
64 Wu WZ, Sun HC, Shen YF *et al.* Interferon alpha 2a down-regulates VEGF expression through PI3 kinase and MAP kinase signaling pathways. *J Cancer Res Clin Oncol* 2005; 131: 169–78.
65 Wada H, Nagano H, Yamamoto H *et al.* Combination therapy of interferon alpha and 5-fluorouracil inhibits tumor angiogenesis in human hepatocellular carcinoma cells by regulating vascular endothelial growth factor and angiopoietins. *Oncology Rep* 2007 (in press).
66 Yoshikawa H, Matsubara K, Qian GS *et al.* SOCS-1, a negative regulator of the JAK/STAT pathway, is silenced by methylation in human hepatocellular carcinoma and shows growth-suppression activity. *Nat Genet* 2001; 28: 29–35.
67 Ota H, Nagano H, Doki Y *et al.* Expression of type I interferon receptor as a predictor of clinical response to interferon-alpha therapy of gastrointestinal cancers. *Oncol Rep* 2006; 16: 249–55.
68 Damdinsuren B, Nagano H, Wada H *et al.* Interferon alpha receptors are important for antiproliferative effect of interferon alpha against human hepatocellular carcinoma cells. *Hepatol Res* 2007; 37 (1): 77–83.
69 Eguchi H, Nagano H, Yamamoto H *et al.* 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: 2881–90.
70 Moriyama M, Hoshida Y, Kato N *et al.* Genes associated with human hepatocellular carcinoma cell chemosensitivity to 5-fluorouracil plus interferon-alpha combination chemotherapy. *Int J Oncol* 2004; 25: 1279–87.
71 Kondo M, Nagano H, Wada H *et al.* Combination of IFN-alpha and 5-fluorouracil induces apoptosis through IFN-alpha/beta receptor in human hepatocellular carcinoma cells. *Clin Cancer Res* 2005; 11: 1277–86.
72 Schwartz EL, Baptiste N, Wadler S, Makower D. Thymidine phosphorylase mediates the sensitivity of human colon carcinoma cells to 5-fluorouracil. *J Biol Chem* 1995; 270: 19073–7.
73 Dou J, Iwashita Y, Sasaki A *et al.* Consensus interferon enhances the anti-proliferative effect of 5-fluorouracil on human hepatoma cells via downregulation of dihydropyrimidine dehydrogenase expression. *Liver Int* 2005; 25: 148–52.
74 Wadler S, Horowitz R, Mao X, Schwartz EL. Effect of interferon on 5-fluorouracil-induced perturbations in pools of

deoxynucleotide triphosphates and DNA strand breaks. *Cancer Chemother Pharmacol* 1996; 38: 529–35.
75 Braybrooke JP, Propper DJ, O'Byrne KJ *et al.* Induction of thymidine phosphorylase as a pharmacodynamic endpoint in patients with advanced carcinoma treated with 5-fluorouracil, folinic acid and interferon alpha. *Br J Cancer* 2000; 83: 219–24.
76 Nagano H, Miyamoto A, Wada H *et al.* Interferon-α and 5-fluorouracil combination therapy following palliative hepatic resection in patients with advanced hepatocellular carcinoma, portal venous tumor thrombus in the major trunk and multiple nodules. *Cancer* 2007 (in press).
77 Yamamoto T, Nagano H, Sakon M *et al.* Partial contribution of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) /TRAIL receptor pathway to antitumor effects of interferon-alpha/5-fluorouracil against Hepatocellular Carcinoma. *Clin Cancer Res* 2004; 10: 7884–95.
78 Nakamura M, Nagano H, Sakon M *et al.* Role of the Fas/FasL pathway in combination therapy with interferon-alpha and fluorouracil against hepatocellular carcinoma in vitro. *J Hepatol* 2007; 46: 77–88.
79 Takaoka A, Hayakawa S, Yanai H *et al.* Integration of interferon-alpha/beta signalling to p53 responses in tumour suppression and antiviral defence. *Nature* 2003; 424: 516–23.
80 Chang JC, Wooten EC, Tsimelzon A *et al.* Gene expression profiling for the prediction of therapeutic response to docetaxel in patients with breast cancer. *Lancet* 2003; 362: 362–9.
81 Kurokawa Y, Matoba R, Nagano H *et al.* Molecular prediction of response to 5-fluorouracil and interferon-alpha combination chemotherapy in advanced hepatocellular carcinoma. *Clin Cancer Res* 2004; 10: 6029–38.
82 Damdinsuren B, Nagano H, Wada H *et al.* Stronger growth-inhibitory effect of interferon (IFN)-beta compared to IFN-alpha is mediated by IFN signaling pathway in hepatocellular carcinoma cells. *Int J Oncol* 2007 Jan; 30 (1): 201–8.

Interferon-α and 5-Fluorouracil Combination Therapy After Palliative Hepatic Resection in Patients With Advanced Hepatocellular Carcinoma, Portal Venous Tumor Thrombus in the Major Trunk, and Multiple Nodules

Hiroaki Nagano, MD, PhD
Atsushi Miyamoto, MD
Hiroshi Wada, MD
Hideo Ota, MD
Shigeru Marubashi, MD
Yutaka Takeda, MD
Keizo Dono, MD
Koji Umeshita, MD
Masato Sakon, MD
Morito Monden, MD

Department of Surgery, Graduate School of Medicine, Osaka University, Osaka, Japan.

Supported by a Grant-in-Aid for Cancer Research from the Ministry of Culture and Science and the Ministry of Health and Welfare in Japan.

We thank Dr. Yasukazu Ohmoto from the First Institute of New Drug Research and Otsuka Pharmaceutical Company, Ltd. for providing antihuman interferon-α receptor 2 antibody (OCT4813).

Address for reprints: Hiroaki Nagano, MD, PhD, Department of Surgery, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka E-2, Suita 565-0871 Osaka, Japan; Fax: (011) 81-6-6879-3259; E-mail: hnagano@surg2.med.osaka-u.ac.jp

Received February 26, 2007; revision received July 3, 2007; accepted July 9, 2007.

BACKGROUND. The authors reported previously the beneficial effects of interferon (IFN)-α/5-fluorouracil (5-FU) combination therapy for patients with advanced hepatocellular carcinoma (HCC) who have tumor thrombi in the major portal branches. In this report, the authors describe the results from IFN/5-FU chemotherapy for patients who underwent palliative hepatic resection for advanced HCC with tumor thrombus in the main trunk of the portal vein and multiple nodules in the whole liver. In addition, they evaluated the correlation between the response to such therapy and expression of IFN-α type 2 receptor (IFNAR2).

METHODS. From October 1999 to December 2004, 30 patients with advanced HCC, tumor thrombi in the main trunk of the portal vein, and multiple nodules in the whole liver (Vp4 and grade 3 intrahepatic metastases) were recruited for this study. They underwent palliative hepatic resection followed by at least 2 courses of IFN/5-FU. IFNAR2 expression levels were determined by immunohistochemistry.

RESULTS. No major treatment-related complications were noted. An objective response was noted in 10 patients (33.3%) and included a complete response in 6 patients (20%), a partial response in 4 patients (13.3%), no response in 1 patient (3.3%), and progressive disease in 19 patients (63.4%). IFNAR2 expression was detected in 20 of 30 patients (66.7%). There was a significant difference in overall survival between patients with positive and negative IFNAR2 expression cases ($P < .0025$), and a significant correlation was observed between IFNAR2 expression and response to IFN/5-FU combination therapy ($P = .0199$).

CONCLUSIONS. Adjunct IFN/5-FU therapy is a promising modality for patients with advanced HCC, tumor thrombi in the major trunk, and multiple nodules after palliative hepatic resection. The results from this study indicated that the response to such therapy seemed to be correlated with IFNAR2 expression. *Cancer* **2007;110:2493–501.** © *2007 American Cancer Society.*

KEYWORDS: hepatocellular carcinoma, hepatic resection, interferon type 2 receptor, portal vein thrombosis, arterial infusion chemotherapy.

The prognosis for patients with advanced hepatocellular carcinoma (HCC) remains poor, particularly in patients who have tumor thrombi in the major trunk of the portal vein (Vp4).[1–3] The mortality rate is very high in patients with unresectable tumors, and their quality of life is poor because of intractable ascites or esophageal bleeding. Even in patients who have resectable HCC, the prognosis is extremely

DOI 10.1002/cncr.23033
Published online 16 October 2007 in Wiley InterScience (www.interscience.wiley.com).

poor despite aggressive surgery.[4,5] In such a situation, conventional therapies generally have no clinical effect on HCC associated with portal vein tumor thrombi (PVTT) because of poor efficacy and possible complications.[6,7] Arterial infusion chemotherapy also has been attempted, but its effectiveness still is unsatisfactory.[9,10] Therefore, a new strategy is required for patients who have intractable HCC and tumor thrombi in the major branch of the portal vein.

Several recent studies have indicated the beneficial effects of interferon (IFN)-α-based combination chemotherapies for HCC[10–15] despite the lack of satisfactory results from IFN-α monotherapy.[16] We also reported on the clinical efficiency of IFN-α and 5-fluorouracil (5-FU) combination therapy for advanced HCC with PVTT and intrahepatic metastasis.[17–20] IFN-α suppresses the proliferation of all type I IFN receptor type 2 (IFNAR2)-positive cancer cell lines in vitro, an effect that is mediated through its high affinity to IFNAR2. Studies from our laboratories demonstrated that IFNAR2 expression in HCC tissues may be a useful predictor of response to IFN/5-FU combination therapy.[19]

The current study was an extension to our previous work,[18–20] in which we examined the clinical effects of the combination therapy of subcutaneous IFN-α and arterial infusion of 5-FU after palliative hepatic resection in 30 patients who had HCC associated with Vp4 and multiple intrahepatic metastases (IM3). We also investigated the correlation between response to this therapy and expression of IFNAR2.

MATERIALS AND METHODS

Patients and Selection Criteria

The current investigation was a single-arm, open-label study that was based on our previous reports.[18–20] Between October 1999 and December 2004, 30 patients with advanced HCC were enrolled. All patients had radiologically confirmed tumor thrombi in the main trunk of the portal vein (Vp4) and IM3. The diagnosis was based on liver function tests, serum α-fetoprotein (AFP), serum protein induced by vitamin K absence or antagonist-II (PIVKA-II), and imaging techniques, which included computed tomography (CT) scans, magnetic resonance imaging (MRI), hepatic angiography, and arterial portography. Consequently, these 30 patients underwent palliative reduction surgery with tumor thrombectomy in the main trunk of the portal vein to reduce tumor volume and to reopen the portal blood flow. IFN-α and 5-FU combination therapy for remnant multiple hepatomas was carried out after surgery. We used the following eligibility criteria for the selection of patients. 1) absence of extrahepatic metastases, 2) granulocyte count > 2500/μL or < 12,000/μL, 3) erythrocyte count > 8.0 g/dL, 4) platelet count > 8×10^4/μL, 5) glutamic oxaloacetic and pyruvic transaminase levels < 100 IU/L, 6) total bilirubin < 1.4 g/dL, 7) blood urea nitrogen < 30 mg/dL, 8) serum creatinine < 1.5 mg/dL, 9) successful implantation of intra-arterial catheter and drug delivery system, and 10) an Eastern Cooperative Oncology Group (ECOG) performance status from 0 to 2.[21] These eligibility criteria were based on our previous studies.[18,19] All patients signed informed consent documents that were approved by the Institutional Review Board attesting that they were aware of the investigational nature of the study and were willing to try the combination therapy.

Treatment Protocol of IFN/5-FU Combination Therapy

In each of 30 patients, an intra-arterial catheter was inserted through the gastroduodenal artery during surgery or through the subclavian or femoral artery with a subcutaneously implanted drug-delivery system.[22] Each patient received subcutaneous IFN-α (OIF; Otsuka Pharmaceutical Company, Tokushima, Japan) and an intra-arterial infusion of 5-FU (Kyowa Hakko Company, Tokyo, Japan). One cycle of treatment consisted of 4 weeks. IFN-α (5×10^6 U [5 MU]) was administered subcutaneously on Days 1, 3, and 5 of each week, resulting in a total dose of 60 MU per cycle. Continuous infusion chemotherapy (5-FU, 300 mg/m^2 per day) through the proper hepatic artery was administered in the first and second weeks through a catheter connected to a subcutaneously implanted drug-delivery system. A 2- or 3-week rest period (cessation of drug therapy) separated the treatment cycles. All anticancer therapies were discontinued when adverse effects reached level 2 of the ECOG classification[21] (with the exception of platelet and leukocyte counts < 40,000/mm^3 and < 2000/mm^3, respectively, because these parameters often were low before treatment because of associated cirrhosis).[18]

Evaluation of Response to IFN/5-FU Combination Therapy

A pretreatment evaluation was conducted at the commencement of the IFN-α/5-FU protocol, and a posttreatment evaluation was conducted after the completion of 2 cycles of treatment, almost 3 months later. The evaluation included CT or MRI studies and an assessment of changes in serum tumor markers, such as AFP and PIVKA-II. All patients had their results compared at these 2 time points for the evaluation of antitumor effects. The objective response was classified according to ECOG criteria.[21] A complete response (CR) was defined as normalization of tumor marker

levels and disappearance of all tumors and portal vein thrombosis on CT and/or MRI studies. A partial response (PR) was defined as a decrease in tumor marker levels and a decrease between 50% and 99% in 2-dimensional tumor measurement. No change (NC) represented a decrease < 50% or an increase < 25% in tumor measurements, and progressive disease (PD) represented an increase > 25%. In addition, we evaluated progression-free and overall survival rates. Follow-up was from 15 to 75 months.

Reagents and Immunohistochemistry

Rabbit polyclonal antihuman IFNAR2 antibody (OCT4813; Otsuka Pharmaceutical Company) and its blocking peptide were prepared according to the report by Novick et al.[23] The expression of IFNAR2 was examined in all 30 resected tumor samples by immunohistochemistry, which was carried out according to the method described previously by investigators in our laboratories.[19,24–26] All slides were interpreted by 1 of 2 investigators (H.W. or H.O.) in a blinded manner without knowledge of the clinical or pathologic parameters.

Statistical Analysis

The Breslow-Gehan-Wilcoxon univariate test was used to examine the possible correlations between the effect of therapy (CR/PR vs NC/PD), Child-Pugh score, serum AFP, serum PIVKA-II, Okuda score, Cancer of the Liver Italian Program (CLIP) score,[3] and the expression of IFNAR2. Survival curves were constructed using the Kaplan-Meier method. Differences in distribution between groups were compared using the chi-square test, and differences in mean values were calculated with the Student t test. All data were expressed as the mean ± standard error of the mean (SEM). A P value < .05 denoted a statistically significant difference.

RESULTS

Patient Characteristics

The preoperative clinical characteristics of the participating patients are summarized in Table 1. The median age of patients was 56 years (range, 29–71 years). AFP and/or PIVKA-II expression levels were abnormal in all 30 patients. Preoperative liver function tests (mean ± SEM values) were as follows; serum albumin, 3.6 ± 0.4 g/dL; serum total bilirubin, 1.0 ± 0.1 mg/dL; prothrombin time, 71.7% ± 12.3%; hepaplastin test, 75.4% ± 16.2%; and indocyanine green retention rate at 15 minutes, 18.5% ± 11.0%.

Clinical Response to Combination Therapy

Table 2 summarizes the operative procedure, postoperative pathologic diagnosis, and clinical response to IFN/5-FU combination therapy. All patients completed at least ≥2 cycles of IFN/5-FU combination therapy after palliative surgery. For patients who had a clinical response, we continued this combination therapy; whereas, for patients who had no response, we stopped treatment after the completion of the second cycle because of extensive progression of HCC.

At the start of IFN/5-FU treatment, all 30 patients had multiple intrahepatic lesions in the residual liver after palliative resection. The average and median tumor size of the largest nodule were 16 mm and 15 mm, respectively (n = 30 patients; range, 10–32 mm), as detected on CT or MRI studies. With regard to the clinical effect, 10 patients (33.3%) had an objective response, 6 patients (20%) had a CR, 4 patients (13.3%) had a PR, 1 patient (3.3%) had NC, and 19 patients (63.3%) had PD. With respect to the time to disease progression, the median progression-free survival was 2 months, and the 1-, 2-, and 3-year progression-free survival rates were 20%, 16%, and 0%, respectively. Furthermore, the median overall survival was 9.5 months, and the 1-, 2-, and 3-year survival rates were 40%, 28.5% and 21.4%, respectively. The median progression-free survival for patients who had a response (CR/PR; n = 10 patients) was 17.5 months, and the median progression-free survival of patients who had NC/PD (n = 20 patients) was 2 months. The 1-, 2-, and 3-year progression-free survival rates of patients who had CR/PR were 60%, 48%, and 0%, respectively; and all 3 rates were 0% for the patients who had NC/PD. The tumor burden generally was very small because of the excision of the main tumor by reduction surgery. However, there was no correlation between antitumor effect and tumor size in the remnant liver.

The median survival was 29 months for patients who achieved a CR/PR (n = 10 patients) and 6 months for patients who had NC/PD (n = 20 patients). The median follow-up of the patients who survived was 32 months. The 1-, 2-, and 3-year survival rates for patients who achieved a CR/PR were 100%, 77.8%, and 58.3%, respectively; and the rates for patients who had NC/PD were 10%, 0%, and 0%, respectively. The time to progression and overall survival curves are shown in Figures 1 and 2, respectively. There were significant differences in the time to progression and overall survival between responders (CR/PR) and nonresponders (NC/PD; $P < .0001$).

Adverse Effects

None of our patients developed side effects related to catheter insertion or subcutaneous implantation of the drug-delivery system. Grade 1 leukopenia, thrombocytopenia, or myelosuppression was ob-

TABLE 1
The Demographics of the Patients in the Current Study (n = 30)*

Patient	Age	Sex	T	M	N	Vp	IM	Stage	Alb, g/dL	T.Bil, mg/dL	PT/HPT, %	ICGR-15, %	AFP, ng/mL	PIVKA-II mAU/mL	Virus
1	51	Woman	4	0	0	4	3	IVA	2.9	1.1	69/130	9	138	49,300	—
2	49	Woman	4	0	0	4	3	IVA	4.3	1.2	92/98	16	6741	< 40	B
3	47	Man	4	0	0	4	3	IVA	3.5	0.9	73/100	11	61820	256	B
4	56	Man	4	0	0	4	3	IVA	4.3	1.2	82/92	ND	< 5	4583	B/C
5	69	Man	4	0	0	4	3	IVA	3.5	1.1	68/70	17	59	209,220	B/C
6	66	Man	4	0	0	4	3	IVA	3.7	1.1	55/63	11	555	13,257	B/C
7	65	Man	4	0	0	4	3	IVA	3.4	0.8	75/69	ND	3612	71	C
8	53	Man	4	0	0	4	3	IVA	3.9	1.2	75/70	17	97,000	1110	B
9	52	Man	4	0	0	4	3	IVA	3.6	1.1	62/72	8	15,800	6496	B
10	66	Man	4	0	0	4	3	IVA	3.6	1.1	61/67	ND	1659	181,770	C
11	39	Man	4	0	0	4	3	IVA	3.7	1.3	51/48	14	15,100	37,274	B
12	56	Man	4	0	0	4	3	IVA	4.1	0.9	67/67	1	276	921	B/C
13	53	Man	4	0	0	4	3	IVA	3.7	1.1	80/70	14	450	8365	B
14	67	Man	4	0	0	4	3	IVA	3.9	0.9	84/89	27	3366	3602	C
15	47	Man	4	0	0	4	3	IVA	4.1	0.9	71/79	3	423,300	49,392	B
16	70	Man	4	0	0	4	3	IVA	3.5	1.1	88/82	25	20	5820	C
17	70	Man	4	0	0	4	3	IVA	3.5	1.2	103/83	17	108,990	239,409	B/C
18	70	Man	4	0	0	4	3	IVA	4.1	1.1	66/68	43	198	498	B/C
19	29	Man	4	0	0	4	3	IVA	3.7	0.8	59/62	11	471,000	357,528	B
20	53	Man	4	0	0	4	3	IVA	3.3	0.9	71/70	29	113,660	205,074	B
21	42	Man	4	0	0	4	3	IVA	3.5	0.9	67/60	30	268	3023	B
22	53	Man	4	0	0	4	3	IVA	3.8	1.2	75/78	13	1710	298,176	B
23	32	Man	4	0	0	4	3	IVA	4.1	1.2	75/62	23	1,280,000	48,636	B
24	53	Man	4	0	1	4	3	IVA	3.5	1.1	97/89	20	17920	3872	C
25	54	Man	4	0	0	4	3	IVA	4.2	0.9	65/66	40	20	99	B
26	51	Woman	4	0	1	4	3	IVA	3	0.9	54/76	32	209	497	—
27	65	Man	4	0	0	4	3	IVA	3.6	1.2	65/58	7	332	7346	B
28	67	Man	4	0	0	4	3	IVA	3.8	1.1	61/54	11	330	20,256	—
29	68	Man	4	0	0	4	3	IVA	3.7	1.1	67/74	14	786	85,974	B
30	71	Man	4	0	0	4	3	IVA	2.8	0.9	74/78	37	137,300	10,200	C

T indicates tumor classification; N, lymph node status; M, metastasis; Vp, grade of portal vein thrombus; IM, intrahepatic metastases; Alb, serum albumin; T.Bil, serum total bilirubin; PT, prothrombin time; HPT, hepaplastin test; ICGR-15, indocyanine green retention rate at 15 minutes; AFP, α-fetoprotein; PIVKA-II, protein induced by vitamin K absence; ND, not determined.
* TNM stage and the grade of portal vein thrombus were classified according to the 4th edition of the *General Rules for the Clinical and Pathological Study of Primary Liver Cancer* (Liver Cancer Study Group of Japan, 2000[1]).

served in 8 patients, but none of those episodes forced the termination of therapy or required treatment with granulocyte-colony stimulating factor. Other adverse effects, including stomatitis or diarrhea, were mostly grade 1 and clinically manageable in general. Fever was observed commonly but was controlled easily by nonsteroidal anti-inflammatory drugs before IFN injection. No depression because of IFN administration was observed in any of the 30 patients.

Correlation Between IFNAR2 Immunostaining Pattern and Prognosis

For each tissue section, the intensity of IFNAR2 immunostaining was scored on a scale from 0 to 2, in which 0 represented no or faint immunostaining, 1 represented moderate staining, and 2 indicated strong staining, based on our previous reports.[19,24,25] Table 2 shows the IFNAR2 expression level in each of the 30 patients. IFNAR2 expression was noted in 10 of 30 patients (33.3%). The median progression-free survival rate was 8 months for IFNAR2-positive patients and 2 months for IFNAR2-negative patients. The time to progression survival rates at 1, 2, and 3 years for IFNAR2-positive patients (n = 20 patients) were 30%, 24%, and 0%, respectively, and were significantly higher than the respective rates for IFNAR2-negative patients (n = 10 patients; 0% for all 3 rates; $P = .0038$) (Fig. 3A). The median overall survival rate was 16 months for IFNAR2-positive patients and 5.5 months for IFNAR2-negative patients. The overall survival rates at 1 year, 2 years, and 3 years among IFNAR2-positive patients (n = 20 patients; 60%, 42.8%, and 32.1%, respectively) were

TABLE 2
Operation, Postoperative Histology, Response to Interferon-α/5-Fluorouracil and Expression of Type I Interferon Receptor 2 in Patients With Hepatocellular Carcinoma

Patient	Operation	Histology		Response to IFN/5-FU	IFNAR2
		Cancer	Noncancer		
1	Extended left lobectomy	Ed III (poor)	Chr glisonitis	PR	1
2	Right lobectomy	Ed IV (undiff)	Normal	PD	1
3	Extended posterior segmentectomy	Ed III (poor)	B′−	PD	1
4	Extended posterior segmentectomy	Ed II (mod)	B′−	PR	1
5	Extended left lobectomy	Ed III (poor)	CIH	CR	1
6	Extended posterior segmentectomy	Ed III (poor)	B′+	CR	1
7	Left lobectomy	Ed III (poor)	B′+	PD	0
8	Right lobectomy	Ed III (poor)	B−	PD	1
9	Extended right lobectomy	Ed III (poor)	CIH	CR	1
10	Anterior segmentectomy	Ed III (poor)	B+	PD	1
11	Extended left lobectomy	Ed III (poor)	B′+	PD	1
12	Left lobectomy	Ed III (poor)	B−	PR	2
13	Left lobectomy	Ed IV (undiff)	B′−	PD	1
14	Right lobectomy	Ed III/IV (poor)	B+	PD	0
15	Extended left lobectomy	Ed III/IV (poor)	CIH	PD	0
16	Right lobectomy	Ed III/IV (poor)	CIH	CR	2
17	Right lobectomy	Ed III (poor)	CIH	CR	1
18	Right lobectomy	Ed III (poor)	CIH	PD	0
19	Extended right lobectomy	Ed III (poor)	CIH	PD	1
20	Right lobectomy	Ed III (poor)	B′−	PD	0
21	Right lobectomy	Ed III (poor)	CIH	PD	0
22	Left lobectomy	Ed III (poor)	CIH	CR	1
23	Right lobectomy	Ed III (poor)	CIH	PD	0
24	Right lobectomy	Ed III (poor)	B′−	PR	1
25	Extended left lobectomy	Ed III (poor)	B+	NC	1
26	Right lobectomy and pancreatoduodenectomy	Ed IV (undiff)	Normal	PD	0
27	Right lobectomy	Ed III (poor)	CIH	PD	0
28	Right lobectomy	Ed III/IV (poor)	B′−	PD	1
29	Right lobectomy	Ed III (poor)	CAH+	PD	1
30	Right lobectomy	Ed III (poor)	CAH+	PD	0

IFN/5-FU indicates interferon-α/5-fluorouracil; IFNAR2, type I interferon receptor 2; Ed, Edmondson grade; poor, poorly differentiated; Chr, chronic; PR, partial response; undiff, undifferentiated; PD, progressive disease; mod, moderately differentiated; CIH, chronic inactive hepatitis; CR, complete response; NC, no change; CAH, chronic active hepatitis.

significantly higher than the respective rates among IFNAR2-negative patients (n = 10 patients; 0% for all 3 rates; $P < .0025$) (Fig. 3B).

Clinical and Pathologic Correlations

Finally, we compared the responders (CR/PR; n = 10 patients) with the nonresponders (NC/PD; n = 20 patients) in terms of serum AFP (within normal range; < 5 ng/mL), serum PIVKA-II (normal range; < 45 mAU/mL), Child-Pugh scores, Okuda scores, CLIP scores, and IFNAR2 expression in univariate analysis (Table 3). Serum AFP, PIVKA-II, Child-Pugh scores, Okuda scores, and CLIP scores did not correlate with the response to combination therapy. Conversely, IFNAR2 expression correlated significantly with the response to IFN/5-FU combination therapy ($P = .0199$). Thus, the expression level of IFNAR2 was the sole factor that influenced the response to the combination therapy.

DISCUSSION

With regard to the patient selection criteria followed in the current study[27] and in our previous investigations,[18–20] we considered the presence of 3 types of advanced HCC with PVTT in the main trunk for the analysis of tumor progression (Fig. 3). The 3 types were defined as follows: type I, PVTT with multiple nodules in the bilateral lobes; type II, PVTT with a huge mass in 1 lobe and no intrahepatic metastatic nodules in the other lobe; and type III, PVTT with a huge mass in 1 lobe and multiple intrahepatic metastatic nodules in the other lobe. Patients with type I PVTT received IFN/5-FU combination treatment: An antitumor effect was noted in 43.7% of patients, and

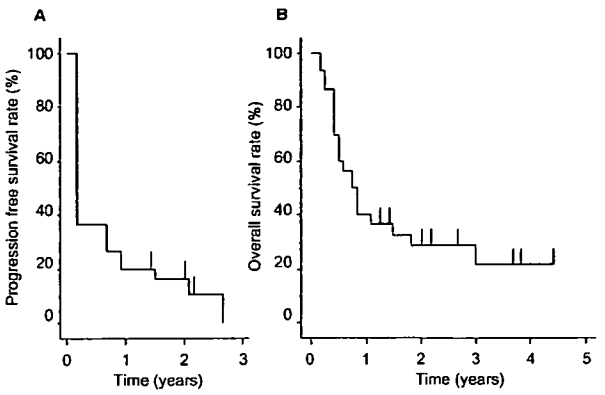

FIGURE 1. Kaplan-Meyer analysis of the efficiency of interferon-α/5-fluorouracil combination therapy. (A) The median progression-free survival was 2.0 months, and the 1-, 2-, and 3-year progression-free survival rates were 20%, 16%, and 0%, respectively. (B) The median overall survival was 9.5 months, and the 1-, 2-, and 3-year survival rates were 40%, 28.5%, and 21.4%, respectively.

a significant survival benefit was noted in 55 patients from our previous study.[19] Patients with type II PVTT underwent hepatic surgery to remove the huge mass followed by IFN/5-FU combination treatment as a postoperative adjunct. In this series, 100% survival rate at 1 year was achieved in 15 patients.[20] The patients in the current study had type III PVTT, which is considered the most advanced stage of HCC. In such patients, the main trunk of the portal vein already is packed with PVTT, and they have rapid worsening to liver failure because of the decrease in portal blood flow. These patients are prone to rupture of esophageal varices because of increased portal venous pressure. In general, most patients with advanced HCC can be treated only with best supportive care. However, for selected patients with type III PVTT who have liver function good enough to endure hepatic lobectomy, a multimodal treatment that includes surgery may be possible. Consequently, the 30 patients in the current

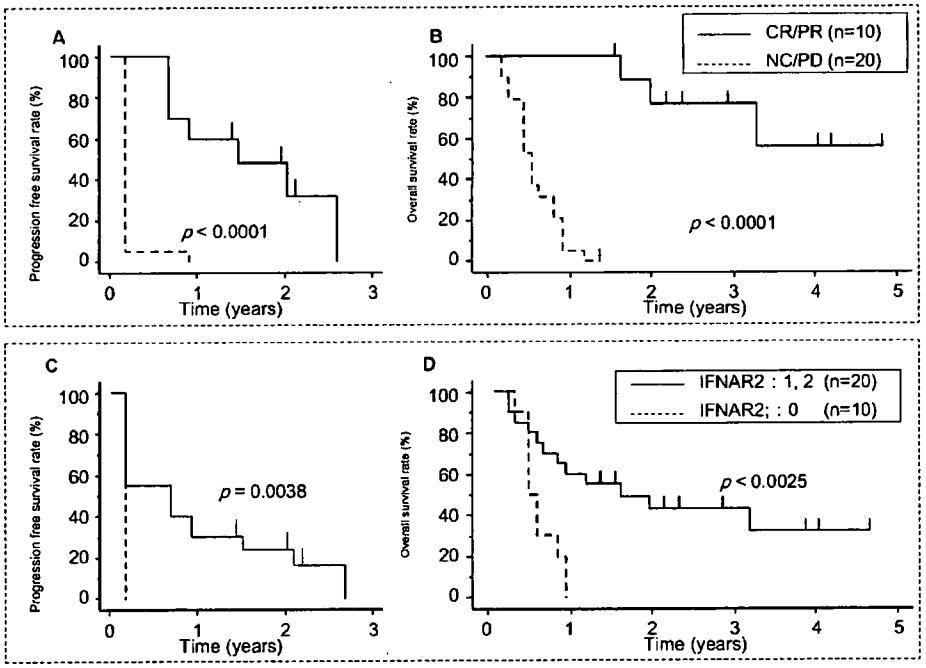

FIGURE 2. (A,B) Kaplan-Meyer analysis of the efficiency of interferon-α(IFN-α)/5-fluorouracil combination therapy. (A) The 1-, 2-, and 3-year progression-free survival rates of patients who attained a complete response/partial response (CR/PR) were 60%, 48%, and 0%, respectively; and all 3 rates were 0% for patients who had no change/progressive disease (NC/PD). (B) The 1-, 2-, and 3-year survival rates for patients who attained a CR/PR were 100%, 77.8%, and 58.3%, respectively; and the rates for patients who had NC/PD were 10%, 0%, and 0%, respectively. (C,D) Kaplan-Meyer analysis of the expression of IFN-α/type 2 interferon receptor (IFNAR2). (C) The 1-, 2-, and 3-year progression-free survival rates were 30%, 24%, and 0%, respectively, for IFNAR2-positive patients; and all 3 rates were 0% for IFNAR2-negative patients. There was a significant correlation between positive patients and negative patients ($P = .0038$). (D) The overall 1-, 2-, and 3-year survival rates for IFNAR2-positive patients (n = 20; 60%, 42.8%, and 32.1%, respectively) were significantly higher than the rates (0% for all 3 periods) for IFNAR2-negative patients (n = 10). There was a significant difference between positive patients and negative patients ($P = .0025$).