2010 Health and Labour Science Research Grants for Clinical Cancer Research

2010－Clinical Cancer－General－015

Study Protocol

Phase III Trial to Establish Combination Therapy with Hepatic Arterial Infusion Chemotherapy and a Molecularly Targeted Drug as a Novel Therapy for Advanced and Recurrent Hepatocellular Carcinoma and an Exploratory Study on a Biomarker Predicting its Efficacy

Randomized Controlled Trial Comparing Efficacy of Sorafenib Versus Sorafenib in Combination with Low-dose Cisplatin /Fluorouracil Hepatic Arterial InfUSion Chemotherapy in Patients with Advanced Hepatocellular Carcinoma and an Exploratory Study to Predict Efficacy of a Biomarker (SILIUS Phase III trial)

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ClinicalTrials.gov ID: NCT01214343

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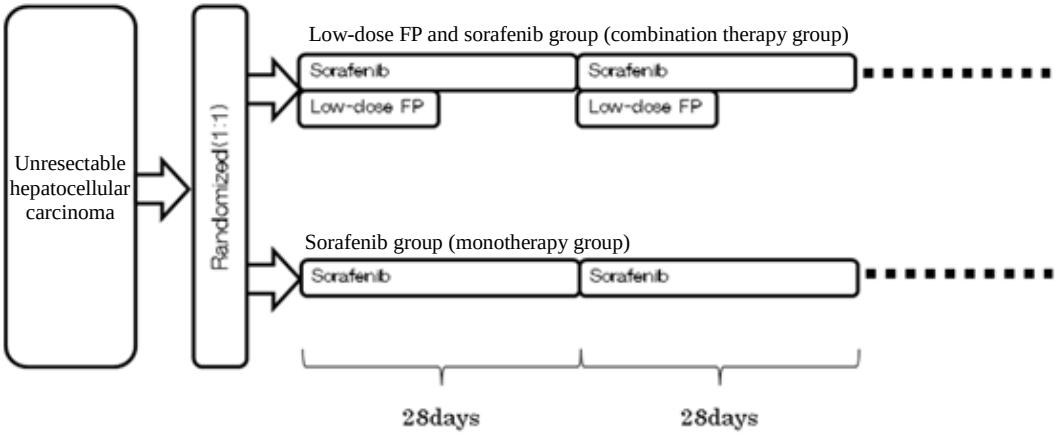
0. SYNOPSIS

0.1. Study Synopsis

A prospective, randomized, open-label, multicenter, parallel-group, phase III, comparative study to verify the superiority of combination therapy with sorafenib and low-dose FP hepatic arterial infusion chemotherapy compared with sorafenib monotherapy in patients with advanced hepatocellular carcinoma who are not candidates for surgical resection, local ablation therapy, or transcatheter arterial chemoembolization.

Figure 1. Schematic of the study design



0.2. Objective

To investigate the superiority of combination therapy with sorafenib and low-dose cisplatin/fluorouracil hepatic arterial infusion over the standard treatment of sorafenib monotherapy in terms of the primary endpoint of prolongation of overall survival (OS) in patients with advanced hepatocellular carcinoma who are not candidates for surgical resection, local ablation therapy, or transcatheter arterial chemoembolization.

0.3. Endpoints

0.3.1. Primary endpoint

The primary endpoint will be overall survival (OS).

0.3.2. Secondary Endpoints

Time to progression (TTP), progression-free survival (PFS), objective response rate (ORR), changes in tumor markers, and safety will be compared. OS will also be compared by tumor markers, Vp grade, and response rate. OS will be compared among responders and biomarkers that predict response will be explored as a supplementary study.

0.4. Subjects (main inclusion criteria)

1) At least 20 years of age (male or female)

2) Life expectancy of at least 12 weeks

3) Advanced hepatocellular carcinoma diagnosed as typical hepatocellular carcinoma histologically, cytologically, or by diagnostic imaging such as dynamic CT (MDCT), dynamic MRI, or CTHA/CTAP

*Patients who meet any of the following criteria are considered to have advanced hepatocellular carcinoma.

(1) Multiple hepatocellular carcinoma with at least 4 tumors

(2) Hepatocellular carcinoma with vascular invasion

(3) Hepatocellular carcinoma with extrahepatic spread that does not affect the prognosis

4) Not a candidate for complete resection by hepatectomy, local ablation therapy, or transcatheter arterial chemoembolization, or is unlikely to respond to these treatments

5) ECOG performance status (PS) of 0 or 1

6) Child-Pugh score of 7 or lower (PT calculated as INR)

7) Laboratory test values within prescribed ranges

8) Provided written informed consent to participate in the study before the start of the study

0.5. Treatments

0.5.1. Combination therapy with low-dose FP and sorafenib

Low-dose cisplatin will be administered on day 1, and fluorouracil will be administered by hepatic infusion using a reservoir for 5 days from day 1 through day 5, followed by 2 days off. This sequence will be repeated for 2 weeks, followed by 2 weeks off. Sorafenib will be administered continuously at a dose of 400 mg twice daily for 28 days from day 1 to day 28. This 4-week period constitutes one cycle, and cycles will be repeated until discontinuation of the protocol treatment. The first cycle of treatment will be started within 28 days of randomization. Earlier treatment with sorafenib will be allowed. However, patients must be off sorafenib for 2 days before and 7 days after reservoir placement. The next cycle of treatment will be started within 14 days. However, sorafenib will be continued during that time. This combination therapy will be repeated until progressive disease (PD) according to the modified RECIST criteria is documented [1]. (However, observation of a new intrahepatic lesion will not be considered PD.)

0.5.2. Sorafenib monotherapy

Sorafenib will be administered continuously at a dose of 400 mg twice daily for 28 days from day 1 to day 28. This 4-week period constitutes one cycle, and cycles will be repeated

until discontinuation of the protocol treatment. The first cycle of treatment will be started within 28 days of randomization. Treatment will be repeated until progressive disease (PD) is diagnosed by the modified RECIST criteria [1]. (However, observation of a new intrahepatic lesion will not be considered PD.)

0.6. Planned Enrollment and Study Period

Planned enrollment: 190 patients (95 in each group)
Enrollment period: 45 months (starting December 2010)
(to be completed in June 2014)
Overall study period: 57 months (to be completed in June 2015)

0.7. Contact Information

Correspondence regarding eligibility criteria and criteria for adjusting treatments, and inquiries requiring clinical decision-making: Study coordinator
Correspondence regarding enrollment procedures and case report forms: J-CRSU

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1. OBJECTIVE

To investigate the superiority of combination therapy with sorafenib and low-dose cisplatin/fluorouracil hepatic arterial infusion over the standard treatment of sorafenib monotherapy in terms of the primary endpoint of prolongation of overall survival (OS) in patients with advanced hepatocellular carcinoma who are not candidates for surgical resection, local ablation therapy, or transcatheter arterial chemoembolization.

Primary endpoint

Overall survival (OS)

Secondary endpoints

Time to progression (TTP)

Progression-free survival (PFS)

Objective response rate (ORR)

Changes in tumor markers

Safety

OS by tumor markers

OS by Vp

OS by response

Biomarkers that predict treatment efficacy will be explored as a supplementary study.

2. INTRODUCTION AND RATIONALE FOR STUDY DESIGN

2.1. Subjects

Hepatocellular carcinoma (HCC) is the fifth most prevalent cancer and the third leading cause of cancer-related death in the world [2]. In Europe and the United States, it is the primary cause of death in patients with cirrhosis of the liver [3-5]. In Japan, primary liver cancer is the fourth leading cause of cancer death, causing about 34,000 deaths each year. It is estimated that about 62,000 cases were newly diagnosed in 2002 [6].

Unlike in the United States and Europe, hepatocellular carcinoma in Japan is associated with chronic hepatitis caused by persistent infection with hepatitis C virus (HCV) or hepatitis B virus (HBV) and subsequent cirrhosis of the liver [7]. Specifically, it is believed that hepatocellular carcinoma develops as a result of inflammation, hepatocellular regeneration, liver matrix remodeling, fibrosis, and ultimately cirrhosis, which are chiefly caused by chronic liver damage. In addition, cirrhosis is the most important risk factor for hepatocellular carcinoma regardless of etiology [8].

A variety of treatments including surgical resection, local ablation therapy (e.g., percutaneous ethanol injection therapy and percutaneous radiofrequency ablation),

transcatheter arterial chemoembolization, chemotherapy, and liver transplantation are performed for hepatocellular carcinoma. Surgical resection and local ablation therapy are considered curative treatments for localized lesions, and transcatheter arterial chemoembolization has also produced good outcomes. However, many patients are not candidates due to various factors such as tumor extent and size or hepatic function. Treatments such as hepatic arterial infusion chemotherapy are performed for patients with more advanced disease. Hepatic arterial infusion chemotherapy has been investigated in monotherapy and in combination with effective anticancer drugs, but has not yet shown sufficient efficacy [9-11]. The subjects of this study are patients with hepatocellular carcinoma who are not candidates for surgical resection, local ablation therapy, or transcatheter arterial chemoembolization.

2.2. Standard Treatments for Advanced Hepatocellular Carcinoma

Patients with advanced hepatocellular carcinoma who are not candidates for surgical resection or local ablation therapy (e.g., percutaneous ethanol injection therapy and percutaneous radiofrequency ablation) are generally treated with hepatic arterial infusion chemotherapy or systemic chemotherapy. The evidence-based clinical practice guidelines for liver cancer recommend hepatic arterial infusion chemotherapy for multiple liver cancer with or without vascular invasion.

Hepatic arterial infusion chemotherapy, which involves direct infusion of anticancer drugs into the hepatic artery, is designed to enhance drug efficacy and reduce adverse reactions by increasing the local concentration of the drugs. Drugs used as monotherapy are cisplatin (response rate: 37% [12]) and epirubicin hydrochloride (response rate: 15% [13]). However, multidrug therapy designed to increase the efficacy of each drug is also widely practiced. Regimens used include cisplatin/fluorouracil (response rate: 14-44% [14-21]) and etoposide/cisplatin/fluorouracil (response rate: 46% [22]).

Sorafenib is currently considered the standard systemic chemotherapy for advanced hepatocellular carcinoma outside of Japan. Sorafenib is a multikinase inhibitor that inhibits various kinases including Raf kinases and vascular endothelial growth factor receptors. Specifically, it inhibits serine/threonine kinases in the Raf family as well as vascular endothelial growth factor receptors (VEGFR-2 and 3), platelet-derived growth factor receptor (PDGFR−β), and receptor tyrosine kinases such as Flt-3, kit, and Ret [23]. Sorafenib suppresses tumor proliferation and angiogenesis by inhibiting target molecules such as these. When the SHARP study demonstrated that sorafenib improved the overall survival rate in patients with unresectable hepatocellular carcinoma [24], sorafenib became the first oral drug approved to treat hepatocellular carcinoma. In the SHARP study, 602 patients with

hepatocellular carcinoma were randomized to either the placebo group or the sorafenib group with the primary objective of comparing overall survival between the sorafenib group and the placebo group. Median overall survival was 10.7 months in the sorafenib group and 7.9 months in the placebo group. Sorafenib significantly prolonged overall survival in patients with hepatocellular carcinoma by 44% compared with the placebo group. These data show that the sorafenib group had a significantly lower mortality risk with a hazard ratio of 0.69 ($p = 0.0006$). Based on the above data, Nexavar® (sorafenib) was approved by the European Medicines Agency (EMA) on October 30, 2007 and by the United States Food and Drug Administration (FDA) on November 19, 2007. As of October 2008, it is in use in 67 countries. In Japan, the indications were expanded to include unresectable hepatocellular carcinoma on May 20, 2009, and the drug is now considered the standard therapy for unresectable advanced hepatocellular carcinoma.

2.3 Rationale for Treatment Plans

As explained above, it would be desirable to develop new effective drugs and treatment methods for hepatocellular carcinoma due to the high prevalence of the disease, the absence of effective treatments, and need for curative treatment, prevention of recurrence, and improved overall survival.

In Japan, hepatic arterial infusion chemotherapy is selected for patients with advanced hepatocellular carcinoma who are not candidates for surgical resection, local ablation therapy, or transcatheter arterial chemoembolization. However, the disease commonly begins to progress again even after the treatment shrinks the tumor, and the cancer recurs or the tumor starts growing again. Thus, treatment is often repeated as long as liver function will allow. Because of these issues, it would be desirable to develop a more effective treatment for this disease. Various multidrug therapies have been attempted to that end. The efficacy of low-dose cisplatin/fluorouracil hepatic arterial infusion chemotherapy has been demonstrated in large groups of patients with advanced hepatocellular carcinoma. However, sorafenib is the only drug demonstrated to increase the overall survival rate in patients with incurable advanced hepatocellular carcinoma in a clinical trial, and is hoped to be effective in clinical practice. The combination of two different types of treatment, specifically, hepatic arterial infusion chemotherapy and sorafenib, is hoped to better suppress recurrence and regrowth of hepatocellular carcinoma, and determining whether it can truly do so would be highly clinically significant. Therefore, this study was designed to assess the additive effects of low-dose cisplatin/fluorouracil hepatic arterial infusion chemotherapy on the current standard therapy of sorafenib monotherapy and to establish this new therapy as the standard therapy for this patient population. If this study demonstrates the clinical effectiveness of the treatment, it will be the

first time that the clinical effectiveness of hepatic arterial infusion chemotherapy (low-dose FP) is objectively demonstrated in a prospective comparative study.

2.4. Drugs Used in the Study

2.4.1. Infusional cisplatin and fluorouracil

Many studies have demonstrated the efficacy of low-dose cisplatin/fluorouracil hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma [14-21]. Tanioka et al. performed 4 cycles, with each cycle consisting of 3 mg/m^2/0.5 h of cisplatin and 170 mg/m^2/24 h of fluorouracil administered by hepatic arterial infusion on days 1 through 5 followed by 2 days off [19]. Itamoto et al. performed 3 or 4 seven-day cycles, with each cycle consisting of 10 mg/1 h of cisplatin and 250 mg/24 h of fluorouracil administered by hepatic arterial infusion on days 1 through 5 followed by 2 days off [16]. Okuda et al. [18] and Lai et al. [17] performed 4 seven-day cycles, with each cycle consisting of 10 mg/1 h of cisplatin and 250 mg/5 h of fluorouracil administered by hepatic arterial infusion on days 1 through 5 followed by 2 days off. Ando et al. performed 4 cycles, with each cycle consisting of 7 mg/m^2/1 h of cisplatin and 170 mg/m^2/5 h of fluorouracil administered by hepatic arterial infusion for 5 days followed by 2 days off [14]. Reported adverse events were neutropenia, hepatotoxicity, and nausea/vomiting. Ando et al. also treated 8 patients who received the 5-day cisplatin/fluorouracil hepatic arterial infusion chemotherapy regimen (4 cycles) with an additional hepatic arterial infusion chemotherapy regimen (cisplatin, fluorouracil, cisplatin plus fluorouracil, or epirubicin, once a week or once every other week) and reported positive results.

2.4.2. Sorafenib

The tolerability, safety, and pharmacokinetic properties of sorafenib when administered at continuous twice-daily doses of 100 mg, 200 mg, 400 mg, and 600 mg have been demonstrated in Japanese patients with advanced solid tumors [25]. Adverse events observed at a repeated twice-daily dose of 400 mg or less were judged not clinically severe enough to necessitate dose reduction. At a twice-daily dose of 600 mg, however, skin toxicity (hand-foot skin reaction and rash/desquamation) was observed in all 6 patients who completed the first cycle. Severe signs and symptoms not observed at a twice-daily dose of 400 mg were also observed. These included palmar and plantar pain, chapping, and skin thickening. One patient also experienced grade 3 fatigue. These results were similar to those reported in the non-Japanese phase I study.

The safety profile in the Japanese phase I study in Japanese patients with hepatocellular carcinoma was also similar to the results of the phase I study in patients with advanced solid

tumors other than hepatocellular carcinoma. The most common adverse reactions were skin toxicity (hand-foot skin reaction and rash/desquamation), abnormal hematology results (lipase and amylase), and gastrointestinal toxicity (anorexia and diarrhea) [26]. The dose was reduced due to skin toxicity in 1 of the 12 patients receiving 400 mg twice daily, but all other adverse events were judged not clinically severe enough to necessitate dose reduction. Therefore, a repeated twice-daily dose of 400 mg was determined to be within the tolerable range for Japanese patients with hepatocellular carcinoma.

Sorafenib has also been investigated in combination therapy. A phase I study on combination therapy with sorafenib and doxorubicin hydrochloride was conducted outside Japan [27]. The regimen consisted of a maximum of six 3-week cycles, with 60 mg/m^2 of doxorubicin hydrochloride administered on the first day of each cycle. Sorafenib was administered continuously in twice-daily doses of 100 mg, 200 mg, or 400 mg starting on day 4 of the first cycle. Patients were taken off sorafenib from 48 h before administration of doxorubicin hydrochloride to 24 h after administration of doxorubicin hydrochloride in the second cycle. The main drug-related toxicities were neutropenia, hand-and-foot skin reaction, stomatitis, and diarrhea. The dose level did not reach the maximum tolerated dose, and thus the tolerability of continuous administration of 400 mg twice daily was confirmed.

Based on the above findings, continuous administration of 400 mg twice daily was determined to be an appropriate dosing regimen for sorafenib in this study in Japanese patients with hepatocellular carcinoma.

2.4.3. Combination therapy with sorafenib and low-dose FP

Combination therapy with sorafenib and low-dose FP was previously investigated in a Japanese phase I study (ClinicalTrials.gov Identifier: NCT00933816). Sorafenib was administered continuously at a twice-daily dose of 400 mg, and was combined with low-dose FP consisting of cisplatin and fluorouracil escalated in stages from 14 mg/m^2 to 20mg/m^2 and 170 mg/m^2 to 330 mg/m^2, respectively, and DLT was observed. At level 1 (sorafenib: 400 mg twice daily administered continuously, cisplatin: 14 mg/m^2 [day 1, day 8], fluorouracil: 170 mg/m^2 [days 1-5, days 9-12]), 2 of 6 patients showed grade 3 erythema multiforme (1 also had grade 4 thrombocytopenia). At level 2 (sorafenib: 400 mg twice daily administered continuously, cisplatin: 14 mg/m^2 [day 1, day 8], fluorouracil: 330 mg/m^2 [days 1-5, days 9-12]), 1 of 6 patients showed grade 3 erythema multiforme. At level 3 (sorafenib: 400 mg twice daily administered continuously, cisplatin: 20 mg/m^2 [day 1, day 8], fluorouracil: 330 mg/m^2 [days 1-5, days 9-12]), none of the 6 patients showed DLT. Therefore, level 3 was selected as the recommended dose. The adverse events observed were a combination of known adverse events of sorafenib and low-dose FP. No synergistic or unknown adverse

events were observed.

In the efficacy analysis, TTP was 9.7 months, longer than the 5.4 months for sorafenib monotherapy (in Kindai University patients) and 4.1 months for hepatic arterial infusion chemotherapy [21]. The treatment yielded a good antitumor effect, with 7 patients showing PR, 7 showing SD, 2 showing PD, and 2 showing NE. The response rate and disease control rate (DCR) were a favorable 38.9% and 77.8%, respectively. The results of the phase I study suggested this treatment has good efficacy, and thus the phase II efficacy study was skipped.

2.5 Study Treatment Regimens

2.5.1. Combination therapy with low-dose FP and sorafenib

The following doses demonstrated as safe in the phase I study of sorafenib and low-dose cisplatin/fluorouracil hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma will be used in this study. Cisplatin will be administered at a dose of 20 mg/m^2 on day 1, and fluorouracil will be administered at a dose of 330 mg/m^2/day by hepatic infusion using a reservoir for 5 days from day 1 through day 5, followed by 2 days off. This sequence will be repeated for 2 weeks, followed by 2 weeks off. Sorafenib will be administered continuously at a dose of 400 mg twice daily for 28 days from day 1 to day 28. This 4-week period constitutes one cycle, and cycles will be repeated until discontinuation of the protocol treatment.

2.5.2. Sorafenib monotherapy

Sorafenib will be administered continuously at a dose of 400 mg twice daily for 28 days from day 1 to day 28 (Fig. 1). This 4-week period constitutes one cycle, and cycles will be repeated until discontinuation of the protocol treatment.

2.6. Study Design

Prospective, randomized, open-label, multicenter, parallel-group, phase III, comparative study

2.6.1. Primary endpoint

The primary endpoint will be overall survival (OS).

2.6.2. Rationale

Sorafenib monotherapy is the standard therapy for unresectable hepatocellular carcinoma. The combination of sorafenib with low-dose FP was demonstrated as safe in a phase I/II study. The objective of this study is to assess response to treatment when low-dose FP is added to sorafenib by comparing overall survival. This study is a randomized controlled trial

with overall survival as the primary endpoint. Overall survival was selected as the primary endpoint because it is the most widely accepted direct and objective measure of clinical effectiveness.

2.6.3. Secondary endpoints

Time to progression (TTP), progression-free survival (PFS), objective response rate (ORR), changes in tumor markers, and safety will be compared. Biomarkers that predict treatment efficacy will be explored in a supplementary study.

2.6.4. Dose selection

The doses used in this study are based on doses used in a previously conducted phase I study of sorafenib and low-dose cisplatin/fluorouracil hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma (ClinicalTrials.gov Identifier: NCT00933816).

See Section 2.4.3. Combination therapy with sorafenib and low-dose FP.

2.6.5. Rationale for sample size

Planned number of patients: 190 patients (95 in each group)

The number of patients was determined based on the primary efficacy endpoint, OS.

If event incidence shows an exponential distribution, that median for sorafenib monotherapy OS is 10 months, and that adding hepatic arterial infusion chemotherapy improves median OS by 70% [28] (hazard ratio: 0.59 [combination therapy with HAIC versus sorafenib monotherapy]), then 112 events would be necessary to achieve a power of 80% for a one-sided α of 0.05 and 1:1 group assignment. The necessary number of events should be observed if 164 patients are followed over a study period of 36 months (enrollment period of 24 months and follow-up period of 12 months). Target enrollment was set at 190 patients (95 in each group) on the assumption that about 15% of patients will be unfollowable.

2.7. Summary of Potential Benefits and Risks of Participation in the Study

2.7.1. Potential benefits

Sorafenib, one of the drugs that will be used in this study, is covered by health insurance for the indication of unresectable hepatocellular carcinoma. Cisplatin and fluorouracil are also covered by health insurance for the indication of hepatocellular carcinoma, and therapy with any of these drugs is covered as part of routine care. All drug costs and other treatment expenses incurred by study participants during the study period will be paid by the patient’s health insurance or out of pocket. Participation in this study provides no additional financial or therapeutic benefits over routine care.

2.7.2. Potential risks

Regular monitoring of severity and incidence of toxicity will be performed in this study. If the risks are determined to exceed the expected level, protocol amendments including study termination will be considered. Unexpected adverse events will be reported through the safety information reporting system, reviewed by relevant parties, and reported to participating institutions as necessary. Study staff will make the utmost effort to minimize risks to patients. Criteria in Section 4. CRITERIA FOR PATIENT SELECTION, Section 6.3. Criteria for Treatment Adjustment, and Section 6.4. Concomitant Treatment and Supportive Care were established to minimize risk and discomfort from adverse events.

2.8 Significance of the Study

As explained above, it would be desirable to develop new effective drugs and treatment methods for hepatocellular carcinoma due to the high prevalence of the disease, the absence of effective treatments for advanced disease, and the need for curative treatment, prevention of recurrence, and improved survival time.

In Japan, hepatic arterial infusion chemotherapy is selected for patients with advanced hepatocellular carcinoma who are not candidates for surgical resection, local ablation therapy, or transcatheter arterial chemoembolization. However, although some studies have shown a response rate higher than 50%, these studies either had a small sample size or were uncontrolled. Therefore, no evidence-based recommendations for drug combinations currently exist. Prolongation of survival time has not been confirmed, either. Sorafenib yields only a low response rate in patients with incurable advanced hepatocellular carcinoma, but is the only drug demonstrated to increase the overall survival rate in this patient population in a clinical trial. These results indicate that combining sorafenib with low-dose cisplatin/fluorouracil hepatic arterial infusion chemotherapy should produce the additive effects of prolonging survival time compared with sorafenib alone and increasing the response rate to hepatic arterial infusion chemotherapy. This would be highly clinically significant, and this treatment could become the standard treatment in the near future. If this study demonstrates the additive effect of low-dose FP, it will be the first time that the clinical effectiveness of hepatic arterial infusion chemotherapy (low-dose FP) is objectively demonstrated in a prospective comparative study.

3. CRITERIA AND DEFINITIONS USED IN THIS STUDY

3.1. Diagnostic Criteria for Advanced Hepatocellular Carcinoma

Patients who meet any of the following criteria will be considered to have advanced hepatocellular carcinoma.

(1) Multiple hepatocellular carcinoma with at least 4 tumors

(2) Hepatocellular carcinoma with vascular invasion

(3) Hepatocellular carcinoma with extrahepatic spread that does not affect the prognosis

3.2. Tumor Assessment

Tumor response will be assessed using version 1.0 of the modified RECIST criteria (mRECIST) [1]. Assessments will be made based on changes in the diameter of surviving tumors deemed viable by a modality such as contrast CT that are observed until

completion or discontinuation of the protocol treatment.

At the time of enrollment for this study (before treatment), the 5 largest tumors among intrahepatic lesions with a large maximum diameter will be selected as target lesions. Lesions other than the target lesions will be included as non-target lesions, and the largest 5 of those will be selected and evaluated.

However, observation of a new intrahepatic lesion will not be considered PD in this study. Determinations of the best overall response will not require confirmation.

Table 2 Assessment of Target Lesion Response: Conventional RECIST and mRECIST Assessment for HCC Following the AASLD-JNCI Guideline

RECIST	mRECIST for HCC
CR = Disappearance of all target lesions	CR = Disappearance of any intratumoral arterial enhancement in all target lesions
PR = At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum of the diameters of target lesions	PR = At least a 30% decrease in the sum of diameters of viable (enhancement in the arterial phase) target lesions, taking as reference the baseline sum of the diameters of target lesions
SD = Any cases that do not qualify for either partial response or progressive disease	SD = Any cases that do not qualify for either partial response or progressive disease
PD = An increase of at least 20% in the sum of the diameters of target lesions, taking as reference the smallest sum of the diameters of target lesions recorded since treatment started	PD = An increase of at least 20% in the sum of the diameters of viable (enhancing) target lesions, taking as reference the smallest sum of the diameters of viable (enhancing) target lesions recorded since treatment started

AASLD, American Association for the Study of Liver Diseases; JNCI, Journal of the National Cancer Institute; HCC, hepatocellular carcinoma; mRECIST, modified Response Evaluation Criteria in Solid Tumors; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

Table 3 Overall Response Assessment in mRECIST: Responses for All Possible Combinations of Tumor Responses in Target and Nontarget Lesions with or without the Appearance of New Lesions

Target Lesions	Nontarget Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	IR/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or no	PD
Any	PD	Yes or no	PD
Any	Any	Yes	PD

mRECIST, modified Response Evaluation Criteria in Solid Tumors; CR, complete response; PR, partial response; IR, incomplete response; SD, stable disease; PD, progressive disease.

3.3. Child-Pugh Score

		Point score calculated from observations		
		1	2	3
Grade of encephalopathy [a]		Absent	1-2	3-4
Ascites		Absent	Slight	Moderate
Serum bilirubin	(mg/dL)	< 2	2-3	> 3.0
	(mcmol/L)	< 34	34-50	> 50
Serum albumin (g/dL)		> 3.5	2.8-3.5	< 2.8
INR		< 1.7	1.7-2.3	> 2.3
Prothrombin time (sec above normal)		< 4	4-6	> 6
Increase in prothrombin time (%)		> 70	40-70	< 40

a Grade of encephalopathy:

Grade 0: Lucid, normal personality, normal neurological test results, normal electroencephalogram

Grade 1: Restlessness, sleep disorder, irritability/agitation, tremors, dysgraphia, 5 cps waves

Grade 2: Lethargy, disorientation (temporal), inappropriateness, difficulty maintaining stable posture, ataxia, slow triphasic waves

Grade 3: Somnolence, confusional state, disorientation (spatial), hyperreflexia, rigidity, slow waves

Grade 4: Coma, no personality/unresponsive, cessation of cerebral activity, slow 2–3 cps delta activity

A: 5–6 points, B: 7–9 points, C: 10–15 points

*Ascites: Patients with a history of ascites who are currently using a diuretic are allotted 2 points even if they do not have ascites.

*Encephalopathy: Patients with a history of hepatic encephalopathy who are using a drug to treat encephalopathy are allotted 2 points even if they are asymptomatic.

3.4. Eastern Cooperative Oncology Group Performance Status (PS)

Grade	Definition
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light house work or office work).
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.

3.5. Staging by the General Rules for the Clinical and Pathological Study of Primary Liver Cancer

The overall stage of a cancer will be determined by calculating the stages for each component and selecting the highest one.

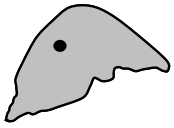
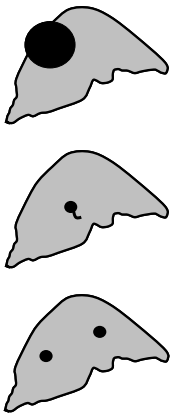
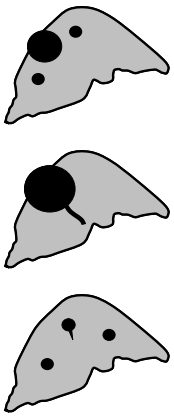
The overall stage will be determined from the following four components.

Stages of hepatocellular carcinoma

	T component	N component	M component
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
Stage IVA	T4	N0	M0
	T1, T2, T3, T4	N1	M0
Stage IVB	T1, T2, T3, T4	N0, N1	M1

T component: Determined from three factors: the number, size, and vascular invasion of tumors. Multiple tumors can be multicentric tumors or intrahepatic metastases. An S3 ruptured hepatocellular carcinoma is classified as T4.

T component of hepatocellular carcinoma

	T1	T2	T3	T4
(1) Number of tumors: Single (2) Tumor diameter: ≤2 cm (3) No vascular invasion (Vp0, Vv0, B0)	Meets all (1) (2) (3) 	Meets 2 	Meets 1 	Meets none 

N component:

N0： No lymph node metastasis

N1： Lymph node metastasis

M component:

M0： No extrahepatic spread

M1： Extrahepatic spread

4. CRITERIA FOR PATIENT SELECTION

This study will enroll patients with hepatocellular carcinoma who are not candidates for surgical resection, local ablation therapy, or transcatheter arterial chemoembolization. Patients must sign the informed consent form, meet all the inclusion criteria, and not meet any of the exclusion criteria. Before patient enrollment, investigators will collect all information about prescription of the study drugs and will familiarize themselves with safety information in the package inserts.

4.1. Inclusion Criteria

Patients who meet all of the following criteria in screening tests and observations within 28 days before enrollment will be included in the study.

1) At least 20 years of age (male or female)

2) Life expectancy of at least 12 weeks

3) Advanced hepatocellular carcinoma diagnosed as typical hepatocellular carcinoma histologically, cytologically, or by diagnostic imaging such as dynamic CT (MDCT), dynamic MRI, or CTHA/CTAP

*Patients who meet any of the following criteria are considered to have advanced hepatocellular carcinoma.

(1) Multiple hepatocellular carcinoma with at least 4 tumors

(2) Hepatocellular carcinoma with vascular invasion

(3) Hepatocellular carcinoma with extrahepatic spread that does not affect the prognosis

4) Not a candidate for complete resection by hepatectomy, local ablation therapy, or transcatheter arterial chemoembolization, or is unlikely to respond to those treatments

5) ECOG performance status (PS) of 0 or 1

6) Child-Pugh score of 7 or lower (PT calculated as INR)

7) Laboratory test values are within the prescribed ranges below. Patients can be re-tested during the screening period within 28 days before enrollment.

a) Hemoglobin ≥ 8.5 g/dL

b) Neutrophil count ≥ 1,500/μL

c) Platelet count ≥ 50,000/μL

d) PT-INR ≤ 2.3

e) Total bilirubin ≤ 2 mg/dL

f) ALT and AST ≤ 6 times the institutional upper limit of normal

g) Serum creatinine ≤ 1.5 times the institutional upper limit of normal

h) Amylase ≤ 2 times the institutional upper limit of normal

8) Provided written informed consent to participate in the study

4.2. Exclusion Criteria

Patients who meet any of the following criteria in screening tests and observations within 28 days before enrollment will be excluded from the study.

1) Another previous or current malignancy, with the following exceptions:

a) Curatively treated intraepithelial cervical cancer, basal cell carcinoma, superficial bladder cancer (Ta, Tis, or T1), early gastric cancer, or other early cancers with low risk of recurrence after curative therapy

b) Disease-free for more for than 3 years after curative therapy at the time of enrollment

2) Renal failure requiring hemodialysis or peritoneal dialysis

3) Any of the following heart diseases:

a) Congestive heart failure classified as NYHA (appendix 1) class III or IV

b) Active coronary artery disease or ischemic heart disease such as cardiac infarction within 6 months prior to screening

c) Serious cardiac arrhythmia (≥ grade 3 according to the Japanese translation of CTCAE v4.0: arrhythmia for which nonsurgical emergency treatment is indicated)

4) Poorly controlled hypertension

5) Active clinically serious infections (≥ grade 3 according to the Japanese translation of CTCAE v4.0) other than HBV and HCV

6) Active chicken pox

7) Hearing impairment

8) History of HIV infection

9) Known meningeal tumor (including meningeal metastasis)

10) Extensive extrahepatic tumor spread (except that which does not affect the prognosis)

11) History of seizure disorder requiring treatment (may have a seizure)

12) Clinically significant gastrointestinal bleeding within 4 weeks prior to study enrollment

13) Thrombosis or embolization (e.g., transient ischemic disease, deep vein thrombosis, or pulmonary embolization) within 6 months prior to study enrollment, except portal vein thrombosis

14) Any history of the following treatments:

a) Currently using a CYP3A4 inhibitor (e.g., rifampicin)

b) Invasive surgical procedure (e.g., thoracotomy or laparotomy) within 4 weeks

prior to starting the study treatment

c) History of organ allograft

15) Unable to swallow oral medications

16) Gastrointestinal disease that may affect drug absorption or pharmacokinetics

17) Using medication that may affect drug absorption or pharmacokinetics

18) Disease or disorder that may affect metabolism, and thus evaluation of the study drugs

19) Enrolled in another clinical study within 4 weeks prior to study enrollment

20) Currently pregnant, may be pregnant, plans to become pregnant, or is breastfeeding

21) Allergy to any of the study drugs

22) Substance abuse or a medical, psychological, or social condition that is likely to interfere with the patient's participation in the study or in terms of evaluation of study results, in the investigator’s judgment

23) Any other condition that is unstable or could jeopardize the safety of the patient or their compliance with the protocol, in the investigator's judgment

5. ENROLLMENT

5.1. Enrollment and Randomization Procedures

After confirmation that a prospective participant meets all of the inclusion criteria and none of the exclusion criteria, the required sections of the Enrollment Eligibility Form will be completed and the form faxed to the data center.

Contact information and hours for patient enrollment office

Data Center Japan Clinical Research Support Unit (J-CRSU)

Yushima D&A Building 1F, 1-10-5 Yushima, Bunkyo-ku, Tokyo 113-0034, Japan

FAX: 03-5298-8536

TEL: 03-5297-7771

Hours: Weekdays from 10:00 AM to 5:00 PM (excluding holidays and New Year’s holiday period)

Direct correspondence about criteria for patient selection to:

Study coordinator Kazuomi Ueshima

Department of Gastroenterology and Hepatology, Kindai University Faculty of Medicine

TEL: 072-366-0221
FAX: 072-367-2880
e-mail: kaz-ues@med.kindai.ac.jp

5.2. Notes Regarding Enrollment

1) No patients will be enrolled after the start of the protocol treatment, with no exceptions

2) Patients will be enrolled by faxing the Enrollment Eligibility Form to the data center

3) If the data center is unable to fully review information on the Enrollment Eligibility Form, the patient will not be enrolled until they are confirmed to meet all requirements

4) Once the data center confirms eligibility, patients will be issued an enrollment number and informed of their assigned treatment

5) The data center will fax to the institution a Confirmation of Enrollment and Notification of Assigned Treatment form with the patient's enrollment number

6) Enrollment will be concluded with the sending of this form. The Confirmation of Enrollment and Notification of Assigned Treatment forms shall be retained by each institution.

7) Except for patients who withdraw their consent (including patients who decline use of their information for research purposes), patients who have been enrolled cannot be unenrolled (i.e., removed from the database). If the same patient is enrolled multiple times, the information from the first enrollment (enrollment number and treatment group) will always be used.

8) Erroneous enrollments and multiple enrollments should be reported to the data center soon after they are noticed

5.3. Randomization and Stratification Factors

Patients will be randomized into the low-dose FP plus sorafenib group (combination therapy) or the sorafenib group (monotherapy) in a 1:1 ratio. Group assignments will be made using a computer program.

The following items will be used as stratification factors for randomization with the minimization method.

(1) Institution

(2) Macroscopic vascular invasion (MVI) (Vp0, Vp1-3, Vp4)

(3) Presence/absence of extrahepatic spread (EHS)

6. TREATMENT PLANS AND CRITERIA FOR ADJUSTING TREATMENT

6.1. Protocol Treatments

6.1.1. Low-dose FP and sorafenib group (combination therapy group) (Table 1)

This study will use the following doses demonstrated as safe in a phase I/II study of sorafenib and low-dose cisplatin/fluorouracil hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma. Cisplatin will be administered at a dose of 20 mg/m^2 on day 1 (within 30 days), and fluorouracil will be administered at a dose of 330 mg/m^2/day by hepatic infusion using a reservoir for 5 days from day 1 through day 5 (continuous infusion over 24 h), followed by 2 days off. This sequence will be repeated for 2 weeks, followed by 2 weeks off. Sorafenib will be administered continuously at a dose of 400 mg twice daily for 28 days from day 1 to day 28. This 4-week period constitutes one cycle.

The first cycle of treatment will be started within 28 days of randomization. Treatment with sorafenib before hepatic arterial infusion chemotherapy will be allowed. However, patients must be off sorafenib for 2 days before and 7 days after reservoir placement. The next cycle of treatment will be started within 14 days. Sorafenib will be continued until the start of the next cycle. This combination therapy will be continued until discontinuation of the protocol treatment.

If an adverse event is observed, treatment will be interrupted or the dose will be reduced as appropriate in accordance with **6.3.2. Criteria for adjusting the sorafenib dose (dose interruption and reduction)** and **Section 6.3.3. Criteria for Hepatic Arterial Infusion Chemotherapy (Low-dose FP) Dose Adjustment (Dose Interruption and Reduction)**. After the protocol treatment is discontinued, appropriate treatment as described in **Section 6.5 Post-trial Treatment** will be instituted.

The arterial catheter should be placed in a manner that allows for proper drug distribution throughout the liver, and the flow should always be checked by contrast CT through the port or by DSA before starting treatment.

6.1.2. Sorafenib group (monotherapy group) (Table 2)

Sorafenib will be administered continuously at a dose of 400 mg twice daily for 28 days from day 1 to day 28. This 4-week period constitutes one cycle, and cycles will be repeated until discontinuation of the protocol treatment.

The first cycle of treatment will be started within 28 days of randomization. If an adverse event is observed during treatment with sorafenib, treatment will be interrupted or the dose will be reduced as appropriate in accordance with **6.3.2. Criteria for adjusting the sorafenib dose (dose interruption and reduction)** After the protocol treatment is discontinued, appropriate treatment as described in **Section 6.5 Post-trial Treatment** will be performed.

Table 1 Treatment schedule (low-dose FP plus sorafenib combination therapy group)

*Patients must be off sorafenib for 2 days before and 7 days after reservoir placement.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Sorafenib	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
CDDP	↓							↓						
5-FU	←――――――→							←――――――→						

	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Sorafenib	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
CDDP														
5-FU														

Table 2 Treatment schedule (sorafenib monotherapy group)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
sorafenib	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓

	15	16	17	18	19	20	21	22	23	24	25	26	27	28
sorafenib	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓

6.2. Criteria for Discontinuation of the Protocol Treatment

In general, in situations such as the following, the protocol treatment will be discontinued, a note to that effect will promptly be written on the patient's medical records, and a Treatment Completion Report will be faxed to the data center.*

1) Progressive disease

a) Progressive disease (PD) as defined by the modified RECIST criteria is confirmed by imaging**

b) Worsening of general condition indicates a lack of clinical response to treatment

2) Patient cannot continue the study due to an adverse event

a) Patient cannot resume sorafenib after 8 weeks of interruption due to an

adverse event

b) Patient cannot start the next cycle of treatment within 8 weeks after completing the previous cycle due to an adverse event

c) An adverse event that meets the criteria for dose reduction occurs after the dose was already reduced to the lowest level

(Reducing the dose to below the lowest level in the dose reduction criteria stipulated in the protocol will be considered discontinuation of the protocol treatment, and all treatment after that point will be considered post-trial treatment.)

3) Hepatic arterial infusion chemotherapy becomes technically infeasible

4) Patient requests to discontinue the study

5) Patient can no longer make continuous visits due to moving or changing doctors

6) Death

7) An investigator determines that discontinuation is necessary for any other reason

8) The patient shows a CR

***Notes regarding discontinuation of the protocol treatment**

The protocol treatment should be continued for as long as possible by reducing doses and interrupting treatment as appropriate. If discontinuation is being considered after a dose reduction or during treatment interruption, the study coordinator or study chair should be promptly notified using the designated fax form. Whether to discontinue the protocol treatment will be determined upon discussion with the study chair. It is particularly important to promptly consult the study chair when considering discontinuation for a patient who does not meet the above criteria for discontinuation of the protocol treatment. However, it is acceptable to not follow this procedure if emergency discontinuation is required for safety reasons.

Contact information:

Study chair: Masatoshi Kudo, Department of Gastroenterology and Hepatology, Kindai University Faculty of Medicine

TEL：072-366-0221（Ext3149）

FAX：072-367-2880

E-mail：m-kudo@med.kindai.ac.jp

Study coordinator: Kazuomi Ueshima, Department of Gastroenterology and Hepatology, Kindai University Faculty of Medicine

TEL：072-366-0221（Ext3525）

FAX：072-367-2880

E-mail：kaz-ues@med.kindai.ac.jp

**The protocol treatment should generally be discontinued if the patient shows PD according to the modified RECIST criteria during the protocol treatment period. However, continuation of the protocol treatment is allowed if the investigator determines that the patient is responding clinically to the protocol treatment. It should be noted that observation of a new hepatic lesion is not considered PD in this study, and protocol treatment should be continued in these patients.

***The protocol treatment should be discontinued if the patient shows a CR according to the modified RECIST criteria. If recurrence is detected, appropriate treatment will be provided at that time. Treatment during this time will follow Section 6.5. Post-trial Treatment.

6.3. Criteria for Treatment Adjustment

6.3.1. Criteria for starting the next cycle (for both groups)

The next cycle will be started if the investigator confirms the following criteria are met within 1 week before the scheduled start date. If any of these criteria are not met, the interval to the next cycle will be extended and the next cycle will be started as soon as the criteria are met. The next cycle cannot be started if the patient is off sorafenib. The start date of the next cycle can be extended for up to 8 weeks from the end of the previous cycle. If the criteria for starting the next cycle are still not met after that period, the protocol treatment will be discontinued.

1) Neutrophil count ≥ 1,000/μL
2) Platelet count ≥ 50,000/μL
3) Total bilirubin ≤ 2 mg/dL
4) ALT and AST ≤ 6 times the institutional upper limit of normal
5) Serum creatinine ≤ 1.5 times the institutional upper limit of normal
6) Amylase ≤ 2 times the institutional upper limit of normal

6.3.2. Criteria for adjusting the sorafenib dose (dose interruption and reduction)

The dose of sorafenib will be adjusted (by interruption or reduction) in the event of clinically significant hematological or non-hematological toxicity attributed to sorafenib. When grading events according to the Japanese translation of the CTCAE v4.0, the grade whose definition most closely matches the event will be assigned. When grading events, investigators will consider not only the fact that the patient received a certain treatment, but also whether that treatment was indicated for the patient's condition. However, they will also consider whether it is possible to continue treatment with sorafenib by increasing the frequency of tests. The following criteria will be followed for each dose reduction level.

Table 3 Dose reduction method

Standard dose	Dose level 1	Dose level 2	Dose level 3
400 mg twice daily	400 mg once daily	400 mg every other day	200 mg every other day
Two 200 mg tablets of sorafenib per dose Twice daily (morning and evening)	Two 200 mg tablets of sorafenib per dose Once daily (morning)	Two 200 mg tablets of sorafenib per dose Once every other day (morning)	One 200 mg tablet of sorafenib per dose Once every other day (morning)

When an adverse event occurs, the dose will be reduced as outlined above. When the adverse event resolves, treatment will be resumed whenever possible, but generally at a reduced dose. After that, the dose may be increased up to 400 mg twice daily at the discretion of the investigator or subinvestigator. When the dose is increased, it will be increased by one level at a time. The protocol treatment will be discontinued if the dose is reduced below level 3.

Table 4 Criteria for adjusting the sorafenib dose for hypertension

Grade	Feasibility of continuing treatment	Dose adjustment
Grade 1	Consider increasing frequency of blood pressure measurements	No change
Grade 2 asymptomatic increase in diastolic blood pressure to < 100 mmHg	Start treatment with an antihypertensive. Continue treatment with sorafenib. If previously untreated, start treatment with one antihypertensive. If patient is already using an antihypertensive, increase the dose of the antihypertensive they are using.	No change
Grade 2 increase that is symptomatic/persistent, Grade 2 increase in diastolic blood pressure to ≥ 100 mmHg, or grade 3/4 increase in blood pressure	1. Interrupt treatment with sorafenib and start or add an antihypertensive. Restart sorafenib at a reduced dose if symptoms resolve and diastolic blood pressure decreases to < 100 mmHg. 2. If diastolic blood pressure cannot be reduced to below 100 mmHg after sorafenib is resumed at a reduced dose, even with the use of antihypertensives, further reduce the dose of sorafenib.	Reduce dose by one level– Interrupt treatment

*: Standard antihypertensives used in clinical studies are calcium channel blockers, angiotensin II receptor antagonists, and beta-blockers.

Table 5 Criteria for adjusting the sorafenib dose for skin toxicity

Grade		Whether treatment can be continued	Dose adjustment
1		Continue treatment	No change
2	1st occurrence	Interrupt treatment until toxicity resolves to grade 1	No change
	2nd occurrence	Interrupt treatment until toxicity resolves to grade 1	Reduce dose by one level
	3rd occurrence	Interrupt treatment until toxicity resolves to grade 1	Reduce dose by one level
	4th occurrence	Interrupt treatment until toxicity resolves to grade 1	No change
	5th occurrence onward	Interrupt treatment until toxicity resolves to grade 1	Reduce dose by one level
3	1st occurrence	Interrupt treatment until toxicity resolves to grade 1	Reduce dose by one level
	2nd occurrence	Interrupt treatment until toxicity resolves to grade 1	Reduce dose by one level
	3rd occurrence	Interrupt treatment until toxicity resolves to grade 1	No change
	4th occurrence onward	Interrupt treatment until toxicity resolves to grade 1	Reduce dose by one level

Table 6 Grades for hand-and-foot skin reaction

Grade 1	Dysesthesia, numbness, tingling, painless swelling, or erythema of the hands or feet, or any other uncomfortable skin condition that does not disrupt the patient's normal activities
Grade 2	Painful erythema or swelling of the hands or feet, or any other uncomfortable skin condition that affects the patient's normal activities
Grade 3	Moist desquamation, ulceration, blistering, or severe pain of the hands or feet, or severe discomfort causing inability to work or perform activities of daily living

Table 7 Criteria for adjusting the sorafenib dose for elevated blood pancreatic enzymes

Grade	Whether treatment can be continued	Dose adjustment
0-2	Continue treatment	No change
3, 4	Carefully monitor the patient by blood tests (including amylase, lipase, and CRP), physical examination, and CT or abdominal ultrasound until toxicity	No change

resolves to grade 2.		
Grade 4 elevated pancreatic enzymes lasting 14 days or longer, grade 4 elevated pancreatic enzymes accompanied by pancreatitis diagnosed clinically or by imaging, or life-threatening or disabling grade 4 elevated pancreatic enzymes	Discontinue the protocol treatment	—

Table 8 Criteria for adjusting the sorafenib dose for hematological toxicity

Grade	Whether treatment can be continued	Dose adjustment
0-2	Continue treatment	No change
3	Continue treatment	Reduce dose by one level
4	Interrupt treatment until toxicity resolves to grade 2	Reduce dose by one level

Table 9 Criteria for adjusting the sorafenib dose for hematological and non-hematological toxicities (other than elevated blood pancreatic enzymes, hypertension, and skin toxicity)

Grade	Whether treatment can be continued	Dose adjustment
1, 2	Continue treatment	No change
3*, 4	1. Temporarily interrupt treatment with sorafenib. Resume treatment at one level lower than the initial dose if toxicity resolves to grade 1 or lower or to baseline within 4 weeks. 2. If treatment cannot be resumed after being delayed or suspended for more than 8 weeks, discontinue the protocol treatment. 3. Consider increasing the dose if no adverse reactions of grade 2 or higher occur for at least 2 weeks after resuming treatment at the reduced dose.	Temporarily interrupt treatment, reduce dose by one level

***For AST and ALT, "6 times the institutional upper limit of normal" is to be used as the criterion instead of "grade 3 or higher".**

6.3.3. Criteria for hepatic arterial infusion chemotherapy (low-dose FP) dose adjustment (dose interruption and reduction)

If clinically significant hematological or non-hematological toxicity attributed to hepatic arterial infusion chemotherapy (low-dose FP) occurs, infusions alone will be interrupted. Sorafenib will be continued. If the event occurs in the middle of a cycle, the infusions for that cycle will be skipped and infusions will not be resumed until the start date of the next cycle. Resumption of infusions at

the next cycle will be decided using criteria in **Section 6.3.1. Criteria for Starting the Next Cycle**.

Treatment may be resumed at the same dose or a lower dose.

Dose reduction levels

Level 1: Fluorouracil 170 mg/m^2, cisplatin 20 mg/m^2

Level 2: Fluorouracil 170 mg/m^2, cisplatin 10 mg/m^2

(It is also acceptable to interrupt infusions of cisplatin alone without reducing the fluorouracil dose if the adverse event is caused by renal dysfunction)

6.4. Concomitant Treatment and Supportive Care

All concomitant medications will be documented in medical records. (Including start dates, end dates, and indications.)

6.4.1. Permitted concomitant treatments and supportive care

The following concomitant treatments and supportive care may be provided if necessary.

1) Patients may receive non-targeted therapy for the primary disease (e.g., *kampo* medicine and acupuncture) or eat foods fortified with vitamins/minerals if the investigator or subinvestigator determines the treatment or food will not interfere with or influence evaluation of study results.

2) Palliative care or supportive care may be provided for the primary disease as long as prohibited drugs are not used.

3) Palliative radiotherapy may be performed if the cancer is not recurrent or progressing, and target lesions are not in the irradiated area.

4) Analgesics

5) Antiemetics

6) Antihypertensives

7) Topical medication to treat hand-and-foot skin reaction

Patients using drugs known to be metabolized by the liver and drugs with a narrow therapeutic range, like digoxin, will be monitored carefully for adverse reactions.

6.4.2. Prohibited concomitant treatments and supportive care

Patients may not receive the following treatments during the protocol treatment period.

1) *Shosaikoto* (*kampo* medicine)

2) Drugs (e.g., rifampicin) or foods (e.g., St. John’s wort) that induce CYP3A4

3) Antitumor drugs and treatments believed to act on tumors that are currently approved, in development, or under investigation, as well as radiotherapy and surgery

(However, palliative radiotherapy is allowed.)

4) Cytokine formulations including interferons

5) Bone marrow and hematopoietic stem cell transplantation

6) Local therapy for hepatocellular carcinoma (resection, RFA, or TACE)

6.5. Post-trial Treatment

Treatments for hepatocellular carcinoma not described in this protocol will not be performed until criteria for discontinuation of protocol treatment are met. Post-trial treatment for after the protocol treatment is concluded is not defined in this protocol. If the cancer is downstaged, local therapy (e.g., resection, RFA, or TACE) can be performed at the investigator's discretion.

However, **post-trial treatment of the sorafenib monotherapy group with low-dose FP and sorafenib, or post-trial treatment of either group with TACE and sorafenib, is prohibited because these treatments are currently only approved for use in clinical studies.**

The protocol treatment should generally be discontinued if the patient shows PD according to the modified RECIST criteria during the protocol treatment period. However, continuation of the protocol treatment is allowed if the investigator determines that the patient is clinically responding to the protocol treatment. Continuation of treatment in this situation will not be considered post-trial treatment.

Reducing the dose to below the lowest level in the dose reduction criteria stipulated in the protocol will be considered to constitute discontinuation of the protocol treatment, and all treatment after that point will be considered post-trial treatment.

7. EXPECTED ADVERSE EVENTS

An adverse event (AE) is defined as any undesirable medical event that occurs in a patient receiving the study drug (excluding worsening of the primary disease). These events may or may not have a clear causal relationship with the study drug. Essentially, an adverse event is any undesirable or unintended sign (including abnormal laboratory test values), symptom, or condition that arises in a patient receiving the study drug, regardless of whether the event has a causal relationship with the study drug. In this study, adverse events that satisfy the above definition will be identified and recorded on case report forms.

If the grade according to the Japanese translation of CTCAE v4.0 worsens, the change will be reported as an adverse event on a case report form. This may also apply to changes within reference ranges or grades if the site investigator believes the event merits clinical consideration (including changes in test values).

The causality of adverse events will also be recorded on case report forms. The Japanese

translation of CTCAE v4.0 will be used for coding adverse events.

7.1. Adverse Events Expected with Each Drug

The appended latest versions of the package inserts for each drug should be referenced for adverse drug reactions anticipated with each drug.

7.1.1. Adverse Events Caused by Sorafenib

Below is an excerpt from the package insert.

Most common adverse reactions to sorafenib

Adverse reactions were observed in 141 of 145 patients in a Japanese phase II study in renal cell carcinoma and a Japanese phase I study in hepatocellular carcinoma. The most common adverse reactions (number of patients, incidence) included increased lipases (85 patients, 58.6%), hand and foot syndrome (80 patients, 55.2%), increased amylase (59 patients, 40.7%), rash (59 patients, 40.7%), hair loss (53 patients, 36.6%), diarrhea (51 patients, 35.2%), hypertension (40 patients, 27.6%), fatigue (23 patients, 15.9%), anorexia (21 patients, 14.5%), pruritus (21 patients, 14.5%), weight loss (18 patients, 12.4%), hoarseness (16 patients, 11.0%), and increased AST (GOT) (15 patients, 10.3%).

Clinically significant adverse reactions to sorafenib

1) Hand and foot syndrome (≥ 10%) and exfoliative dermatitis (1-10%): Hand and foot syndrome and exfoliative dermatitis may occur. If skin symptoms appear, consider symptomatic treatment, dose reduction, or interruption or discontinuation of sorafenib.

2) Stevens-Johnson syndrome (frequency unknown) and erythema multiforme (0.1-less than 1%): Stevens-Johnson syndrome and erythema multiforme may occur. Monitor patients carefully, discontinue treatment, and take appropriate therapeutic measures if Stevens-Johnson syndrome or erythema multiforme is suspected.

3) Hypertensive crisis (0.1-less than 1%): Hypertensive crisis may occur. Carefully monitor changes in blood pressure during use. Discontinue sorafenib and take appropriate therapeutic measures if hypertensive crisis occurs.

4) Reversible posterior leukoencephalopathy (0.1-less than 1%): Reversible posterior leukoencephalopathy may occur. Monitor patients carefully, discontinue sorafenib, and take appropriate therapeutic measures (e.g., blood pressure control and anticonvulsants) if reversible posterior leukoencephalopathy is suspected.

5) Gastrointestinal perforation (0.1-less than 1%): Gastrointestinal perforation may occur, and deaths from this reaction have been reported. Discontinue sorafenib and take appropriate therapeutic measures if gastrointestinal perforation is suspected.

6) Hemorrhage (gastrointestinal hemorrhage, pulmonary hemorrhage, cerebral hemorrhage, oropharyngeal hemorrhage, epistaxis, subungual hemorrhage, and hematoma) (≥ 10%): Severe hemorrhage such as gastrointestinal hemorrhage, pulmonary hemorrhage, and

cerebral hemorrhage may occur, and deaths from this reaction have been reported. Monitor patients carefully during treatment, discontinue sorafenib, and take appropriate therapeutic measures if severe hemorrhage is suspected.

7) Myocardial ischemia/infarction (0.1-less than 1%): Myocardial ischemia/infarction may occur, and deaths from this reaction have been reported. Monitor patients carefully, discontinue sorafenib, and take appropriate therapeutic measures if any abnormalities are observed.

8) Congestive heart failure (0.1-less than 1%): Congestive heart failure may occur, and deaths from this reaction have been reported. Monitor patients carefully, discontinue sorafenib, and take appropriate therapeutic measures if any abnormalities are observed.

9) Liver dysfunction/jaundice (0.1-less than 1%), hepatic failure (frequency unknown), hepatic encephalopathy (frequency unknown): Liver dysfunction, jaundice, hepatic failure, and hepatic encephalopathy accompanied by elevated AST (GOT) and ALT (GPT) may occur. Monitor patients carefully, reduce the dose of sorafenib, interrupt treatment or discontinue sorafenib, and take appropriate therapeutic measures if any abnormalities are observed. Hepatic encephalopathy has primarily been reported in patients with hepatocellular carcinoma or cirrhosis of the liver. As such, patients in these groups should be carefully monitored for clinical symptoms such as impaired consciousness.

10) Pancreatitis (0.1-less than 1%): Pancreatitis may occur. Monitor patients carefully, interrupt or discontinue treatment with sorafenib, and take appropriate therapeutic measures if symptoms suggestive of pancreatitis (e.g., abdominal pain) or persistent elevation of pancreatic enzymes is observed.

11) Acute lung injury and interstitial lung disease (frequency unknown): Acute lung injury and interstitial lung disease may occur. Monitor patients carefully for clinical symptoms such as dyspnea, fever, and cough, and promptly take a chest X-ray if any abnormalities are observed. Discontinue sorafenib and take appropriate therapeutic measures (e.g., treatment with corticosteroids) if acute lung injury or interstitial lung disease is suspected.

12) Leukopenia, neutropenia, lymphopenia, thrombocytopenia, and anemia (frequency unknown): Leukopenia, neutropenia, lymphopenia, thrombocytopenia, and anemia may occur. Monitor patients carefully, and reduce the dose of sorafenib, interrupt treatment or discontinue sorafenib, and take appropriate therapeutic measures if any abnormalities are observed.

13) Renal failure (frequency unknown): Renal failure may occur. Monitor patients carefully, discontinue sorafenib, and take appropriate therapeutic measures if any abnormalities are observed.

7.1.2. Adverse Events Caused by Cisplatin

Below is an excerpt from the package insert.

Summary

The incidence of adverse reactions and laboratory abnormalities in the 104 patients in Japanese clinical studies of patients with hepatocellular carcinoma was 99.0%. The most common adverse reactions included anorexia (79.8%), nausea/vomiting (76.0%), fever (63.5%), malaise (26.9%), leukopenia (78.8%), neutropenia (77.2%), thrombocytopenia (76.9%), elevated AST (GOT) (56.7%), hypochromia (51.0%), ALT (GPT) (45.2%), increased serum bilirubin (36.5%), decreased serum albumin (31.7%), decreased serum total protein (29.8%), increased LDH (27.9%), increased BUN (25.0%), and increased blood creatinine (21.2%). (At time of approval)

Clinically significant adverse reactions

1) Acute kidney injury (frequency unknown): Serious kidney injury such as acute kidney injury may occur. Monitor patients carefully, discontinue cisplatin, and take appropriate therapeutic measures if any abnormalities in BUN, serum creatinine, or creatinine clearance are observed. Hematuria, proteinuria, oliguria, and anuria may also occur.

2) Myelosuppression such as pancytopenia (frequency unknown): Pancytopenia, anemia, leukopenia, neutropenia, and thrombocytopenia may occur. Monitor patients carefully (e.g., by frequent hematology tests), reduce the dose of cisplatin, interrupt treatment or discontinue cisplatin, and take appropriate therapeutic measures if any abnormalities are observed.

3) Thrombocytopenia (frequency unknown): Sudden thrombocytopenia may occur 1-4 days after using cisplatin. Monitor patients carefully (e.g., by frequent hematology tests) after starting cisplatin and take appropriate therapeutic measures if any abnormalities are observed.

4) Fulminant hepatitis (frequency unknown), liver dysfunction (< 1%), and jaundice (frequency unknown): Serious liver dysfunction, fulminant hepatitis, and jaundice accompanied by elevated AST (GOT), ALT (GPT), Al-P, LDH, γ-GTP, or serum bilirubin may occur. Monitor patients carefully, reduce the dose of cisplatin, interrupt treatment or discontinue cisplatin, and take appropriate therapeutic measures if any abnormalities are observed. Abnormalities in serum albumin, serum total protein, and ICG may also occur. Repeated dosing with cisplatin may also cause cholestasis.

5) Myocardial infarction (< 1%), angina pectoris (frequency unknown), heart failure (frequency unknown), and arrhythmia (frequency unknown): Myocardial infarction, angina pectoris (including atypical angina), congestive heart failure, and arrhythmias (e.g., ventricular fibrillation, cardiac arrest, atrial fibrillation, and bradycardia) may occur.

Monitor patients carefully, discontinue cisplatin, and take appropriate therapeutic measures if chest pain, syncope, shortness of breath, palpitations, or abnormal ECG findings are observed.

6) Pulmonary tuberculosis (< 1%): Serious infectious diseases such as pulmonary tuberculosis may occur. Monitor patients carefully and discontinue cisplatin if any abnormalities are observed.

7) Hearing impairment (3.8%): Loss of high frequency hearing, hearing difficulty, and tinnitus may occur. The frequency of hearing impairment is known to increase as the dose of infusional cisplatin is increased, and is particularly high at a daily dose of 80 mg/m^2 or higher or a total dose of 300 mg/m^2. Thus, patients receiving these doses should be monitored carefully.

8) Shock and anaphylactoid symptoms (frequency unknown): Shock and anaphylactoid symptoms may occur. Monitor patients carefully, discontinue cisplatin, and take appropriate therapeutic measures if symptoms such as facial edema, bronchospasm, cyanosis, dyspnea, chest pain, or hypotension appear.

9) Papillary edema, retrobulbar neuritis, and cortical blindness (frequency unknown for all): Visual impairment such as papillary edema, retrobulbar neuritis, and cortical blindness may occur. Discontinue cisplatin if any abnormalities are observed.

10) Cerebral infarction (frequency unknown): Cerebral infarction may occur. Discontinue cisplatin if any abnormalities are observed.

11) Hemolytic uremic syndrome (frequency unknown): Hemolytic uremic syndrome characterized by thrombocytopenia, hemolytic anemia, and renal failure may occur. Monitor patients carefully by regular hematology tests (e.g., platelet and red blood cell counts) and renal function tests, discontinue cisplatin, and take appropriate therapeutic measures if any abnormalities are observed.

12) Hemolytic anemia (frequency unknown): Coombs-positive hemolytic anemia may occur. Discontinue cisplatin if any abnormalities are observed.

13) Interstitial lung disease (frequency unknown): Interstitial pneumonia accompanied by symptoms such as fever, coughing, dyspnea, and abnormalities on chest X-ray may occur. Monitor patients carefully, discontinue cisplatin, and take appropriate therapeutic measures (e.g., treatment with corticosteroids) if any abnormalities are observed.

14) Syndrome of inappropriate antidiuretic hormone secretion (frequency unknown): Syndrome of inappropriate antidiuretic hormone secretion (SIADH) characterized by hyponatremia, hypoosmolality, increased urinary sodium secretion, hypersthenuria, convulsions, and impaired consciousness may occur. If such symptoms appear, discontinue cisplatin, and take appropriate therapeutic measures (e.g., limiting fluid intake).

15) Gastrointestinal hemorrhage, gastrointestinal ulcer, and gastrointestinal perforation (frequency unknown for all): Gastrointestinal hemorrhage, gastrointestinal ulcer, and gastrointestinal perforation may occur. Monitor patients carefully, reduce the dose of cisplatin, interrupt treatment or discontinue cisplatin, and take appropriate therapeutic measures if any abnormalities are observed.

16) Acute pancreatitis (frequency unknown): Acute pancreatitis may occur. Monitor patients carefully and discontinue cisplatin if any abnormalities such as increased serum amylase or serum lipase are observed.

17) Hyperglycemia (frequency unknown) and worsening of diabetes (frequency unknown): Hyperglycemia and worsening of diabetes may occur. Complications such as coma and ketoacidosis have also been reported with infusional cisplatin. Monitor patients carefully by blood glucose and urine glucose tests, discontinue cisplatin, and take appropriate therapeutic measures if any abnormality is observed.

18) Rhabdomyolysis (frequency unknown): Rhabdomyolysis may occur. Discontinue cisplatin and take appropriate therapeutic measures if an increase in CK (CPK) or blood/urine myoglobin is observed.

7.1.3. Adverse events caused by fluorouracil

Below is an excerpt from the package insert.

Summary

The most common adverse reactions reported in 1936 patients at the time of approval and in adverse reaction studies up to February 1970 were anorexia (295 cases, 15.2%), diarrhea/loose stools (239 cases, 12.3%), general malaise (172 cases, 8.9%), nausea/vomiting (159 cases, 8.2%), leukopenia (153 cases, 7.9%), stomatitis (129 cases, 6.7%), hyperpigmentation (92 cases, 4.8%), and hair loss (74 cases, 3.8%).

Clinically significant adverse reactions

(1) Severe diarrhea may occur and may cause dehydration symptoms. Monitor patients carefully, discontinue fluorouracil, and take appropriate therapeutic measures (e.g., fluid replacement) if such symptoms appear.

(2) Serious enterocolitis (e.g., hemorrhagic enterocolitis, ischemic enterocolitis, and necrotizing enterocolitis) may occur. Monitor patients carefully, discontinue fluorouracil, and take appropriate therapeutic measures if symptoms such as severe abdominal pain and diarrhea appear.

(3) Myelosuppression (e.g., pancytopenia, leukopenia, neutropenia, anemia, and thrombocytopenia) may occur. Monitor patients carefully and take appropriate therapeutic measures (e.g., dose reduction or interruption) if any abnormalities are observed.

(4) Shock and anaphylactoid symptoms may occur. Monitor patients carefully,

immediately discontinue fluorouracil, and take appropriate therapeutic measures if symptoms such as rash, dyspnea, and hypotension are observed.

(5) Leukoencephalopathy (early symptoms: gait abnormality, numbness in the distal extremities, impaired tongue movement, etc.) or neuropsychiatric symptoms such as extrapyramidal symptoms, speech impairment, ataxia, nystagmus, impaired consciousness, convulsions, facial paralysis, disorientation, numbness in the distal extremities, delirium, memory loss, loss of motivation, and urinary incontinence may occur. Monitor patients carefully and discontinue fluorouracil if such symptoms appear.

(6) Congestive heart failure, myocardial infarction, and variant angina may occur. Monitor patients carefully and take appropriate therapeutic measures (e.g., dose reduction or interruption) if any abnormalities are observed.

(7) Serious renal impairment such as acute kidney injury may occur. Monitor patients carefully, discontinue fluorouracil, and take appropriate therapeutic measures if any abnormalities are observed. Be particularly cautious during coadministration of fluorouracil with antitumor agents known to cause renal impairment (e.g., cisplatin and methotrexate).

(8) Interstitial pneumonia may occur. Discontinue fluorouracil, perform tests such as a chest X-ray, and take appropriate therapeutic measures (e.g., treatment with corticosteroids) if respiratory symptoms such as fever, cough, and dyspnea appear.

(9) Liver dysfunction and jaundice associated with elevated AST (GOT), ALT (GPT), Al-P, and γ-GTP may occur, and some cases may result in hepatic failure. Monitor patients carefully, discontinue fluorouracil, and take appropriate therapeutic measures if any abnormalities are observed.

(10) Gastrointestinal ulcers and serious stomatitis may occur. Monitor patients carefully, and discontinue fluorouracil and take appropriate therapeutic measures if any abnormalities are observed.

(11) Acute pancreatitis may occur. Monitor patients carefully, discontinue fluorouracil, and take appropriate therapeutic measures if abdominal pain or increased serum amylase is observed.

(12) Hyperammonemia with impaired consciousness may occur. Monitor patients carefully, and discontinue fluorouracil and take appropriate therapeutic measures if any abnormalities are observed.

(13) Hepatic arterial infusion of fluorouracil can cause hepatobiliary impairment (e.g., cholecystitis, bile duct necrosis, and hepatic parenchymal impairment). Check the distribution of drugs carefully by imaging with contrast. Discontinue fluorouracil and take appropriate therapeutic measures if any abnormalities are observed.

(14) Hand and foot syndrome (e.g., palmar/plantar erythema, painful redness and swelling, and hyperesthesia) may occur. Monitor patients carefully and take appropriate therapeutic measures (e.g., dose reduction or interruption) if any abnormalities are observed.

(15) Olfactory impairment may occur (most often with long-term use), and can lead to anosmia. Monitor patients carefully and take appropriate therapeutic measures (e.g., discontinuation of fluorouracil) if any abnormalities are observed.

(16) Serious hepatic impairment (e.g., fulminant hepatitis), cirrhosis of the liver, ventricular tachycardia, nephrotic syndrome, Stevens-Johnson syndrome, toxic epidermal necrolysis (Lyell syndrome), and hemolytic anemia have been reported with similar drugs. Monitor patients carefully, discontinue fluorouracil, and take appropriate therapeutic measures if any abnormalities are observed.

7.2. Adverse Events Caused by Catheters and Reservoirs

Events such as bleeding associated with arterial puncture, hematoma, intimal injury, neuropathy, aneurysm, arteriovenous fistula, infection, pain (all rare), blood vessel perforation by the catheter/guidewire (rare), spasms and obstruction (rare), infection and pain (rare), nausea/vomiting (20%), vagal response (rare), shock caused by iodine contrast medium (very rare), and foreign matter left in blood vessels after equipment breakage (very rare) are anticipated to occur during catheterization before the start of the study. These events may also occur later. Events such as hemorrhage, hematoma, intimal injury, neuropathy, infection, and pain are also anticipated with reservoir placement.

7.3. Adverse Events Expected with Combination Chemotherapy

Sorafenib may exacerbate hemorrhage, hematoma, and intimal injury associated with reservoir placement. Patients will be monitored carefully for these.

7.4. Evaluation of Adverse Events/Adverse Reactions

Site investigators will look out for adverse events throughout the entire study period. Therefore, patients should be monitored carefully during the study and even after the end of the study. All adverse events (observed events, unsolicited events, and events described in medical interviews) must be described in detail on case report forms. The following items must be recorded.

1) Date of occurrence

2) Grade (Japanese translation of CTCAE v4.0)

3) Causal relationship of adverse event with each study drug (**causality definitions from Table 10**)

4) Assessment of the adverse event as serious* or non-serious

5) Outcome of the adverse event (resolved/not resolved)

Site investigators will determine the seriousness of each event that occurs. If the event is judged as serious*, they will submit necessary institutional reports in accordance with institutional procedures. In the event of a serious adverse reaction, site investigators will make an unsolicited report of necessary details to the manufacturer.

Table 10 Causality definitions

Category	Definition
No relationship	The event has no causal relationship with the protocol treatment, and can be clearly explained by other factors such as progression of the primary disease, comorbid disease, or other drugs or treatments
Possible relationship	The event could possibly have a causal relationship with the protocol treatment, but could also be explained by other factors such as progression of the primary disease, a concomitant disease, or other drugs or treatments
Relationship	The event has a clear causal relationship with the protocol treatment and cannot be explained by other factors such as progression of the primary disease, comorbid disease, or other drugs or treatments

*: Events that meet the following criteria are defined as serious.

1) Death

2) Disability (dysfunction severe enough to interfere with ADL)

3) Life-threatening

4) Risk of disability

5) Hospitalization or prolongation of existing hospitalization for treatment is indicated

6) Equivalently serious to 1) through 5)

7) Congenital anomaly or birth defect

Site investigators will properly diagnose and treat events to minimize patient risks. They will also perform appropriate diagnostic tests to collect evidence that clarifies the causality of serious adverse events.

8. PARAMETERS ASSESSED, CLINICAL TESTS, AND ASSESSMENT

SCHEDULE

8.1. Parameters Assessed Before Enrollment

Data on the following parameters will be collected within 4 weeks before enrollment for pre-enrollment evaluation.

1) Patient characteristics: Sex, height, pathological diagnosis, treatment history, disease stage (using the General Rules for the Clinical and Pathological Study of Primary Liver Cancer, see Section 21.3), ECOG-PS (see Section 21.2), allergies, and concomitant diseases

2) Signs and symptoms and blood pressure

3) Body weight

4) Chest X-ray

5) Electrocardiogram

6) Target lesion measurements (dynamic CT is preferred, but dynamic MRI is also acceptable)

7) Hematology parameters: Hemoglobin, white blood cell count, neutrophil count, red blood cell count, platelet count

8) Blood biochemistry: AST, ALT, total bilirubin, direct bilirubin, ALP, γ-GTP, albumin, creatinine, Na, K, Cl, amylase, lipase, blood glucose

9) Coagulation: PT (INR)

10) Tumor markers: AFP, AFP-L3 fraction, PIVKA-II

11) Hepatitis virus: HBs antigen/HBs antibody/Hbc antibody,*[1] HCV antibody*[2]

*1 If the patient tests positive for Hbs antibody and/or Hbc antibody, HBV DNA will be analyzed following the methods in the “Guidelines for Management of Hepatitis B Virus Caused by Immunosuppressive Therapy and Chemotherapy” [28] (**see 21.5 Guidelines for Management of Hepatitis B Virus Caused by Immunosuppressive Therapy and Chemotherapy**).[[not a link]] Patients who test positive for HBV DNA will be provided appropriate treatment.

*2 Patients who test positive will be tested for HCV RNA and serotype.

8.2. Tests and Evaluations during the Study Period

8.2.1. Parameters evaluated during the first cycle

Data on the following parameters will be collected each week during the first cycle.

1) Signs and symptoms* and blood pressure

2) Hematology parameters: Hemoglobin, white blood cell count, neutrophil count, red blood cell count, platelet count

3) Blood biochemistry: AST, ALT, total bilirubin, direct bilirubin, ALP, γ-GTP, albumin, creatinine, Na, K, Cl, amylase, lipase, blood glucose

*: Signs and symptoms will be recorded using terms from the Japanese translation of CTCAE v4.0.

4) Coagulation: PT (INR)

5) Adherence to study treatment

8.2.2. Parameters evaluated from the second cycle onward

Data on the following parameters will be collected right before each cycle from the second cycle onward.

1) Signs and symptoms* and blood pressure

2) Body weight

3) Target lesion measurements (dynamic CT is preferred, but dynamic MRI is also acceptable)

4) Hematology parameters: Hemoglobin, white blood cell count, neutrophil count, red blood cell count, platelet count

5) Blood biochemistry: AST, ALT, total bilirubin, direct bilirubin, ALP, γ-GTP, albumin, creatinine, Na, K, Cl, amylase, lipase, blood glucose

*: Signs and symptoms will be recorded using terms from the Japanese translation of CTCAE v4.0.

6) Coagulation: PT (INR)

7) Tumor markers: AFP, AFP-L3 fraction, PIVKA-II

8) Adherence to study treatment

8.3. Tests and Evaluations after Discontinuation of the Protocol Treatment

As much of the following data as possible will be collected in order to evaluate efficacy and safety (within 1 month of the date of discontinuation of the protocol treatment).

1) Signs and symptoms and blood pressure

2) Body weight

3) Target lesion measurements (dynamic CT is preferred, but dynamic MRI is also acceptable)

4) Hematology parameters: Hemoglobin, white blood cell count, neutrophil count, red blood cell count, platelet count

5) Blood biochemistry: AST, ALT, total bilirubin, direct bilirubin, ALP, γ-GTP, albumin, creatinine, Na, K, Cl, amylase, lipase, blood glucose

6) Coagulation profile: PT (INR)

7) Tumor markers: AFP, AFP-L3 fraction, PIVKA-II

8) Adherence to sorafenib

8.4. Follow-up after Discontinuation of the Protocol Treatment

The following items will be monitored to the greatest extent possible from the time protocol treatment is discontinued until the end of the entire study. Tests will be performed at the investigator's discretion depending on the patient's condition and will not be defined as part of this study.

1) Survival: Date survival was last confirmed or date of death; if dead, cause of death

2) Disease progression*: Whether the disease has progressed, date of last follow-up regarding progression or date progression was confirmed, site of progression

3) Post-trial treatment: Whether patient is receiving post-trial treatment, treatments received, start date

*Patients who enter the follow-up stage before progression is confirmed will be followed until progression. Reporting is not necessary after progression.

8.5. Imaging

Lesions will be imaged by dynamic CT or dynamic MRI 4 weeks (± 1 week) after the start of treatment and every 8 weeks thereafter until progression is confirmed.

8.6. Study Schedule

Observations and tests for evaluating safety and efficacy will be performed following the schedule below (Table 11).

Table 11 Schedule of observations and tests

		Before treatment	During protocol treatment					At termination	After discontinuation of the protocol treatment
Cycle			1				Right before the start of each cycle		
Week			1	2	3	4			
Patient characteristics*1		○							
Signs and symptoms, and blood pressure		○	○	○	○	○	○	○	
Body weight		○	○	○	○	○	○	○	
Chest X-ray		○							
Electrocardiography		○							
Lesion measurements*2		○				○	○	○	○
Clinical assessments	Hematology*3	○	○	○	○	○	○	○	
	Blood biochemistry*4	○	○	○	○	○	○	○	
	Coagulation*5	○	○	○	○	○	○	○	
	Tumor markers*6	○				○	○	○	
	Hepatitis virus	○							
Adherence to study treatment			○	○	○	○	○	○	
CRFs	Enrollment Eligibility Form	○							
	Patient Characteristics Report	○							
	Progress Report						○		
	Antitumor Effect Report						○	(○)	
	Treatment Completion Report							○	
	Treatment Suspension (Considering Discontinuation) Report							○	
	Follow-up Form								○

*1 Patient characteristics: Sex, height, pathological diagnosis, treatment history, disease stage, ECOG-PS, allergies, concomitant diseases

*2 Imaging: Lesions will be imaged by dynamic CT or dynamic MRI 4 weeks (±1 week) after the start of treatment and every 8 weeks thereafter until progression is confirmed.

*3 Hematology parameters: Hemoglobin, white blood cell count, neutrophil count, red blood cell count, platelet count

*4 Blood biochemistry: AST, ALT, total bilirubin, direct bilirubin, ALP, γ-GTP, albumin, creatinine,

Na, K, Cl, amylase, lipase, blood glucose

*5 Coagulation profile: PT (INR)

*6 Tumor markers: AFP, AFP-L3 fraction, PIVKA-II

9. DATA COLLECTION

9.1. Types of Case Report Form (CRF) and Submission Deadlines

The types of case report forms used in this study and their submission deadlines are as follows.

1) Enrollment Eligibility Form: Fax to data center at enrollment

2) Patient Characteristics Report: Fax to data center within 2 weeks before the start of the study

3) Progress Report (each cycle): Fax to data center within 1 week after eligibility to start next cycle is confirmed

4) SAE Report (Expedited Primary Report): Fax to **study coordinator** within 72 hours of SAE onset

5) SAE Report (Expedited Secondary Report): Fax to **study coordinator** within 7 days of learning of the event

6) Adverse Event Report (Normal Report): Fax to **study coordinator** within 15 days of learning of the event

7) Treatment Response Report: Fax to data center within 4 weeks after the end of the protocol treatment

8) Treatment Completion Report: Fax to data center within 4 weeks after the end of the protocol treatment

9) Follow-up Form: Fax to data center within 2 weeks after receiving a request*

10) Treatment Suspension Report: Fax to the study chair when considering discontinuation for a patient who does not clearly meet the specified criteria for discontinuation of the protocol treatment

*Will be requested every 4 months following the monitoring schedule of the data center after the end of the study treatment.

9.2. Submission of Imaging Data

When submitting imaging data (CT or MRI) for interim analysis, each study site will mask personal information (e.g., ID number, name, date of birth) on data from enrollment and after discontinuation of the protocol treatment, write in the patient's enrollment number for this

study, and send the data to the study coordinator. DICOM data recorded on CD-R or DVD-R is generally preferred, but films are also acceptable. These should be submitted after discontinuation of the protocol treatment. Imaging data from enrollment will be collected for patients who did not start the protocol treatment.

9.3. Where to Direct Inquiries

1) Eligibility criteria, criteria for adjusting treatments, or imaging assessment, and inquiries requiring clinical judgment: Study Coordinator

2) Enrollment procedures or completion of CRFs: Data Center

3) Serious Adverse Event Reports: Study Chair/Study Coordinator

9.4. Data Management

Data sent to the data center will be anonymized in a linkable fashion at each study site. These data will be strictly managed in accordance with institutional standards. The data center will notify each participating institution of the serial numbers assigned to each enrolled patient. Data collected by the data center will be kept under strict control using these serial numbers.

When study results are presented at academic conferences or published in academic journals, measures will be taken to ensure study subjects cannot be identified. (See **Section 17. PUBLICATION OF STUDY RESULTS**). Patient data will be deleted if they withdraw their consent. However, results of analysis will not be deleted if study results have already been published.

If data from this study is used for secondary purposes, such as meta-analysis, personal information will be kept strictly confidential and measures will be taken to ensure study subjects cannot be identified.

10. REPORTING OF ADVERSE EVENTS

If a serious adverse event or unexpected adverse event occurs, the investigator will report the event to the study chair. Reports to institutional directors, drug safety reports for the Ministry of Health, Labour and Welfare, and unsolicited reports from medical institutions to the manufacturer through the manufacturer reporting system defined in the Pharmaceutical Affairs Law will be made as appropriate in accordance with institutional regulations and will be the responsibility of individual investigators.

10.1. Adverse Events Subject to Mandatory Reporting

10.1.1. Adverse Events Subject to Expedited Reporting

Adverse events that meet any of the below criteria are subject to expedited reporting.

1) All deaths that occur during the protocol treatment or within 30 days* of the last day of the protocol treatment

This applies regardless of causality. In the case of patients who discontinued the protocol treatment, all deaths within 30 days of the last day of the protocol treatment are subject to expedited reporting, even if the patient already started post-trial treatment.

*: Here, “30 days” refers to 30 days beginning from the day after the last day of the protocol treatment, with the last day of treatment considered as day 0.

**: Deaths of patients who did not receive the protocol treatment after enrollment are not subject to expedited reporting. However, evaluation of eligibility at enrollment and other necessary evaluations will be made as appropriate through regular monitoring.

2) Unexpected grade 4 adverse events

Grade 4 adverse events that are not listed on the package inserts for the study drugs and are judged to have a causal relationship (definite, probable, or possible) with the protocol treatment are subject to expedited reporting.

10.1.2. Adverse events subject to regular reporting

Adverse events that meet any of the criteria in 1) through 4) below and are judged to have a causal relationship (definite, probable, or possible) with the protocol treatment are subject to regular reporting.

1) Deaths that occur 31 days or more after the last day of the protocol treatment

This applies to deaths suspected to be treatment-related. It does not apply to deaths clearly caused by the primary disease.

2) Expected grade 4 non-hematological toxicity (adverse event not in the blood/bone marrow categories in CTCAE v4.0)

Grade 4 non-hematological toxicity listed in the package inserts for the study drugs. Note that serious adverse events are subject to regular reporting even if expected.

3) Unexpected grade 2 and grade 3 adverse events

Grade 2 or 3 adverse events not listed in the package inserts for the study drugs.

4) Other clinically significant medical events

10.2. Reporting Obligations of Investigators and Reporting Procedures

10.2.1. Expedited reporting

If an adverse event subject to expedited reporting occurs, the investigator will promptly notify the lead site investigator and the medical representative of the manufacturer. If the lead

site investigator cannot be reached, the site coordinator or the investigator must perform this duty in place of the lead site investigator.

Primary reporting

Within 72 hours of learning of the adverse event, the lead site investigator will complete the designated items on the SAE Report (Expedited Primary Report), fax the report to the study coordinator, and notify the study coordinator by phone. They will also contact the medical representative of the manufacturer by phone or another appropriate means.

Secondary reporting

Investigators will prepare an SAE Report (Expedited Secondary Report) (appended) and a separate Case Details Report (appended) with more detailed information and mail or fax the report to the study chair within 7 days of learning of the event. As prompt submission is of the highest priority, it is acceptable if some items on the report cannot be completed due to uncertainty.

Additional reporting

Investigators will prepare an Adverse Event Report (Additional Report) (appended) only if there were issues with the secondary report.

10.2.2. Regular reporting

Within 15 days of learning of the adverse event, the lead site investigator will complete the designated items on the Adverse Event Report (Regular Report) (appended), and mail or fax the report to the study coordinator.

10.3. Responsibilities of the Study Chair/Study Coordinator

Upon receiving a report from the lead site investigator, the study coordinator will report this to the study chair and research group representatives and consult with them about the report. The urgency, importance, and impact of the report contents will be determined, and measures such as temporary suspension of enrollment (the data center and all participating institutions would be contacted) or urgent notification of participating institutions to inform them of the matter will be taken if necessary. The data center and participating institutions may be contacted by phone if the matter is very urgent, but the phone call should be promptly followed by written correspondence (sent by fax, mail, email, or in person).

10.4. Evaluation by the Data and Safety Monitoring Committee

If an adverse event reported by a participating institution by expedited reporting or regular reporting is judged to meet the criteria in **10.1. Adverse Events Subject to**

Mandatory Reporting after the study coordinator consults with the study chair about the event, the study coordinator will consult with the research group representative and send a written report of the event to the Data and Safety Monitoring Committee office within 15 days of learning of the event. At the same time, they will request that the committee review the opinion of the study chair regarding the adverse event and the appropriateness of measures to be taken.

When they do so, they will include information such as the results of evaluation by the study coordinator/study chair and measures to be taken (including the decision on whether to continue or suspend the study) on SAE Reports (Expedited Primary Reports) and Adverse Event Reports (Regular Reports) sent by participating institutions. In the event of a death within 30 days (**Section 10.1.1-1**), a death after 31 days or later judged to be treatment-related (**Section 10.1.2-1**), or an expected grade 4 non-hematological toxicity (**Section 10.1.2-2**), these notes will include not only the course of individual patients, but also discussion regarding whether the incidence was within the expected range. If the observed incidence is determined to be outside the expected range, this fact will be also be recorded on the Adverse Event Report.

10.5. Data and Safety Monitoring Committee

Kiwamu Okita （Chairman）
Japan Community Health care Organization, Shimonoseki Medical Center

Shigeki Arii Hamamatsu Rosai Hospital

Norihiro Kokudo Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, The University of Tokyo

(Titles omitted)

11. ANALYSIS AND ENDPOINT DEFINITIONS

11.1. Analysis

The additive effect of low-dose FP on sorafenib monotherapy will be evaluated by overall survival (OS). The distribution of time to death in each group will be calculated using the Kaplan-Meier method. The Cox proportional hazards model will be used to evaluate efficacy. Interim analysis will not be performed in this study.

Analysis by average sorafenib dose will also be performed as a subgroup analysis. Infusion frequency and overall survival will also be compared in the HAIC group. Overall survival will also be compared by baseline patient characteristics (hepatic function, age, sex, and tumor markers).

11.2. Imaging Interpretation

Interpretation of imaging results at two points, enrollment and discontinuation or completion of the protocol treatment, will be performed by central review by the **Central Imaging Interpretation Committee (Section 16.7.)**.

10.3. Definitions of Analysis Populations

The analysis populations for regular monitoring and final analysis are defined as follows.

All enrolled patients

The "all enrolled patients" population consists of all patients enrolled in accordance with **Section 5.1. Enrollment and Randomization Procedures** excluding duplicate enrollments and erroneous enrollments.

Ineligible patients

The "ineligible patients" population consists of enrolled patients who did not meet the eligibility criteria (ineligibility was discovered at a later date) or were unable to start the protocol treatment.

All eligible patients

The "all eligible patients" population consists of all enrolled patients except patients determined to be ineligible by the research group. Patients judged to be ineligible at the sole discretion of an investigator, site coordinator, or lead site investigator are included in "all eligible patients". In order for the research group to judge a patient ineligible, the chair of the research group must approve the designation during the final analysis. For analysis for regular monitoring or when presenting data at an academic conference before submission of the final analysis report, the data center may choose to not include patients judged to be ineligible by the study coordinator with the understanding of the study coordinator.

Full analysis set

The full analysis set consists of all enrolled patients who completed the protocol treatment in whole or in part. Patients who did not start the protocol treatment at all are considered "untreated patients".

11.4. Patients Included in Analysis

Analysis populations

Safety evaluation: Randomized patients will be included in the safety evaluation.

Efficacy evaluation: The full analysis set (FAS) defined in Section 11.3 will be used.

11.5. Subgroup Analysis

Stratified analysis by average and total sorafenib dose will also be performed.

11.6. Endpoint Definitions

11.6.1. Primary endpoint

Overall survival (OS)

The length of time from the date of randomization until death from any cause. The date survival was last confirmed will be used to censor surviving patients. Unfollowable patients will be censored by the date survival was last confirmed before they became unfollowable.

11.6.2. Secondary endpoints

Progression-free survival (PFS)

The length of time from the date of randomization until diagnosis of recurrence or death from any cause, whichever is sooner.

Time to progression (TTP)

The length of time from the date of randomization until diagnosis of progression (PD) by the modified RECIST criteria. The date on which the investigator evaluates a patient and performs tests will be considered the date of progression as assessed by imaging. In the case of clinical progression, the date of the examination in which clear evidence of disease progression was observed will be considered the date of progression. Investigators will record the date progression was diagnosed and the reason on medical records. Patients whose disease does not progress as of the final follow-up point will be censored by the later of the last examination date and the last imaging test date for analysis. Patients who die before disease progression will be censored if the death was clearly unrelated to hepatocellular carcinoma or was due to rupture of gastroesophageal varices.

Overall response rate (ORR):

Will be determined using the modified RECIST criteria. Assessments will be made based on changes in the diameter of surviving tumors deemed viable by a modality such as contrast CT that are observed until completion or discontinuation of the protocol treatment.

Tumor markers:

Associations of baseline tumor marker levels and changes from baseline with efficacy will be explored.

Safety:

The Japanese translation of CTCAE v4.0 will be used to tabulate data for the safety population. The incidence and causality of each adverse event and serious adverse event will be tabulated by group and by CTCAE v4.0 grade. Blood pressure and other safety parameters will also be tabulated descriptively.

OS by tumor markers

Overall survival will be compared by tumor marker levels (AFP, AFP-L3, and PIVKA-II).

OS by Vp

Overall survival will be compared by Vp stage.

OS by response

OS will be compared between responders and non-responders

12. STATISTICAL ANALYSES

12.1. Sample Size

Planned number of patients: 190 patients (95 in each group)

The sample size was determined based on the primary efficacy endpoint, OS.

Assuming that event incidence shows an exponential distribution, that median for sorafenib monotherapy OS is 10 months, and that adding hepatic arterial infusion chemotherapy improves median OS by 70% [28] (hazard ratio: 0.59 [combination therapy with HAIC versus sorafenib monotherapy]), 112 events would be necessary to achieve power of 80% for a one-sided α of 0.05 and 1:1 group assignment. The necessary number of events should be observed if 164 patients are followed over a study period of 36 months (enrollment period of 24 months and follow-up period of 12 months). Target enrollment was set at 190 patients (95 in each group) on the assumption that about 15% of patients will be unfollowable.

12.2. Enrollment Period and Follow-up Period

Enrollment period: **45 months**, Follow-up period: 12 months

Initially, the study was started in October 2010 with a planned enrollment period of 24 months. However, the actual pace of enrollment was slower than expected, so the enrollment period was extended by one year in January 2012. As of August 2013, 180 patients (94.7%) have enrolled. Many patients have been unable to start the protocol treatment. Therefore, upon consultation with the research group, it was decided to extend the enrollment period, which was to conclude at the end of September 2013, by 9 months to the end of June 2014.

12.3. Subgroup Analysis

Stratified analysis by average and total sorafenib dose will be performed.

Infusion frequency and overall survival will be compared in the arterial infusion group.

Overall survival will also be compared by baseline patient characteristics (hepatic function, age, sex, and tumor markers).

12.4. Interim Analysis

Interim analysis of efficacy will not be performed in this study.

13. ETHICAL CONSIDERATIONS

13.1. Protection of Patients' Rights

All researchers involved in this study will conduct the study in accordance with the Declaration of Helsinki and the Ethical Guidelines for Clinical Studies (415th Notification of the Ministry of Health, Labour and Welfare, 2008).

Relevant website:

http://www.mhlw.go.jp/general/seido/kousei/i-kenkyu/rinsyo/dl/shishin.pdf

13.2. Informed Consent

13.2.1. Informed consent discussion

Prior to enrollment, investigators will give an Informed Consent Form approved by the participating institution (either the appended form or a modified version created by the participating institution) directly to the patient along with a thorough verbal explanation of the following items. In this protocol, "approval by the participating institution" means that the matter was reviewed by the advisory body of the institution (institutional review board or ethics committee) and a written letter of approval was sent to the applicant by the director of the participating institution or the chair of the reviewing committee.

1) Explanation of the diagnosis, stage, and expected prognosis

2) That this study is a clinical trial

The difference between a clinical trial and clinical practice

3) Study design and rationale (e.g., significance, number of enrolled patients, need for the study, objective, and treatment assignment)

4) Protocol treatments

Drug names, routes of administration, dose, treatment schedule, duration of the entire protocol treatment, etc.

5) Anticipated effects of the protocol treatment

Prolongation of survival, tumor shrinkage, symptom alleviation, etc.

6) Expected adverse events, complications, and sequelae and measures to be taken if they occur

Explanation of the severity and incidence of expected adverse events (including complications, sequelae, and treatment-related death) and measures to be taken if an event occurs

7) Study-related costs and compensation

Explanation that the study will be similar to routine care in that treatment costs (both for the protocol treatment and treatment for any adverse events) will be covered by health insurance and compensation for illness or injury will be consistent with that awarded in normal clinical practice

8) Alternative treatments

Current typical treatments (including palliative care) and the procedures, effectiveness, and toxicity of standard therapies

Advantages and disadvantages of selecting alternative treatment

9) Potential benefits and potential risks

Explanation of potential benefits and risks of participating in the study

10) Direct access of medical history

Explanation that medical records may be reviewed, for example, that healthcare providers from another institution may directly access medical history records and other such records with the permission of the institution's director to ensure accuracy

11) Declining to consent and withdrawal of consent

That patients are free to decline to participate in this study before participating, are also free to withdraw consent even after providing consent, and that these decisions will not adversely impact their care

12) Protection of patients' rights

That the utmost efforts will be made to keep names and other personal information confidential

13) Freedom to ask questions

Written notification of contact information of not only their assigned investigator but also the site investigator and the study chair (or study coordinator) and explanation that patients are free to ask questions about the study or treatment

13.2.2. Informed consent

A patient's participation in the study will be requested after they have been given an explanation of the study and sufficient time to consider the decision, and it is confirmed that they have a firm understanding of what the study entails. If the patient personally consents to

participate in the study, it will be confirmed that the name of the doctor who conducted the informed consent discussion, the name of the patient giving informed consent, and the date of informed consent are recorded on the appended Informed Consent Form or an informed consent form in a format chosen by the study site.

The informed consent form will be copied two times. One copy will be given to the patient directly and one copy will be retained by the site coordinator. The original will be stored with medical records.

13.3. Protection of Personal Information and Identification of Patients

To protect the privacy of individual patients, enrollment numbers issued on enrollment will be used to identify or refer to enrolled patients. All researchers will make the utmost effort to protect personal information.

13.4. Adherence to the Protocol

Researchers participating in this study will adhere to this protocol as long as it does not infringe on the safety or rights of patients.

13.5. Conflicts of Interest

This study is being conducted with funds from a Health and Labour Science Research Grant. The study has no commercial affiliations with any company.

13.6. Patents

Because the purpose of this study is to benefit all patients with hepatocellular carcinoma and not to pursue profit for the researchers, no patent applications will be made.

13.7. Approval by Institutional Review Boards or Ethics Committees

Before this study is conducted, it will be reviewed and approved by the institutional review board or ethics committee of each participating institution and then approved by institutional directors. On approval by the institutional review board or ethics committee of each participating institution, the approval letter will be faxed to the office. Each participating institution will retain their original approval letter and the study chair will retain a copy.

14. MONITORING AND AUDITS

14.1. Regular Monitoring

Monitoring will consist of central monitoring of data recorded in CRFs collected by the data center. Monitoring by site visits involving checking data against source documents

on-site will not be performed. Regular monitoring reports prepared by the data center will be submitted to the study chair, the study coordinator, and the Data and Safety Monitoring Committee for consideration.

14.2. Aspects of the Study to be Monitored

1) Enrollment progress

2) Eligibility: Patients who are ineligible or may be ineligible

3) Whether protocol treatment is in progress or complete and reasons for discontinuation/completion

4) Pre-treatment background factors

5) Serious adverse events

6) Adverse reactions/adverse events

7) Protocol deviations

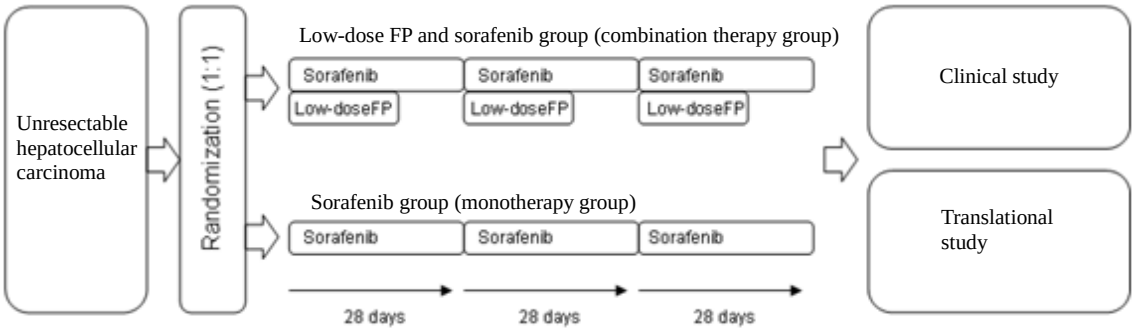
8) Overall survival: All eligible patients

9) Other aspects of study progress and safety problems

15. SUPPLEMENTARY STUDY

15.1. Overview

15.1.1. Schema



15.1.2. Objective

To identify stratification biomarkers for sorafenib-based treatment for hepatocellular carcinoma.

15.1.3. Subjects

Patients who are enrolled in the main study and have consented to participate in the

translational study

15.1.4. Samples

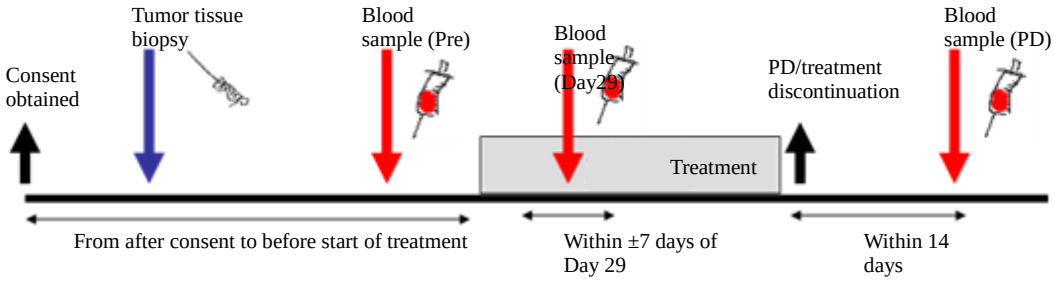
(1) Peripheral blood (3 time points)

A 4-mL sample of peripheral blood will be collected and serum will be separated by centrifugation and stored. The serum will be used as the sample.

Blood samples will be collected at 3 time points: (1) Before treatment (Pre), (2) 1 month after treatment (day 29), and (3) when treatment is discontinued due to PD (no response).

(2) Tumor tissue biopsy specimen (one specimen)

One tumor tissue biopsy specimen will be carefully collected by liver biopsy (ultrasound-guided needle biopsy) to avoid including non-tumor tissue as much as possible. DNA extracted from the tumor tissue will be used as the sample.



15.1.5. Analysis

Serum collected before treatment will be analyzed using an antibody suspension bead array system (Luminex® system) or normal ELISA. Serum humoral factors and growth factors related to sorafenib target genes will be quantified, and proteins related to response to treatment will be identified.

Tumor tissue biopsy specimens will be evaluated and changes in the copy number of various genes in tumor tissue will be analyzed by comparative genomic hybridization (CGH). If the DNA sample is sufficient, somatic cell gene mutations in genes such as sorafenib target genes will be detected by semiglobal gene mutation analysis (OncoCarta) or direct sequencing.

15.1.6. Enrollment period

Same as the main study.

15.1.7. Potential benefits

Identification of protein or gene biomarkers that could predict response to standard

sorafenib-based treatment for hepatocellular carcinoma would make a major contribution to the potential treatment options for hepatocellular carcinoma in the future.

15.1.8. Potential risks and discomforts

Peripheral blood samples (total of 12 mL) and a single tumor tissue specimen from normal liver biopsy (ultrasound-guided needle biopsy) will be analyzed in the translational study, and thus risks and discomforts associated with sample collection should be minimal. Risks related to patient rights and privacy are also very low because (1) the study will be conducted with the informed consent of the patient in accordance with the study protocol, (2) personal information will be anonymized and kept under strict control, (3) “genomic information or gene structures/functions possibly inherited by descendants” will not be analyzed, and (4) the genome of normal tissues will not be analyzed.

15.1.9. Contact information

Inquiries about the translational study:

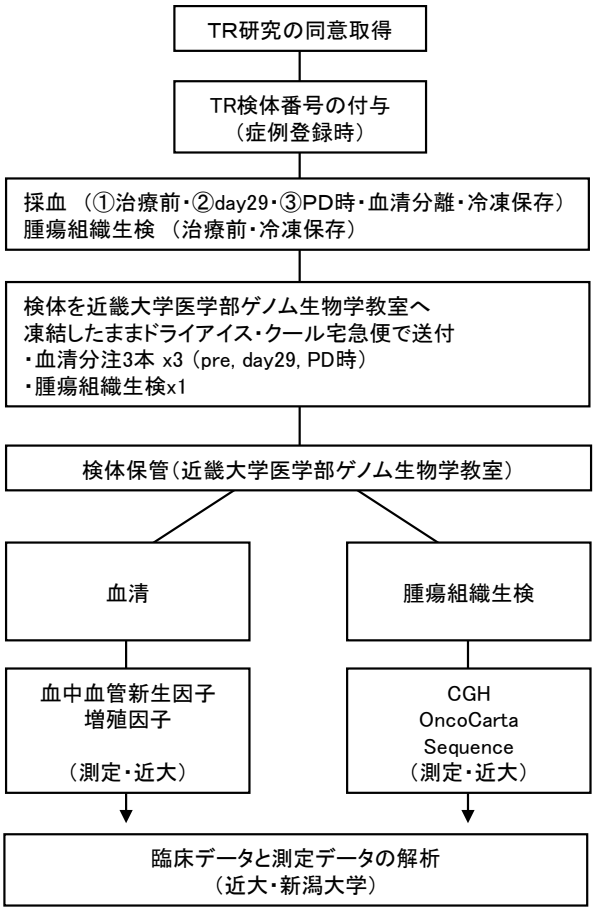
SILIUS-TR Data Center

Yoshihiko Fujita SILIUS Phase III Data Center

Inquiries about samples:

Translational study manager **Yoshihiko Fujita** (Department of Genome Biology, Kindai University Faculty of Medicine)

15.1.10. Study workflow



Consent for the translational study	
Translational study sample number assigned (at patient enrollment)	
Blood collected (1. Pre-treatment, 2. Day 29, 3. PD: serum isolated and stored frozen) Tumor tissue biopsy (pre-treatment, stored frozen)	
Samples sent to Department of Genome Biology, Kindai University Faculty of Medicine Packed frozen with dry ice and sent by frozen delivery service ・Three tubes of serum × 3 (pre, day 29, PD) ・Tumor tissue biopsy × 1	
Samples stored (Department of Genome Biology, Kindai University Faculty of Medicine)	
Serum	Tumor tissue biopsy
Blood angiogenic factors Growth factors (Measured at Kindai)	CGH OncoCarta Sequence (Measured at Kindai)
Analysis of clinical data and measurement data (Kindai and Niigata University)	

15.2. Background

The concept of the angiogenic switch was first proposed by Folkman in the 1970s. He found that when tumors spread to more than 2 mm^3 in diameter, their growth is limited by oxygen supply and nutrition, indicating that angiogenesis is required for further growth. This

concept that angiogenesis by the host is required for tumors to grow in size beyond that point led to the idea that inhibiting tumor angiogenesis could be a target for cancer pharmacotherapy. Angiogenesis inhibitors have been proven clinically effective for many types of cancer, and are now becoming increasingly important.

Sorafenib is a multikinase inhibitor that inhibits angiogenic signal transduction, particularly VEGFR and PDGFR. It is generally classified as an angiogenesis inhibitor because this is its main effect. However, it also has a characteristic inhibitory effect on RAF kinase, and thus also inhibits tumor proliferation signal transduction in cancer cells. When investigated as an antitumor drug for hepatocellular carcinoma, sorafenib was demonstrated to improve overall survival in patients with hepatocellular carcinoma in a phase III clinical trial. Therapeutic use of sorafenib as an angiogenesis inhibitor is also widely practiced in Japan.

It is now clear that copy number abnormalities and activating mutations in genes targeted by molecularly targeted cancer drugs are clinically effective biomarkers for those drugs. The importance of these biomarkers is widely recognized due to findings such as the effect of HER2 expression on trastuzumab, the effect of k-ras mutation on anti-EGFR antibody, and the effect of EGFR gene mutations on EGFR tyrosine kinase inhibitors. Specifically, identifying predictors of response to treatment with antineoplastic agents and stratifying treatment is not only advantageous because it reduces healthcare expenses, but also because it offers clear benefits for the patient such as avoiding adverse events from an ineffective drug and time wasted on ineffective treatment. The importance of biomarkers in predicting response to treatment with angiogenesis inhibitors is widely recognized by basic and clinical researchers alike, and is now the subject of much research. Some blood angiogenic proteins such as VEGF, tumor microvessel density, immunohistological assay of tumor angiogenic factors, and SNP of VEGFRs (the target molecules of multi-tyrosine kinase inhibitors) have also been investigated, but no clinically useful biomarker has been identified.

Two approaches were devised for this translational study. The goal of the first approach, which focuses on the effects of sorafenib as an angiogenesis inhibitor, is to measure the serum concentrations of multiple proteins that act as angiogenic factors and identify predictors of response and factors in drug resistance. The goal of the second approach, which focuses on the direct effect of sorafenib on hepatocellular carcinoma, is to analyze changes in cancer cell genome copy numbers and mutations in VEGFR2-like receptor families (e.g., VEGFRs, FGFRs, PDGFRs, and KIT) and BRAF, which are target molecules for sorafenib.

(1) Blood angiogenic factors and growth factors

Various cytokines, chemokines, and growth factors in blood, mainly VEGF, FGF, IL-8, IL-6, and TNF-alpha, are believed to be potent angiogenic factors.[1] Past studies relating to

predictors of response to sorafenib have investigated factors such as blood VEGF and VEGFRs, tumor vessel density, and tumor VEGF expression, but no useful biomarkers have been identified. Therefore, evaluation of the clinical utility of various blood cytokines, chemokines, and growth factors could lead to the identification of new biomarkers that predict response to treatment and resistance to treatment. The antibody suspension bead arrays system (Bio-Rad Laboratories) can assess 30 to 50 cytokines and chemokines at once under the same conditions and its clinical utility for biomarker discovery has been established.[2,3] Blood cytokines, chemokines, and growth factors other than VEGF can reduce sensitivity to a drug through the phenomenon of escape, and thus would be significant to include in a study investigating potential predictors of response to sorafenib. This research group has previously shown that the pre-treatment serum concentration of various growth factors is a useful noninvasive biomarker of response to molecularly targeted drugs.[4] Predictors of response to sorafenib will be identified using methods established in that clinical study.

(2) Cancer cell genome copy number variations

Amplifications and losses in copy number that affect the physical size of the cancer cell genome at a chromosomal level can be the essential etiology of many solid cancers and leukemia. However, copy number variations in certain loci seen in examples such as leukemia and brain cancers are known to define the malignancy and prognosis of the tumor. Genome copy number variations of sorafenib target genes in cancer cells may be useful predictors of response to treatment with sorafenib as tumor factors predicting response.

(3) Cancer cell gene mutations

It is widely recognized that activation of target molecules of molecularly targeted drugs through gene mutations can be a predictor of response. Therefore, mutations in VEGFR2-like receptor families (e.g., VEGFRs, FGFRs, PDGFRs, and KIT) and BRAF, which are target molecules for sorafenib, will be analyzed.

15.3. Objective

To identify stratification biomarkers for sorafenib-based treatment for hepatocellular carcinoma.

This comprises the following three specific goals:

(1) Identify serum proteins that correlate with response to treatment with sorafenib.

(2) Identify cancer cell genome copy number variations that correlate with response to treatment with sorafenib.

(3) Identify cancer cell gene mutations that correlate with response to treatment with sorafenib.

15.4. Subjects

Patients who are enrolled in the main study and have consented to participate in the translational study.

15.5. Exclusion Criteria

Not specifically defined.

15.6. Enrollment Procedures

Enrollment process for the translational study

(1) Each participating institution will obtain consent for both the main study and the translational study.

(2) Each participating institution will enroll patients for the translational study at the same time as the main study and will fax forms to the data center. The specific method for enrolling patients in the translational study is to check the box for “enroll in the translational study” on the Enrollment Eligibility Form.

(3) The data center will confirm eligibility and then enroll the patient in both the main study and the translational study.

(4) The data center will record the translational study sample number for the patient on the Enrollment Confirmation and Assignment Notification Form and send it by reply fax. The same number will be used for the patient enrollment number and translational study sample number.

(5) Each participating institution will collect samples, write the translational study sample number on the label, and store the samples at their institution.

Contact information and hours for inquiries about the translational study:

SILIUS-TR Data Center: **Yoshihiko Fujita** (Department of Genome Biology, Kindai University Faculty of Medicine)

TEL: 072-367-6369

FAX: 072-367-6369

E-mail: **fujita@med.kindai.ac.jp**

Hours: Monday through Friday, 9:00 AM to 5:00 PM (except December 29 to January 3)

Contact information for inquiries about samples:

Translational study manager: **Yoshihiko Fujita** (Department of Genome Biology, Kindai University Faculty of Medicine)

TEL: 072-367-6369
FAX: 072-367-6369
E-mail: **fujita@med.kindai.ac.jp**

15.7. Handling of Samples

15.7.1. Types and quantities of samples

(1) Peripheral blood, 4 mL x 3 time points

(2) Tumor tissue biopsy specimen, one specimen

15.7.2. Timing of sample collection

(1) Peripheral blood (serum)

Samples will be collected at three time points: before treatment (Pre), 1 month after treatment (Day 29), and when treatment is discontinued due to progressive disease (PD) (no response).

(1) Pre: The "Pre" sample should be collected during the period after the time of consent but before the start of treatment.

(2) Day 29: The "Day 29" sample may be collected ±7 days from day 29 after starting treatment.

(3) PD: The "PD" sample may be collected up to 14 days after PD is diagnosed. This sample should also be collected if treatment is discontinued for any other reason.

(2) Tumor tissue biopsy specimen

Should be collected during the period after the time of consent but before the start of treatment.

15.7.3. Sample processing

See "Sample Processing Procedures". The data center will send designated blood collection tubes, microcentrifuge tubes, saline, sample labels, and dispensing pipettes in advance to institutions able to enroll patients for the translational study. They will use the Laboratory Request Form if necessary.

Peripheral blood

Four milliliters of peripheral blood will be collected. Each 4-mL sample will be collected using the designated 4-mL blood collection tubes sent by the data center (VP-AS054K50, sealed with red film). Serum will be isolated, the translational study sample number will be recorded, and the sample will be dispensed and stored frozen.

Once frozen, the sample may not be thawed.

Procedures for serum isolation	
1	Collect 4 mL of blood in the designated blood collection tube. Slowly invert 6 times.

2	Let rest at room temperature for 30 min.
3	Centrifuge (1500 g for 10 min at room temperature)
4	Dispense the serum into three microcentrifuge tubes using the dispensing pipette (about 0.5 mL x 3 tubes).
5	Record the translational study sample number in two places on the microcentrifuge tubes (top and side) with a permanent marker.
	Translational study enrollment number (SL3-○○○)-Pre/ -Day29/ -PD)
6	Freeze and store in a freezer at −20°C to −80°C.

Serum will be obtained under the above conditions as far as possible. Samples can be stored at −20°C at participating institutions for up to 6 months. After that point, they will be sent to the sample receiving destination. Samples can be stored at −80°C at participating institutions for an indefinite period of time. The sample number on serum samples will consist of the translational study enrollment number plus a code for the collection time point, as follows: SL3-○○○-Pre or SL3-○○○-Day29 or SL-○○○-PD.

Tumor tissue biopsy specimen

One tumor tissue biopsy specimen will be collected by liver biopsy (ultrasound-guided needle biopsy). A total of 0.5 mL of sent saline will be added to a microcentrifuge tube and the collected biopsy specimen will be added to the saline. The translational study sample number will be recorded on the microcentrifuge tube and the sample stored frozen at −20°C to −80°C. The sample number recorded on the tumor tissue biopsy specimen will be "Translational Study Sample Number-Bps (SL3-○○○-Bps)".

Sample	Collection container	Processing methods/translational study sample number	Storage container	Storage conditions
Blood (serum)	4 mL Vacuum blood collection tube	3 tubes for centrifugation/dispensing SL3-○○○-Pre/Day29/PD	1.5 mL microcentrifuge tube	-20 to -80°C
Tumor tissue biopsy specimen		1 specimen SL3-○○○-Bps	1.5 mL microcentrifuge tube with 0.5 mL of saline	-20 to -80°C

15.7.4. Shipping of samples

Every 6 months after the start of the study, each participating institution will receive a fax

from the SILIUS-TR data center requesting that frozen stored samples be sent. At that time, the corresponding site investigator will be sent Styrofoam for packing samples. They will include a List of Sent Specimens and Samples with the samples and send still-frozen samples to the Department of Genome Biology at Kindai University Faculty of Medicine. However, depending on the sample storage capacity of the institution, samples may also be sent for one patient at a time. The translational study sample number should always be recorded in two places (top and side) on the sample. The following shipping methods apply to both serum and tumor tissue biopsy specimens. It is preferred that they be sent at the same time.

(1) Check that the following day is a weekday.
(2) Prepare the designated Styrofoam box (sent by the data center).
(3) Fill with dry ice and seal the designated Styrofoam box with packing tape.
(4) Be sure to send the box by frozen delivery service. (Frozen serum samples may not be thawed.)
Costs associated with shipping (including dry ice and frozen delivery fees) will be paid by the study coordinator (when shipping, be sure to get a receipt and submit a Sample Shipping Fee Reimbursement Form for reimbursement.)

Where to send samples:
Sample Storage Manager
Department of Genome Biology, Kindai University Faculty of Medicine
Kazuto Nishio
knishio@med.kindai.ac.jp
377-2, Ohno-Higashi, Osaka-Sayama, Osaka, Japan 589-8511
TEL /FAX：072-367-6369

15.7.5. Storage and disposal of samples

Samples will be stored at the laboratory of the Department of Genome Biology, Kindai University Faculty of Medicine. The sample storage manager is Kazuto Nishio of the Department of Genome Biology, Kindai University Faculty of Medicine. Serum and tumor tissue biopsy specimens will be stored in a deep freezer at –80°C. The storage location will be secured by two or three levels of locks, including the entrance to the research building and the constantly locked laboratory door and deep freezer door.

Samples will be stored for up to 3 years after the final analysis for the study. Once the storage period has elapsed, samples will be disposed of if there is no particular reason not to do so. Samples will be disposed of in the event that the patient who provided the sample requests to withdraw their consent, the sample number is unrecognizable due to problems

with the label or computer, the sample was or was suspected to have been collected improperly or contaminated, or a researcher deems disposal necessary for any other reason. Such samples will have the sample number removed before disposal. If the provider of the sample requests to withdraw their consent and the sample is being stored at a participating institution, the responsible party at that institution will be contacted and the sample will be disposed of promptly. If the sample is being stored at Kindai University, it will be disposed of after the study coordinator is contacted.

15.7.6. Management of personal information and anonymization

The personal information manager will anonymize samples in a linkable fashion on patient enrollment. Specifically, they will record the linking code (translational study sample number) on the Enrollment Confirmation and Assignment Notification Form and notify the institution by fax.

Assistant personal information managers are staff members at each institution responsible for linking personal information with the linking code (translational study sample number). Upon notification of linking codes, the assistant personal information manager will record the codes on samples from their institution (no other information besides the linking code and Pre/Day29/PD may be recorded on samples). Therefore, although samples identified by linking code will later be sent from each participating institution to the measuring facility, no identifying personal information will be sent to staff involved in measurements and analysis. The chart for linking personal information to linking codes will be controlled by the data center and each participating institution, disposed of as appropriate, and kept under strict control to ensure no information containing personal information is leaked.

*The same number used for the patient enrollment number will be used as the linking code (translational study sample number).

15.8. Study Methods

15.8.1. Measurement of blood angiogenic factors and growth factors

Angiogenic factors (cytokines and chemokines) and growth factors in patient serum will be quantified by multiplex ELISA or ELISA (ELISA will be used if the measurement range is not appropriate for multiplex ELISA). Measurements will be performed in serum collected before treatment, on day 29, and after PD using a Bioplex suspension array system manufactured by Bio-Rad. Measurements will be performed in the laboratory of the Department of Genome Biology, Kindai University Faculty of Medicine. Data from these measurements will be used to assess correlation with response to treatment with sorafenib (e.g., response, PFS, and OS) and identify factors predicting response. The following are examples of markers that might be measured:

Amphiregulin, Beta-cellulin, EGF, EGFR, Epiregulin, FGF-basic, HB-EGF, PDGF-BB, PIGF, Tenascin C, TGF-alpha, HGF, IL-1αIL-1β, IL-2α, IL-2R, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-16, IL-17, IL-18, basic FGF, CTACK, Eotaxin, G-CSF, GM-CSF, GRO-α, HGF, ICAM1, IFN-α2, IFN-γ, IP-10, KC, LIF, MCP-1, MCP-3, M-CSF, MIF, MIG, MIP-1α, MIP-1β, MIP-2, β-NGF, PDGF-BB, RANTES, SCF, SCGF-β, SDF-1α, TNF-α, TNF-β, TRAIL, VCAM-1, and VEGF. (See Translational Study Table 1)

15.8.2. Measurement of cancer cell genome copy number variations

DNA will be extracted from collected tumor tissue biopsy specimens and cancer cell genome copy number variations will be detected by comparative genomic hybridization (CGH). CGH detects variations in copy number in the whole genome (including excessive genomic DNA, deletions, and amplifications) in a short period of time. Because CGH can detect changes in copy number for genes in the cancer genome, it is now widely used to analyze genomic abnormalities in solid tumors, which were previously difficult to analyze in detail with chromosomal analysis. It can determine what losses and amplifications in copy number have affected the physical size of the genome at the chromosomal level. This method cannot detect reciprocal chromosomal translocations not accompanied by changes in copy number. Copy number variations in sorafenib target genes and genes related to angiogenesis will be analyzed to assess correlation with response to treatment with sorafenib and identify factors predicting response. Measurements will be performed at Kindai University Faculty of Medicine. Results will be verified by PCR.

15.8.3. Measurement of cancer cell gene mutations

Mutations in VEGFR2-like receptor families (e.g., VEGFRs, FGFRs, PDGFRs, and KIT) and BRAF, which are target molecules for sorafenib, will be analyzed. Mutations in major oncogenes and tumor suppressor genes will also be investigated. The OncoCarta panel is an analytical technology based on time of flight mass spectrometry that can measure 239 mutations in 19 genes at once (See Translational Study Table 2). Gene mutations will also be detected by direct sequencing and correlations with response to treatment with sorafenib will be assessed.

15.9. Biostatistical Analysis

Correlations between clinical data provided by the data center and various measured parameters will be analyzed at the Department of Genome Biology at Kindai University Faculty of Medicine and the Department of Medical Informatics at Niigata University Medical and Dental Hospital. Data on the degree of expression of quantified serum proteins,

gene copy numbers at about 250,000 loci in the cancer cell genome, and mutations in sorafenib target genes and major oncogenes will be obtained in this study. The following statistical methods will be used to analyze correlations between these measured data and clinical parameters. CGH analysis will be performed using Partek Genomic Suite 6.4 software (Partek Inc., St Louis, MO). Analysis of correlations of measured data with progression-free survival and overall survival will be performed using the Cox proportional hazards model. The clinical utility of factors as biomarkers will be evaluated by multivariate analysis with adjustment for clinical factors. In addition, differences in survival between subgroups will be compared with the log-rank test. Correlations between biomarkers and response rate will be assessed with Fisher's exact test. Statistical analysis will be performed and graphs created with SAS for Windows (ver. 9.1.3) or Medcalc for Windows (ver. 11.1.1).

15.10. Study Workflow

See Section 15.1.10. Study Workflow.

15.11. Target Sample Size and Period

The enrollment target for the translational study is 80% to 90% of the patients enrolled in the main study. The enrollment period will be the same as that for the main study.

15.12. Potential Benefits and Risks/Discomforts for Patients

Potential benefits

Identification of protein or gene biomarkers that could predict response to standard sorafenib-based treatment for hepatocellular carcinoma could be used for treatment stratification by biomarkers and would make a major contribution to the future treatment of hepatocellular carcinoma. No useful biomarker for angiogenesis inhibitors has yet been found, and thus this discovery could also contribute biomarkers for various other angiogenesis inhibitors.

Potential risks/discomforts for patients

Risks and discomforts associated with the translational study should be minor, as the study only requires blood sampling (peripheral blood, total volume of 12 mL) and collection of a tumor tissue specimen by liver biopsy (ultrasound-guided needle biopsy), which is commonly performed in routine care. There is an extremely small risk of serious complications associated with hemorrhage and other issues with liver biopsy, and thus the procedure will be performed with the informed consent of the patient. Due care will be taken to prevent and manage complications from liver biopsy.

Risks related to patient rights and privacy are also very low because (1) the study will be conducted with the full informed consent of the patient in accordance with the study protocol, (2) personal information will be anonymized and kept under strict control, and (3) “genomic information or gene structures/functions possibly inherited by descendants” will not be analyzed.

15.13. Ethical Considerations

15.13.1. Informed consent

Informed consent from sample providers

The investigator will explain to the sample provider the significance, objective, methods, and expected results and risks of the study before enrollment using written information for patients and the informed consent form, and will obtain the freely given written consent of the sample provider. The investigator and sample provider will sign and date the informed consent form (date of informed consent discussion and date consent obtained). The written information for patients and informed consent form (copy) will be handed to the sample provider and the original will be stored with their medical records.

Items explained to the sample provider using the written information for patients and informed consent form

Before obtaining consent, the following items will be explained using the written information for patients and informed consent form.

・That participation in the study is voluntary. That there are no consequences of declining to participate.

・That patients can withdraw their consent to participate in the study at any time.

・That withdrawal of consent will have no impact on medical care.

・That samples will be disposed of when a patient withdraws their consent. That results of analysis will not be deleted if study results have already been published.

・The reason why the patient was selected to provide samples.

・The objective and methods of the study.

・Potential study results and potential risks faced by sample providers.

・That it is permissible to share information about the study plan and study methods.

・The types of documents the patient is required to provide and details about protection of personal information.

・That samples and genetic information will be provided to collaborating institutions.

・That results of gene expression analysis will not be disclosed.

・That intellectual property rights for this study do not belong to subjects.

15.13.2. Disclosure of analysis results

Because this study is an exploratory molecular biomedical study, the reproducibility of the data obtained must be validated in a clinical translational study and the data is not accurate or reliable enough to return to sample providers. Therefore, results of the analysis will not be disclosed to sample providers. Moreover, they will not receive genetic counseling because the mutations analyzed in this study are acquired somatic cell mutations. If the sample provider requests the data be disclosed, only the data from that sample provider will be explained in consideration of the above.

15.14. Data Collection

Clinical data that can be obtained in the main study, for example, data on treatment efficacy, will be used in this study. Clinical data and sample storage charts (with translational study sample numbers and storage locations recorded at the time of storage) will be stored at the SILIUS-TR data center.

15.15. Translational Study Organization

TR Lead:	Department of Genome Biology, Kindai University Faculty of Medicine Kazuto Nishio
TR resercher:	Department of Genome Biology, Kindai University Faculty of Medicine Yoshihiko Hujita
TR researcher:	Department of Genome Biology, Kindai University Faculty of Medicine Kazuko Sakai
	Department of Genome Biology, Kindai University Faculty of Medicine Kazuko Matsumoto
Sample storer:	Department of Genome Biology, Kindai University Faculty of Medicine Kazuto Nishio
Analysis:	Department of Genome Biology, Kindai University Faculty of Medicine Kazuto Nishio
Statistics:	Department of Medical Informatics, Niigata University Graduate School of Medical and Dental Sciences Kohei Akazawa

15.16. Preparation of the Translational Study Protocol

Department of Genome Biology, Kindai University Faculty of Medicine Tokuzo Arao

15.17. Publication of Study Results

Investigators, the study coordinator, or collaborating researchers will compile study results as soon as possible and determine whether to file a patent application. The applicant and the inventor will be determined separately considering the facts of the matter. The results will be published in a suitable English-language journal or presented at a conference. Co-authors will be only those who reviewed the manuscript before submission and agreed to presentation contents.

15.18. References

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Translational Study Table 1.

Parameters measured by multiplex ELISA

Cytokines Available in Bio-Plex Assays

Singleplex assays, preconfigured multiplex panels, and custom-mixed multiplex panels are available.

Assays	Human	Mouse	Rat
IL-1α	●	●	●
IL-1β	●	●	●
IL-1ra	●		
IL-2	●	●	●
IL-2Rα	●		
IL-3	●	●	
IL-4	●	●	●
IL-5	●	●	
IL-6	●	●	●
IL-7	●		
IL-8	●		
IL-9	●	●	
IL-10	●	●	●
IL-12 (p40)	●	●	
IL-12 (p70)	●	●	
IL-13	●	●	
IL-15	●	●	
IL-16	●		
IL-17	●	●	
IL-18*	●	●	
Basic FGF	●	●	
CTACK	●		
Eotaxin	●	●	
G-CSF	●	●	
GM-CSF	●	●	●
GRO-α	●		

Assays	Human	Mouse	Rat
HGF	●		
ICAM-1	●		
IFN-α2	●		
IFN-γ	●	●	●
IP-10	●		
KC		●	
LIF	●	●	
MCP-1 (MCAF)	●	●	
MCP-3	●		
M-CSF	●	●	
MIF	●		
MIG	●	●	
MIP-1α	●	●	
MIP-1β	●	●	
MIP-2		●	
β-NGF	●		
PDGF-BB	●	●	
RANTES	●	●	
SCF	●		
SCGF-β	●		
SDF-1α	●		
TNF-α	●	●	●
TNF-β	●		
TRAIL	●		
VCAM-1	●		
VEGF	●	●	

* IL-18 is not available as a singleplex assay, only as part of an assay panel.

Parameters measured by WideScreen™ Human Cancer Panel 2

Analyte	Alternate/Full name
Amphiregulin	AREG
Betacellulin	BTC
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
Epiregulin	EREG
FGF-basic	fibroblast growth factor-basic, FGF-2
HB-EGF	heparin-binding epidermal growth factor
PDGF-BB	platelet-derived growth factor-BB
PlGF	placental growth factor
Tenascin C	TnC
TGF-α	transforming growth factor alpha
VEGF	vascular endothelial growth factor

Translational Study Table 2.

Mutations and parameters measured by OncoCarta

ABL1	G250E, Q252H, Y253H, Y253F, E255K, E255V, D276G, F311L, T315I, F317L, M351T, E355G, F359V, strong96R
AKT1; AKT2	V461L, P388T, L357T, E319G, V167A, Q43X, E17del; S302G, R371H
BRAF	G464R, G464V/E, G466R, F468C, G469S, G469E, G469A, G469V, G469R, G469R, D594V/G, F595L, G596R, L597S, L597R, L597Q, L597V, T599I, V600E, V600K, V600R, V600L, K601N, K601E
CDK	R24C, R24H
EGFR	R108K、T263P、A289V、G598V、E709K/H、E709A/G/V、G719S/C、G719A、M766_A767insAI、S768I、V769_D770insASV、V769_D770insCV、D770_N771>AGG/V769_D770insASV/V769_D770insASV、D770_N771insG、N771_P772>SVDNR、P772_H773insV、H773>NPY、H773_V774insNPH/PH/H、V774_C775insHV、T790M、L858R、L861Q、E746_T751del、E746_A750del、E746_T751del、E746_T751del、S752D、L747_E749del、L747_T750del、L747_S752del、L747_T751del、L747_S752del、P753S、A750P、T751A、T751P、T751I、S752I/F、S752_I759del、L747_Q ins、E746_T751del、I ins (combined)、E746_A750del、T751A (combined)、L747_E749del、A750P (combined)、L747_T750del、P ins (combined)、L747_S752del、Q ins (combined)
ERBB2	L755P, G776S/LC, G776VC/VC, A775_G776insYVMA, P780_Y781insGSP, P780_Y781insGSP, S779_P780insVGS
FGFR1;FGFR3	S125L, P252T; G370C, Y373C, A391E, K650Q/E, K650T/M
FLT3	I836del, D835H/Y

16. STUDY ORGANIZATION

16.1. Research Group

2010 Health and Labour Science Research Grants for Clinical Cancer Research

Phase III Trial to Establish Combination Therapy with Hepatic Arterial Infusion Chemotherapy and a Molecularly Targeted Drug as a Novel Therapy for Advanced and Recurrent Hepatocellular Carcinoma and Exploratory Study on Biomarkers Predicting its Efficacy

"2010－Clinical Cancer Research－General－015" Research Group (Kudo Group)

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16.4. Participating Institutions (group members) (in no particular order, titles omitted)

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Ikeda	
Yasuaki Arai	Department of Diagnostic Radiology, National Cancer Center Hospital
Hiroaki	Osaka University Hospital, Dept of Transplantation Medicine
Nagano	
Etsuro	Division Hepato-Billary-Pancreatic Surgey and Transplantation Department,
Hatano	Kyoto University
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Ishikawa	
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Ogawa	
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Shouta Iwado	Department of Internal Medicine, Hiroshima City Hospital

16.5. Statistician

Kouhei Akazawa Department of Medical Informatics Niigata University Medical & Dental Hospital

16.6. Protocol Review Committee

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Yasuaki Arai Department of Diagnostic Radiology, National Cancer Center Hospital

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16.7. Central Imaging Interpretation Committee

Takamichi Murakami（Chairman） Department of Radiology, Kindai University School of Medicine

Seishi Kumano Department of Radiology, Kindai University School of Medicine

Kim Tonsok Department of Diagnostic and Interventional Radiology, Osaka University Graduate School of Medicine

Kazuhiro Yamamoto Department of Radiology, Osaka Medical College

16.8. Data Center

Non-Profit Organization（NPO）Japan Clinical Research Support Unit（J-CRSU）

16.9. Chair for Supplementary Study (Sample Storage/Biomarker Analysis)

Kazuto Nishio Department of Genome Biology, Kindai University Faculty of Medicine

17. PUBLICATION OF STUDY RESULTS

After the end of the study, the research group will compile the results as soon as possible and publish them in a suitable English-language journal and present them at conferences. Co-authors will be determined by degree of contribution to the study (e.g., number of

patients enrolled, participation in biomarker analysis, and participation in statistical analysis) and by discussion, up to the maximum number allowed by the journal in which results are to be published.

18. CLINICAL TRIAL REGISTRATION

This study will be registered in a public database and information made public until the first patient is enrolled.

19. PROTOCOL REVISION HISTORY

Ver.1	Data	17/07/2010
Ver.1.1	Data	27/07/2010
Ver.1.2	Data	03/08/2010
Ver.1.3	Data	30/08/2010
Ver.1.4	Data	17/09/2010
Ver.1.5	Data	07/10/2010
Ver.1.6	Data	19/10/2010
Ver.2.0	Data	14/02/2011
Ver.2.1	Data	15/09/2012
Ver.2.2	**Data**	**20/09/2013**
Ver.2.3	**Data**	**06/06/2015**

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21. REFERENCE MATERIALS

21.1. Child-Pugh Score

		Point score calculated from observations		
		1	2	3
Grade of encephalopathy [a]		Absent	1-2	3-4
Ascites		Absent	Slight	Moderate
Serum bilirubin	(mg/dL)	< 2	2-3	> 3.0
	(mcmol/L)	< 34	34-50	> 50
Serum albumin (g/dL)		> 3.5	2.8-3.5	< 2.8
INR		< 1.7	1.7-2.3	> 2.3
Prothrombin time (sec above normal)		< 4	4-6	> 6
Increase in prothrombin time (%)		> 70	40-70	< 40

b Grade of encephalopathy:

Grade 0: Lucid, normal personality, normal neurological test results, normal electroencephalogram

Grade 1: Restlessness, sleep disorder, irritability/agitation, tremors, dysgraphia, 5 cps waves

Grade 2: Lethargy, disorientation (temporal), inappropriateness, difficulty maintaining stable posture, ataxia, slow triphasic waves

Grade 3: Somnolence, confusional state, disorientation (spatial), hyperreflexia, rigidity, slow waves

Grade 4: Coma, no personality/unresponsive, cessation of cerebral activity, slow 2-3 cps delta activity

A: 5-6 points, B: 7-9 points, C: 10-15 points

*Ascites: Patients with a history of ascites who are currently using a diuretic are given 2 points even if they do not have ascites.

*Encephalopathy: Patients with a history of hepatic encephalopathy who are using a drug to treat encephalopathy are given 2 points even if they are asymptomatic.

21.2. Eastern Cooperative Oncology Group (ECOG) PS

Grade	Definition
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light house work or office work).
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about

	more than 50% of waking hours.
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.

21.3. Staging by the General Rules for the Clinical and Pathological Study of Primary Liver Cancer

The overall stage of a cancer is determined by calculating stages for each component and selecting the highest one.

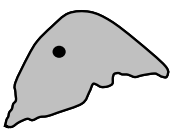
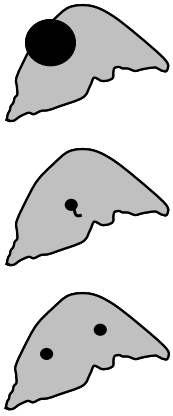
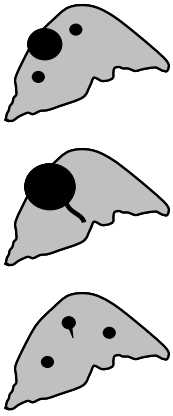
The overall stage is determined from the following four components.

Stages of hepatocellular carcinoma

	T component	N component	M component
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
Stage IVA	T4	N0	M0
	T1, T2, T3, T4	N1	M0
Stage IVB	T1, T2, T3, T4	N0, N1	M1

T component: Determined from three factors: the number, size, and vascular invasion of tumors. Multiple tumors can be multicentric tumors or intrahepatic metastases. An S3 ruptured hepatocellular carcinoma is classified as T4.

T component of hepatocellular carcinoma

	T1	T2	T3	T4
① Number of tumors: Single (2) Tumor diameter: ≤ 2 cm (3) No vascular invasion (Vp0, Vv0, B0)	Meets all (1) (2) (3) 	Meets 2 	Meets 1 	Meets none 

N component:

N0: No lymph node metastasis

N1: Lymph node metastasis

M component:

M0: No extrahepatic spread

M1: Extrahepatic spread

21.4. Modified RECIST

From Lencioni R, Llovet JM.: Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. Semin Liver Dis. 2010 Feb;30(1): 52-60.

Observation of a new intrahepatic lesion will not be considered PD.

Table 2 Assessment of Target Lesion Response: Conventional RECIST and mRECIST Assessment for HCC Following the AASLD-JNCI Guideline

RECIST	mRECIST for HCC
CR = Disappearance of all target lesions	CR = Disappearance of any intratumoral arterial enhancement in all target lesions
PR = At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum of the diameters of target lesions	PR = At least a 30% decrease in the sum of diameters of viable (enhancement in the arterial phase) target lesions, taking as reference the baseline sum of the diameters of target lesions
SD = Any cases that do not qualify for either partial response or progressive disease	SD = Any cases that do not qualify for either partial response or progressive disease
PD = An increase of at least 20% in the sum of the diameters of target lesions, taking as reference the smallest sum of the diameters of target lesions recorded since treatment started	PD = An increase of at least 20% in the sum of the diameters of viable (enhancing) target lesions, taking as reference the smallest sum of the diameters of viable (enhancing) target lesions recorded since treatment started

AASLD, American Association for the Study of Liver Diseases; JNCI, Journal of the National Cancer Institute; HCC, hepatocellular carcinoma; mRECIST, modified Response Evaluation Criteria in Solid Tumors; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

Table 3 Overall Response Assessment in mRECIST: Responses for All Possible Combinations of Tumor Responses in Target and Nontarget Lesions with or without the Appearance of New Lesions

Target Lesions	Nontarget Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	IR/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or no	PD
Any	PD	Yes or no	PD
Any	Any	Yes	PD

mRECIST, modified Response Evaluation Criteria in Solid Tumors; CR, complete response; PR, partial response; IR, incomplete response; SD, stable disease; PD, progressive disease.

21.5. Guidelines for Management of Hepatitis B Virus Caused by Immunosuppressive Therapy

and Chemotherapy

Excerpted from Tsubouchi et al: Prevention of immunosuppressive therapy or chemotherapy-induced reactivation of hepatitis B virus infection: Joint report of the Intractable Liver Diseases Study Group of Japan and the Japanese Study Group of the Standard Antiviral Therapy for Viral Hepatitis. *Kanzo*. 2009; 50: 38-42. [28]

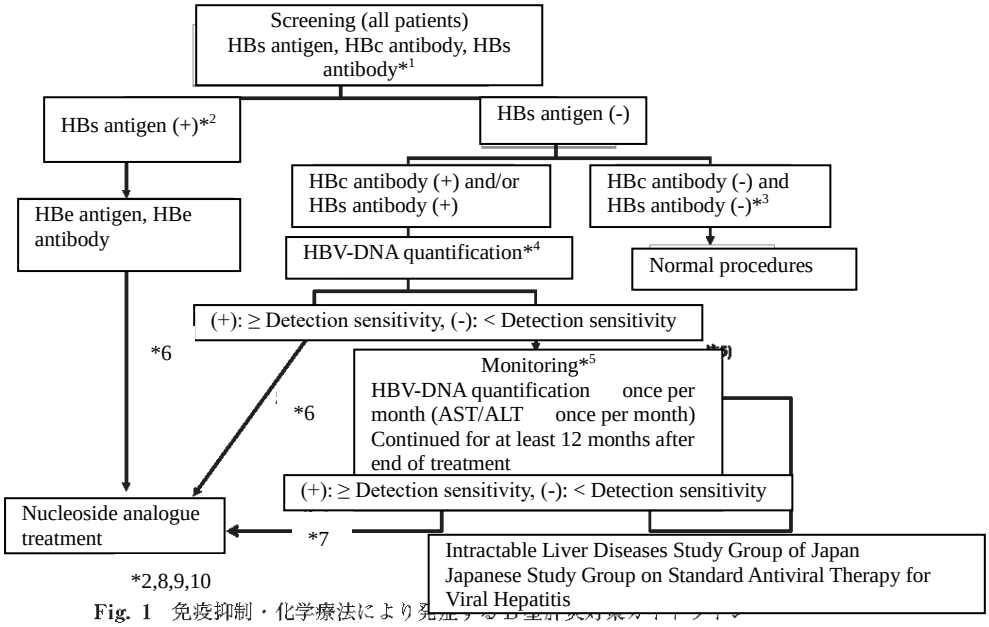


Fig. 1 免疫抑制・化学療法により発症するB型肝炎対策ガイドライン

補足

＊血液悪性疾患に対する強力な免疫･抑制化学療法中あるいは終了後に HBs 抗原陽性あるいは HBs 抗原陰性例の一部に HBV 再活性化により B 型肝炎が発症し，その中には劇症化する症例があり，注意が必要である．その他の疾患においても治療による HBV 再活性化のリスクを考慮して対応する必要がある．また，ここで推奨する核酸アナログ予防投与のエビデンスはなく，劇症化予防効果を完全に保証するものではない．

注 1） CLIA 法で測定することが望ましい．

注 2） HBs 抗原陽性例は肝臓専門医にコンサルトすること．全ての症例で核酸アナログ投与にあたっては肝臓専門医にコンサルトするのが望ましい．

注 3） 初回治療時に HBc 抗体，HBs 抗体未測定の再治療例では抗体価が低下している場合があり，HBV-DNA 定量検査などによる精査が望ましい．

注 4） PCR 法およびリアルタイム PCR 法により実施する．より検出感度の高いリアルタイム PCR 法が望ましい．

注 5） リツキシマブ・ステロイド使用例，造血細胞移植例は HBV 再活性化の高リスクであり，注意が必要である．フルダラビンは強力な免疫抑制作用を有するが，HBV 再活性化のリスクは不明であり，今後注意が必要である．

注 6） 免疫抑制・化学療法を開始する前，できるだけ早期に投与を開始するのが望ましい．

注 7） 免疫抑制・化学療法中は HBV-DNA 定量検査が検出感度以上になった時点で直ちに投与を開始する．

注 8） 核酸アナログはエンテカビルの使用を推奨する．

注 9） 下記の条件を満たす場合には核酸アナログ投与の終了を検討して良い．
スクリーニング時に HBs 抗原（＋）例では B 型慢性肝炎における核酸アナログ投与終了基準を満たす場合．スクリーニング時に HBc 抗体（＋）and/or HBs 抗体（＋）例では，(1) 免疫抑制･化学療法終了後，少なくとも 12 カ月間は投与を継続すること．(2) この継続期間中に ALT（GPT）が正常化していること．(但し HBV 以外に ALT 異常の原因がある場合は除く) (3) この継続期間中に HBV-DNA が持続陰性化していること．

注 10） 核酸アナログ投与終了後 12 カ月間は厳重に経過観察する．経過観察方法は各核酸アナログの使用上の注意に基づく．経過観察中に HBV-DNA 定量検査が検出感度以上になった時点で直ちに投与を再開する．

Figure 1 Guidelines for Management of Hepatitis B Virus Caused by Immunosuppressive Therapy and Chemotherapy

Notes

*Caution should be exercised with patients who test positive for HBs antigen or test negative for HBs antigen during or after receiving intensive immunosuppressive therapy/chemotherapy for a

hematological malignancy because some of these patients develop hepatitis B due to reactivation of HBV, and some cases become fulminant. Some other diseases also pose a risk of treatment-induced reactivation of HBV, and this risk must be considered when managing such patients. There is currently no evidence to support the prophylactic nucleoside analogue treatment recommended here, and it cannot be guaranteed to completely prevent development of fulminant hepatitis.
*1) Measurement by CLIA is preferred.
*2) A hepatologist should be consulted when a patient tests positive for HBs antigen. A hepatologist should preferably be consulted before starting nucleoside analogue treatment in any patient.
*3) In some cases, patients who did not have HBc antibody and HBs antibody measured before initial treatment have reduced antibody titers after retreatment. Therefore, further testing such as HBV-DNA quantification should preferably be performed for these patients.
*4) Performed by PCR or real-time PCR. Real-time PCR is preferable as it has higher detection sensitivity.
*5) Caution should be exercised with patients using rituximab or steroids and patients undergoing hematopoietic stem cell transplantation as they are at high risk for reactivation of HBV. Fludarabine is a potent immunosuppressive drug, but risk of HBV reactivation with this drug is unknown and it will be necessary to stay alert for further information.
*6) Prophylactic treatment should preferably be started as soon as possible before starting immunosuppressive therapy/chemotherapy.
*7) Treatment will be started immediately if HBV-DNA quantification shows levels above the detection sensitivity during immunosuppressive therapy/chemotherapy.
*8) The recommended nucleoside analogue to use is entecavir.
*9) Completion of nucleoside analogue treatment may be considered if the following criteria are met.
Patients who were HBs antigen (+) at screening: Meets criteria for completing nucleoside analogue treatment for chronic hepatitis B
Patients who were HBc antibody (+) and/or HBs antibody (+) at screening:

(1) Continued treatment for at least 12 months after immunosuppressive therapy/chemotherapy.
(2) ALT (GPT) normalized during this period (excluding when ALT is abnormal due to a cause other than HBV).
(3) Repeatedly tested negative for HBV-DNA throughout this period.

*10) Patients will be carefully monitored for 12 months after completing nucleoside analogue treatment. Methods listed in the Precautions section of the nucleoside analogue package insert will be used for monitoring. Treatment will be resumed immediately if HBV-DNA quantification shows levels above the detection sensitivity during follow-up.