Cancer Therapy: Clinical

Clinical Cancer Research

A Phase II Study of the Efficacy and Safety of the Combination Therapy of the MEK Inhibitor Refametinib (BAY 86-9766) Plus Sorafenib for Asian Patients with Unresectable Hepatocellular Carcinoma

Ho Yeong Lim[1], Jeong Heo[2], Hye Jin Choi[3], Cheng-Yao Lin[4], Jung-Hwan Yoon[5], Chiun Hsu[6], Kun-Ming Rau[7,8], Ronnie T.P. Poon[9], Winnie Yeo[10], Joong-Won Park[11], Miah Hiang Tay[12], Wen-Son Hsieh[13], Christian Kappeler[14], Prabhu Rajagopalan[15], Heiko Krissel[14], Michael Jeffers[15], Chia-Jui Yen[16], and Won Young Tak[17]

Abstract

Purpose: There is an unmet need for treatment options in hepatocellular carcinoma (HCC). Sorafenib is currently the only approved systemic treatment for HCC. Refametinib, an oral, allosteric MEK inhibitor, has demonstrated antitumor activity in combination with sorafenib *in vitro* and *in vivo*. A phase II study evaluated efficacy and safety of refametinib plus sorafenib in Asian patients with HCC (NCT01204177).

Experimental Design: Eligible patients received twice-daily refametinib 50 mg plus twice-daily sorafenib 200 mg (morning)/400 mg (evening), with dose escalation to sorafenib 400 mg twice daily from cycle 2 if no grade $\geq$2 hand-foot skin reaction, fatigue, or gastrointestinal toxicity occurred. Primary efficacy endpoint: disease control rate. Secondary endpoints: time to progression, overall survival, pharmacokinetic assessment, biomarker analysis, safety, and tolerability.

Results: Of 95 enrolled patients, 70 received study treatment. Most patients had liver cirrhosis (82.9%) and hepatitis B viral infection (75.7%). Disease control rate was 44.8% (primary efficacy analysis; $n = 58$). Median time to progression was 122 days, median overall survival was 290 days ($n = 70$). Best clinical responders had *RAS* mutations; majority of poor responders had wild-type *RAS*. Most frequent drug-related adverse events were diarrhea, rash, aspartate aminotransferase elevation, vomiting, and nausea. Dose modifications due to adverse events were necessary in almost all patients.

Conclusions: Refametinib plus sorafenib showed antitumor activity in patients with HCC and was tolerated at reduced doses by most patients. Frequent dose modifications due to grade 3 adverse events may have contributed to limited treatment effect. Patients with *RAS* mutations appear to benefit from refametinib/sorafenib combination. *Clin Cancer Res; 20(23); 5976–85. ©2014 AACR.*

Introduction

Worldwide, liver cancer is the fifth most commonly diagnosed neoplasm in men and a leading cause of cancer-related death in both men and women (1). Hepatocellular carcinoma (HCC) is the most common subtype of hepatic neoplasm, accounting for 85% to 90% of liver cancer worldwide (2). Prognosis is poor for patients with HCC, with a 5-year survival rate of only 11% (3). Survival rates may improve following surgery (i.e., 5-year survival of 60% to 70% for postsurgical patients with HCC);

[1]Division of Hematology-Oncology, Department of Medicine, Samsung Medical Center; Sungkyunkwan University School of Medicine, Seoul, Republic of Korea. [2]Department of Internal Medicine, Pusan National University School of Medicine and Medical Research Institute, Busan, Republic of Korea. [3]Yonsei Cancer Center, Yonsei University Health System, Seoul, Republic of Korea. [4]Chi-Mei Medical Center, Tainan, Taiwan. [5]Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea. [6]National Taiwan University Hospital, Taipei, Taiwan. [7]Division of Hematology-Oncology, Department of Internal Medicine, Kaohsiung Chang Gung Memorial Hospital of the Chang Gung Medical Foundation, Kaohsiung, Taiwan. [8]Chang-Gung University, College of Medicine, Tao-Yuan, Taiwan. [9]Department of Surgery, University of Hong Kong, Hong Kong. [10]Department of Clinical Oncology, Chinese University of Hong Kong, Hong Kong. [11]National Cancer Center, Goyang-si, Republic of Korea. [12]OncoCare Cancer Centre, Singapore, Singapore. [13]Cancer Science Institute of Singapore, Singapore. [14]Bayer Pharma AG, Berlin, Germany. [15]Bayer HealthCare Pharmaceuticals, Whippany, New Jersey. [16]Internal Medicine, National Cheng Kung University Hospital, Tainan, Taiwan. [17]Department of Internal Medicine, Liver Research Institute, Kyungpook National University School of Medicine, Daegu, Republic of Korea.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Corresponding Author: Ho Yeong Lim, Samsung Medical Center, School of Medicine, Sungkyunkwan University, No. 50 Irwon-dong, Gangnam-gu, Seoul 135-710, Republic of Korea. Phone: 82-2-3410-3459; Fax: 82-2-3410-1754; E-mail: hoylim@skku.edu

doi: 10.1158/1078-0432.CCR-13-3445

Translational Relevance

Patients with hepatocellular carcinoma (HCC) have poor prognosis and a limited choice of effective treatment. The multikinase inhibitor sorafenib is the only systemic treatment currently approved for these patients. Refametinib (BAY 86-9766, RDEA 119), a MEK1 and MEK2 inhibitor, has demonstrated synergistic antitumor activity *in vitro* and *in vivo* when given with sorafenib. Combining these agents may provide an effective and tolerable therapy for patients with HCC. We report findings from a phase II study evaluating the efficacy and safety of refametinib plus sorafenib in Asian patients with HCC. Although not directly compared in this study, observed disease control rate, time to progression, and overall survival were higher compared with previous sorafenib monotherapy studies, especially in relation to Asian patients. Both refametinib and sorafenib were tolerated; however, most patients required dose modifications, mainly due to frequent grade 3 adverse events. The identification of *RAS* mutations within the best clinical responders of this study suggested a distinct clinical benefit for this patient subgroup which merits further investigation.

however, as the majority of patients present with intermediate/advanced disease, only a small proportion (18%) are eligible for surgical intervention (4). Nonsurgical locoregional treatments are available (5); however, these are associated with a high risk of recurrence, and not all patients with HCC benefit from these interventions (6). The development of systemic treatments for HCC is therefore essential.

The multikinase inhibitor sorafenib (Nexavar; Bayer Pharma AG; Onyx Pharmaceuticals) is currently the only approved systemic treatment for HCC (7). Global guidelines currently recommend sorafenib as first-line therapy for patients with HCC and Child–Pugh A/B status (7). This recommendation was based on the statistically significant improvements observed in overall survival (OS) in two phase III HCC studies investigating sorafenib monotherapy (8, 9). Median OS was 10.7 months in the SHARP study, which enrolled 602 patients mainly from North America and Europe (9), and 6.5 months in the ORIENTAL study, which enrolled 271 patients from the Asia-Pacific region (8). Although there were absolute differences in median OS, the decreased risk of progression of disease (PD) or death was similar in both studies, and this may have been related to patients having more advanced disease in the ORIENTAL study (8). In the ORIENTAL study, a clinically meaningful disease control rate (DCR) of 35.3% was demonstrated in patients receiving sorafenib compared with only 15.8% in those receiving placebo (8, 9). The SHARP study reported a significantly higher DCR in sorafenib patients compared with placebo patients (43.5% vs. 31.7%, respectively; $P = 0.002$; ref. 8).

Despite the clinical improvements associated with sorafenib, additional treatment options are required to address the continuing unmet need of patients with HCC. The MAPK/ERK kinases 1 and 2 (MEK1 and 2) have been identified as potential oncology therapeutic targets (9). These enzymes are central components of the RAS signal transduction cascade, one of the main pathways activated in cancer that controls cellular proliferation, angiogenesis, apoptosis, and metastasis (10). Preclinical studies have demonstrated that overexpression of activated MAPK and MEK1 in HCC cell lines is associated with increased tumor growth and apoptotic resistance (11).

Refametinib (BAY 86-9766, RDEA 119; Bayer Pharma AG; Onyx Pharmaceuticals) is an orally available, potent, nonadenosine triphosphate competitive inhibitor of MEK1 and 2. The antitumor activity of this agent as a monotherapy and in combination with sorafenib has been demonstrated *in vitro* and *in vivo* in preclinical studies (12). A phase I study (NCT00785226) reported that refametinib monotherapy was well tolerated and provided clinical benefit for patients with advanced solid tumors, including HCC (13). The combination of refametinib and sorafenib has shown efficacy in preclinical HCC models by 1 or both of 2 potential mechanisms. The first is the blockade of the MAPK signaling pathway at two different points (RAF with sorafenib and MEK with refametinib), the second is the inhibition of parallel signaling pathways (MAPK with refametinib and VEGF receptor–mediated signaling with sorafenib) which showed increased antitumor activity in HCC (14). In addition, increased phosphorylation of MEK and ERK in HCC cells at low concentrations of sorafenib due to paradoxical activation of RAF signaling (13) argues that dual inhibition by combining sorafenib plus refametinib may be an effective approach in HCC.

The promising findings from these studies led to the design of a phase II study to evaluate the efficacy and safety of the combination therapy in Asian patients with HCC with Child–Pugh A status. The study population and planned evaluations were based on those of the ORIENTAL phase III study, the randomized, placebo-controlled study which evaluated sorafenib monotherapy in patients with advanced HCC (15). Additional inclusion and exclusion criteria were added to reflect adverse events (AE) now associated with sorafenib and to enhance the evaluation of refametinib.

Materials and Methods

Study design and objectives

This was a single-arm, open-label, multicenter phase II study of patients with advanced or metastatic HCC from 14 centers in South Korea, Taiwan, Hong Kong, and Singapore (NCT01204177).

All eligible patients received twice-daily refametinib 50 mg in combination with sorafenib. The treatment period was divided into 3-week cycles, for the purposes of data recording. In cycle 1, patients received daily sorafenib 600 mg (200 mg in the morning and 400 mg in the evening), which was escalated to 800 mg (400 mg both morning and evening) in cycle 2 if there were no occurrences of hand-foot skin reaction, fatigue, or gastrointestinal toxicities of grade 2 or above. The combination dose was selected based on a phase Ib study (NCT00785226) in 62 patients, including 19 patients with HCC in the dose-escalation phase, which found twice-daily refametinib 50 mg in combination with twice-daily sorafenib 400 mg to be the maximum tolerated dose in patients with HCC (manuscript in preparation). However, a slightly lower sorafenib dose was used in cycle 1 to potentially minimize early toxicity associated with sorafenib (8).

Doses of sorafenib or refametinib could be modified (interrupted or reduced) in cases of clinically significant hematologic or other toxicities that were possibly, probably, or definitely related to study medications. Dose modifications followed predefined dose levels (Supplementary Table S1).

Treatment continued until PD (defined by Response Evaluation Criteria in Solid Tumors [RECIST] version 1.1), clinical progression (defined by Eastern Cooperative Oncology Group performance status [ECOG PS] of $\geq$3), or withdrawal from study. A safety follow-up was performed 30 to 35 days after the last study treatment administration. Long-term follow-up was planned for every 3 months after the end of study treatment administration.

The primary efficacy endpoint was DCR, defined by the proportion of patients who had a best response rating over the duration of the study of complete response, partial response (PR), or stable disease, according to RECIST version 1.1. To be included in the DCR, stable disease had to be maintained for at least 28 days from the first occurrence of that rating.

Secondary efficacy endpoints were OS, time to progression (TTP), progression-free survival, response rate, and duration of response. Other secondary endpoints were pharmacokinetic (PK) assessments (in a subset of patients), biomarker analysis, safety, and tolerability.

The study followed the Declaration of Helsinki and conformed to Good Clinical Practice guidelines. All local legal and regulatory requirements were met. An independent ethics committee approved the protocol at each study center. Written, informed consent was provided by all patients before study enrollment.

Patients

All patients were aged 18 years or over and had a histologically or cytologically confirmed diagnosis of unresectable advanced or metastatic HCC. Cirrhotic patients must have had a clinical diagnosis of HCC according to the American Association for the Study of Liver Diseases criteria. All patients must have at least 1 untreated, unidimensional measurable lesion, identified by computed tomography or magnetic resonance imaging according to RECIST version 1.1. Eligible patients had an ECOG PS of 0 or 1, Child–Pugh A status, a life expectancy of at least 12 weeks, and normal-range cardiac function, and had stopped receiving any cancer-related therapy at least 4 weeks before screening. Patients with previous or concurrent cancer other than HCC, treated 3 years or less before study entry, were excluded. Other exclusion criteria were renal failure, history of cardiac disease, previous treatment with either refametinib or sorafenib, and any prior systemic anticancer treatment for HCC.

Assessments

Baseline and demographic data were provided in the full analysis group, which included all patients assigned to study treatment. The per protocol group was defined as all patients with no major protocol deviations. The first 58 patients of the per protocol group, in order of study treatment assignment, were included in the primary efficacy analysis. Secondary efficacy analyses were performed on either the per protocol group (DCR by RECIST and modified RECIST) or the full analysis group (progression-free survival, TTP, and OS). The response rate was defined as the proportion of patients with the best tumor response (i.e., confirmed PR or complete response) achieved during or within 30 days after therapy. Safety variables were assessed from the safety analysis group, defined as all patients in the full analysis group with at least 1 intake of study drug.

Radiological (computed tomography/magnetic resonance imaging) tumor assessments were performed locally by the investigator/study site using both RECIST version 1.1 and modified RECIST at screening, then every 6 weeks during treatment until either PD or end of study treatment. The final analysis for this study was performed 12 months after the last patient had received the first study treatment.

In a subset of patients, single-dose PK of refametinib was characterized on cycle 1 day −3, and multiple-dose PK of refametinib and sorafenib was characterized on cycle 2 day 1. Serial blood samples were collected for 72 hours after dose on cycle 1 day −3 for PK analysis of refametinib and its inactive metabolite M17 (BAY 1085159), and over the 12-hour dosing interval for analysis of refametinib, metabolite M17, sorafenib, and its metabolite M2 (BAY 67-3472) on cycle 2 day 1. Plasma samples were analyzed using validated analytical methods.

Mutational analysis was performed by Inostics GmbH using Beads, Emulsions, Amplification, and Magnetics (BEAMing) technology on DNA isolated from plasma samples collected at baseline. An assay cutoff of 0.02% mutant allele for positivity was used. Mutational status was correlated with clinical outcome using descriptive analyses. Plasma from 69 patients was evaluated for the following mutations: *KRAS* (G12A, C, D, R, S, V; G13D; Q61H; A146T), *NRAS* (Q61H, K, L, R), and *BRAF* (V600E). Plasma from the 18 best responders of the 69 patients was also evaluated for mutations in *CSF-1R* (L301S) and *PIK3CA* (E542K; E545G, K; H1047L, R, Y), but because mutations in these genes were not identified in this patient subset, the remaining 51 patients were not evaluated for these mutations.

Safety evaluations were performed at screening, on the first day of study treatment administration, every week for the first 6 weeks, and 3-weekly thereafter. All treatment-emergent AEs, serious AEs (SAE), and drug-related AEs and

SAEs were recorded and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0. The assessment of a causal relationship between an AE and administration of study treatment was assessed separately for refametinib and sorafenib. Safety was also assessed within 7 days after discontinuation of study treatment and 30 to 35 days after the last study treatment administration.

Statistical analysis

Statistical analysis was performed using SAS version 9.2 (SAS Institute Inc.). All analyses were descriptive only. Assuming a 1-sided α of 0.05, a power of 90%, and an improvement in DCR from 0.35 to 0.55, the estimated required sample size for the primary analysis of this study was 58 patients. The null hypothesis for the primary endpoint was rejected if at least 27 out of the planned fixed sample size of 58 patients were classified as responders contributing to DCR.

Results

Of the 95 patients enrolled onto this study, 70 eligible patients received at least 1 dose of study treatment (Supplementary Fig. S1) and all 70 patients were included in the full analysis and safety groups. The most common reason for patients failing screening was failure to meet the eligibility (inclusion or exclusion) criteria.

The patient demographic and baseline disease characteristics are shown in Table 1. All patients within this study population were Asian, with the majority being male. At enrollment, the mean age was 55.4 ± 12.3 years and most patients (74.3%) were 65 years of age or younger.

Just over half of the study population (54.3%) had an ECOG PS of 0, the remainder had an ECOG PS of 1. A high proportion of patients had Barcelona Clinic Liver Cancer stage C (92.9%). Hepatitis B viral infection was the most commonly reported etiology (53 patients; 75.7%) of HCC in this study population. Only 17.1% (12 patients) had hepatitis C viral infection. The majority of patients (82.9%) had liver cirrhosis. Transarterial chemoembolization was the most commonly received prior locoregional treatment (45.7%) for HCC (Table 1). In total, 66 patients (94.3%) discontinued study treatment due to radiological progression (30 patients), AEs associated with PD (11 patients), withdrawal of consent (9 patients), AEs not associated with PD (9 patients), death (9 patients), and PD (1 patient). Four patients were still receiving study treatment at the time of the database cutoff for the final analysis (Supplementary Fig. S1).

Sixty-five patients were included in the per protocol analysis, and 5 patients were excluded mainly due to termination of study treatment before postbaseline tumor evaluation for reasons other than toxicity or progression (3 patients).

Median actual daily dose of study treatment was 83.4 mg refametinib and 541.4 mg sorafenib. This represents 64.8 ± 21.7% of the planned dose of refametinib and 61.6 ± 20.9% of the planned dose of sorafenib, also taking into account dose interruptions. Median duration of treatment was 7.4 weeks (range, 1.0–61.0) for refametinib and 8.0 weeks (range, 1.0–61.0) for sorafenib. Following cycle 1, the sorafenib dose was escalated to 800 mg daily for only 15 patients, with median duration on full sorafenib dose level (800 mg/day) of 66 days (range, 6–250). The proportion of the study population with any study treatment modification (interruption or reduction) was 91.4% and 92.9% for refametinib and sorafenib, respectively. Dose reductions of refametinib and sorafenib were reported in 52.9% and 50.0% of the study population, respectively. AEs were the main reason for both dose reductions and interruptions.

Efficacy

The overall response rate by RECIST was 6.2%; all PRs (Table 2). In the per protocol analysis, confirmed PR was reported in 4 patients (6.2%) and unconfirmed PR in 1 patient (1.5%). The overall response rate by modified RECIST was 9.2%. Stable disease for 10 weeks or more was seen in an additional 22 patients, for a DCR (PR + stable disease) of 44.8% in the efficacy population (Table 2). Of note, the DCR with 26 responders was 1 shy of the prescribed primary endpoint of 27 responders.

DCR by modified RECIST was analyzed in the 65 patients in the per protocol group (Table 2). Twenty-eight patients were classified as responders contributing to a DCR of 43.1%, 22 patients had stable disease, and 6 patients had confirmed PR.

Duration of response was analyzed only in the 4 patients who had achieved confirmed PR (85, 128, 335, and 382 days for these patients).

In the 70 patients in the full analysis group, median TTP by RECIST was 122 days (95% confidence interval [CI], 84–130), and median progression-free survival by RECIST was 114 days (95% CI, 80–125; Fig. 1). Median TTP and median progression-free survival by modified RECIST were 125 days (95% CI, 84–130) and 114 days (95% CI, 81–126), respectively. At database cutoff, 53 patients (75.7%) had either experienced PD or died.

Median OS for this study was 290 days (95% CI, 198–416; Fig. 2). At the time of database cutoff, 44 patients had died (62.9%). Of the patients who died, survival time ranged from 12 to 416 days.

Pharmacokinetic assessments

Sixteen Korean and Taiwanese patients from the overall study population participated in single-dose PK assessments on cycle 1 day −3, 7 of whom also took part in multiple-dose PK evaluations on cycle 2 day 1. The other 9 patients either discontinued study treatments before cycle 2 day 1 or had dose interruption or reduction due to AEs within 7 days before PK assessment and were therefore ineligible for multiple-dose PK assessment.

Single-dose PK parameters for refametinib and metabolite M17 are shown in Supplementary Table S2. Refametinib was well absorbed in this study subgroup, with a median time to maximum concentration of 3 hours and plasma

Table 1. Patient demographics and baseline disease characteristics

	Total ($N = 70$)
Gender, *n* (%)	
Female	10 (14.3)
Male	60 (85.7)
Mean age at enrollment, years (range)	55.4 ± 12.3 (28–78)
Age group, *n* (%)	
≤65 years	52 (74.3)
>65 years	18 (25.7)
ECOG PS, *n* (%)	
0	38 (54.3)
1	32 (45.7)
Target lesions, *n* (%)	
1	17 (24.3)
2	35 (50.0)
3	12 (17.1)
4	4 (5.7)
5	2 (2.9)
Sites of disease, *n* (%)	
Adrenal gland	3 (4.3)
Bone	5 (7.1)
Liver	61 (87.1)
Lung	33 (47.1)
Lymph node	20 (28.6)
Other	15 (21.4)
BCLC stage, *n* (%)	
B	5 (7.1)
C	65 (92.9)
Symptoms of hepatobiliary cancer present at initial diagnosis[a], *n* (%)	35 (50.0)
Etiology of HCC[b], *n* (%)	
Hepatitis B	53 (75.7)
Hepatitis C	12 (17.1)
Alcohol use	10 (14.3)
TNM grading of hepatobiliary cancer at study entry, *n* (%)	
Stage II	2 (2.9)
Stage III	22 (31.4)
Stage IV	46 (65.7)
Macrovascular invasion/extrahepatic spread, *n* (%)	
Macrovascular invasion present; no extrahepatic spread	17 (24.3)
Extrahepatic spread present; no macrovascular invasion	19 (27.1)
Both conditions present	27 (38.6)
Both conditions absent	7 (10.0)
Liver cirrhosis[c], *n* (%)	58 (82.9)
Alpha fetoprotein (ng/mL)[d], *n* (%)	
<400 ng/mL	36 (51.4)
≥400 ng/mL	32 (45.7)
Prior locoregional treatment for HCC (in ≥5% of patients), *n* (%)	
Transarterial chemoembolization	32 (45.7)
Hepatectomy, partial	10 (14.3)
Radiofrequency ablation	9 (12.9)
Lobectomy	5 (7.1)
Percutaneous ethanol injection	4 (5.7)
Transarterial embolization	4 (5.7)
Median time from initial diagnosis to start of study treatment, weeks	36.1
Median time from first progression to start of study treatment, weeks	29.9
Median time from most recent progression to start of study treatment, weeks	5.5

(Continued on the following column)

Table 1. Patient demographics and baseline disease characteristics (Cont'd)

Abbreviations: BCLC, Barcelona Clinic Liver Cancer; TNM, tumor node metastases.
[a]Unknown, $n = 7$; [b]multiple diseases per patient are possible, unknown, $n = 4$; [c]missing, $n = 1$; [d]missing, $n = 2$.

refametinib concentrations reduced with an average half-life of approximately 16 hours. Refametinib single-dose geometric mean maximum observed drug concentration (C_{max}), area under the curve from 0 to 12 hours ($AUC_{(0-12)}$), and AUC from 0 to infinity were 0.99 mg/L, 7.03 mg × h/L, and 14.98 mg × h/L, respectively, with minimal inter-patient variability. Exposure to metabolite M17 was on average approximately 10% of exposure to the parent compound.

Multiple-dose PK results for refametinib, sorafenib, and their metabolites on cycle 2 day 1 are shown in Supplementary Table S3. Refametinib geometric mean multiple-dose C_{max} and $AUC_{(0-12)}$ values were 1.31 mg/L and 11.61 mg × h/L, respectively. Compared with the single-dose refametinib geometric mean $AUC_{(0-12)}$ value of 6.52 mg × h/L in these 7 patients, multiple-dose $AUC_{(0-12)}$ value was higher by approximately 80%, consistent with accumulation expected based on single-dose refametinib PK profile and half-life. For sorafenib, multiple-dose geometric mean values for C_{max} and $AUC_{(0-12)}$ were 4.38 mg/L and 32.70 mg × h/L, respectively.

Biomarker analysis

DNA isolated from baseline plasma samples collected from 69 patients was evaluated for mutations in *RAS* (K, N) and *BRAF* using BEAMing technology. A *RAS* mutation was identified in 4 patients, 3 of whom were still receiving study treatment at the cutoff date used for the final data analysis. These 3 patients had achieved confirmed PR, with duration responses ranging from 128 to 382 days. The fourth patient with a *RAS* mutation discontinued study treatment after 41 days on therapy due to PD. No mutations in *BRAF* were

Table 2. Best overall response according to RECIST (primary efficacy analysis) and modified RECIST (per protocol analysis)

n (%)	Primary efficacy analysis (by RECIST) ($n = 58$)	Per protocol analysis (by RECIST) ($n = 65$)	Per protocol analysis (by modified RECIST) ($n = 65$)
PR	4 (6.9)	4 (6.2)	6 (9.2)
Stable disease	22 (37.9)	24 (36.9)	22 (33.9)
Unconfirmed PR	1 (1.7)	1 (1.5)	1 (1.5)
Unconfirmed stable disease	12 (20.7)	14 (21.5)	16 (24.6)
Progression of disease	14 (24.1)	16 (24.6)	14 (21.5)
Not applicable	1 (1.7)	1 (1.5)	1 (1.5)
Missing	4 (6.9)	5 (7.7)	5 (7.7)
Response rate	4 (6.9)	4 (6.2)	6 (9.2)
Disease control rate	26 (44.8)	28 (43.1)	28 (43.1)

identified in these 69 samples. Plasma DNA from 18 of these 69 patients was also evaluated for mutations in *CSF-1R* and *PIK3CA*, but none was identified.

Safety

Seventy patients received at least 1 dose of study treatment and were therefore eligible for safety analysis. An exploratory analysis of safety was performed for the 4 patients identified with *RAS* mutations.

At least 1 treatment-emergent AE was recorded for each of the 70 patients within the safety analysis (Table 3). The majority of patients had an AE of worst Common Terminology Criteria for Adverse Events grade 3 (60.0%) or grade 4 (20.0%). SAEs were experienced by 62.9% of patients. Eleven patients (15.7%) died due to SAEs, including PD occurring during treatment or within 30 days after the last dose of study treatment. Dose modifications were reported for 95.7% of patients, leading to permanent discontinuation of at least 1 of the study treatments in 28.4% of these patients.

The most frequently reported treatment-emergent AEs (irrespective of relationship to study treatment) were diarrhea (77.1%), rash acneiform (57.1%), aspartate aminotransferase (AST) elevation (51.4%), nausea (47.1%), anorexia (42.9%), and vomiting (42.9%; Supplementary Table S4). The most common grade 3 or 4 AE was AST elevation, 34.3% grade 3 and 11.4% grade 4 (Supplementary Table S4).

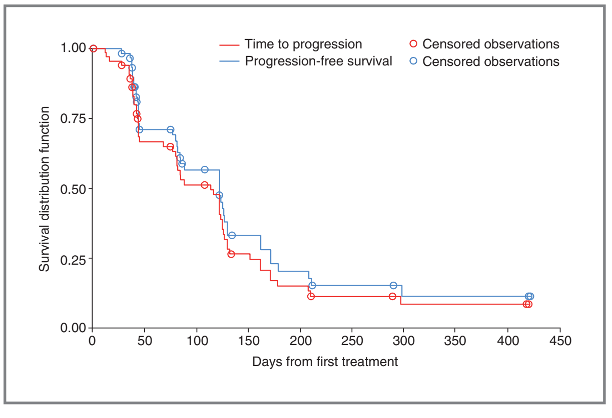

Figure 1. Kaplan–Meier showing time to progression and progression-free survival.

All 70 patients reported at least 1 AE assessed by the investigator as being related to refametinib or sorafenib (Table 4). The relationship of AEs to refametinib or sorafenib was comparable, and most events were considered to be related to both study drugs. A higher proportion of grade 3 or 4 drug-related AEs (81.4% each for refametinib-related AEs and sorafenib-related AEs) was reported than grade 1 or 2 (12.9% each for refametinib-related AEs and sorafenib-related AEs). The most commonly reported AEs attributed to each study drug were diarrhea, rash acneiform, AST elevation, hypertension, vomiting, nausea, alanine aminotransferase elevation, and anorexia (Supplementary Table S5). AST elevation was the most commonly reported grade 3 or 4 AE for both drugs: 28.6% grade 3 and 10% grade 4 for both drugs. SAEs related to each study drug were reported by 42.9% of patients, and all were considered related to both refametinib and sorafenib. The most frequent drug-related SAEs (occurring in 2 or more patients) were increased AST (5 patients; 7.1%), diarrhea (4 patients; 5.7%), and upper gastrointestinal hemorrhage (2 patients; 2.9%; Supplementary Tables S6 and S7). Four patients (5.7%) experienced fatal SAEs that were considered to be related to both study drugs, including death (not otherwise specified), hepatic failure, tumor lysis syndrome, encephalopathy, and sepsis. Refametinib- and sorafenib-related AEs resulting in either dose modification or permanent discontinuation of study treatment were experienced by 90.0% and 17.1% of patients, respectively.

Cardiac, ophthalmic, and neurologic events were considered to be of special interest during this study. Three serious cardiac disorder events (4.3%) of grade 3 were reported as related to both study treatments: acute coronary syndrome, left ventricular systolic dysfunction, and ventricular tachycardia. Sixteen (22.9%) ophthalmic events of grades 1 to 3 were reported (blurred vision, cataracts, dry eye, floaters,

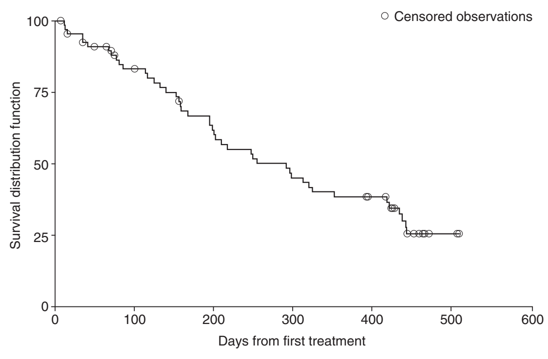

Figure 2. Kaplan–Meier showing OS.

retinal vascular disorders, retinopathy, other eye disorders); 12 of these were considered to be related to the study treatments. Nervous system disorder events were observed in 32 patients (45.7%). The most common neurologic events by Common Terminology Criteria for Adverse Events term were headache in 11 patients (15.7%), dizziness in 10 patients (14.3%), and encephalopathy in 7 patients (10.0%), which reflected the impaired liver function of this study population. Most neurologic events were grade 1 or 2, and the outcome of most events was reported as resolved. Eleven patients had events assessed as related to refametinib and 12 as related to sorafenib. Seven patients had serious neurologic events, 2 of which, grade 4 seizure and grade 5 encephalopathy, were considered related to both study drugs. No significant changes from baseline were reported in electrocardiogram findings, blood pressure, or heart rate at end of study treatment. A continuous decrease in body weight was observed within the study population, which may reflect PD in these patients.

Each of the 4 patients identified with *RAS* mutations experienced at least 1 treatment-emergent AE: diarrhea, alanine aminotransferase elevation, AST elevation, and rash acneiform. All 4 patients had a dose interruption and reduction of study treatment due to AEs, although no patient discontinued study treatment due to AEs. Two of the patients with *RAS* mutations experienced SAEs: grade 3 retinopathy in 1 patient; grade 3 ileus, grade 3 and grade 4 AST elevation, and grade 1 fever in another patient. Except for grade 3 ileus, all SAEs were attributed to the study treatments.

Table 3. Overview of treatment-emergent AEs

n (%)	Total ($N = 70$)
Any AE	70 (100)
Worst CTCAE grade of AE	
Grade 1	0
Grade 2	3 (4.3)
Grade 3	42 (60.0)
Grade 4	14 (20.0)
Any serious AE	44 (62.9)
Death[a]	11 (15.7)
AE leading to dose modification[b]	67 (95.7)
AE leading to permanent discontinuation of study drug[c]	19 (27.1)

Abbreviation: CTCAE, Common Terminology Criteria for Adverse Events.
[a]Including during treatment or within 30 days after last dose of study treatment was taken.
[b]Modifications include delays, interruptions, and reductions.
[c]Including discontinuation due to death.

Discussion

This phase II study was designed to evaluate the efficacy and safety of the combination therapy of refametinib plus sorafenib for Asian patients with HCC. The study population was selected to be as similar as possible to that of the large phase III randomized, placebo-controlled ORIENTAL study, which evaluated sorafenib monotherapy (16, 17). In addition, the methodology for efficacy and safety evaluation was based on the ORIENTAL study. However, our study used a lower starting dose of sorafenib from that used in the ORIENTAL study (600 mg per day instead of 800 mg per day) to potentially reduce the early onset of side effects, with subsequent dose escalation permitted from cycle 2 if there were no occurrences of hand-foot skin reaction, fatigue, or gastrointestinal AEs (grade 2 or above).

The baseline demographic and disease characteristics of our study population are comparable with those reported for the ORIENTAL study population (8). Clinical trials have generally included only patients with stable liver function (Child–Pugh A status), as liver dysfunction events associated with Child–Pugh B/C status may influence results. Similarly to the SHARP and ORIENTAL studies, our study enrolled only patients with Child–Pugh A status.

Although DCR was higher (44.8%) in our study than that reported in the phase III sorafenib monotherapy ORIENTAL study (35.3%; ref. 8), the prescribed primary efficacy endpoint was not reached. The DCR improvements observed in our study compared with previous sorafenib studies are also reflected in the survival findings, especially in relation to Asian patients. Median TTP (4.1 months) and OS (9.7 months) compared favorably with the sorafenib monotherapy ORIENTAL study (median TTP, 2.8 months; median OS, 6.5 months; ref. 8). Notably, in a Japanese prospective study investigating the efficacy and safety of sorafenib in 96 patients with advanced HCC, median OS was higher (11.6 months) but TTP was lower (3.2 months) than in our study (8). Although direct comparison of our findings with previous sorafenib monotherapy studies must be done cautiously as study populations may not be comparable (18), the combination of sorafenib plus refametinib did not result in markedly higher response rates or prolonged survival. The single-arm design of this study, although

Table 4. Overview of drug-related AEs

n (%)	Refametinib-related (n = 70)	Sorafenib-related (n = 70)
Any drug-related AE	70 (100)	70 (100)
Worst CTCAE grade of drug-related AE		
Grade 1	1 (1.4)	0
Grade 2	8 (11.4)	9 (12.9)
Grade 3	46 (65.7)	46 (65.7)
Grade 4	11 (15.7)	11 (15.7)
Any drug-related serious AE	30 (42.9)	30 (42.9)
Death[a]	4 (5.7)	4 (5.7)
Drug-related AE leading to dose modification[b]	63 (90.0)	63 (90.0)
Drug-related AE leading to permanent discontinuation of study drug[c]	12 (17.1)	12 (17.1)

Abbreviation: CTCAE, Common Terminology Criteria for Adverse Events.
[a]Including during treatment or within 30 days after last dose of study treatment was taken.
[b]Modifications include delays, interruptions, and reductions.
[c]Including discontinuation due to death.

appropriate to assess initial efficacy and safety signals, is a source of limitation in that without a direct comparator the primary endpoint can only be assessed in the context of historical data from previous studies.

PK results in Asian patients enrolled in this study were compared with historical data from western patients enrolled in 2 U.S.-based, dose-escalation phase I studies assessing refametinib as monotherapy (NCT00610194; ref. 18) and in combination with sorafenib (NCT00785226) in patients with advanced cancer (data on file; Bayer HealthCare Pharmaceuticals). Overall, average single-dose PK parameters were generally comparable between Asian and western study populations enrolled in the monotherapy study. Refametinib exposure was generally lower in patients enrolled in the sorafenib combination study conducted in the United States. In our study, refametinib $AUC_{(0-12)}$ increased by approximately 80% following twice-daily dosing, which is comparable with the 2-fold increase reported in the refametinib monotherapy phase I study. The ratio of metabolite M17 to refametinib concentration was similar (generally less than 30%) in Asian and U.S. patients. With regard to sorafenib, multiple-dose PK data from our study are comparable with exposure values reported from a phase I study of sorafenib in Japanese patients with advanced refractory solid tumors (14) and other Asian (Chinese and Taiwanese) populations (data on file). Overall, our study suggests that there are no significant ethnic differences in PK of refametinib between Asian and western populations, and that refametinib appears to be well absorbed in Asian patients with HCC.

A biomarker analysis was performed using plasma DNA from 69 patients to investigate a possible correlation between mutational status and clinical outcome. The frequency of mutations for the genes screened was quite low, with only 4 *RAS* mutations detected (5.8%) and no *BRAF* mutations detected. The frequency of patients with HCC with mutant *RAS* identified in our study (5.8%) is similar to the frequency of *RAS* mutations reported in patients with HCC (5%; ref. 19). A *RAS* mutation was identified in 3 of the best clinical responders in this study (i.e., patients who had achieved PR and had received treatment for a duration ranging from 128 to 382 days at database cutoff). In contrast, the majority of poor clinical responders in this study had wild-type *RAS*. These results are consistent with preclinical evidence demonstrating increased activity of MEK inhibitors against cancer cells harboring *RAS* mutations (20). Therefore, in this study, patients with HCC with mutant *RAS* appeared to exhibit a better clinical response to refametinib plus sorafenib compared with patients with wild-type *RAS*. The concordance of *RAS* mutation analysis by BEAMing with tissue analysis has not been confirmed in HCC, although it has been for other solid tumors (20). Thus, as the biomarker analysis in our study was also performed retrospectively in a relatively small uncontrolled patient population, it should be considered to be exploratory. Further investigation is required to assess the clinical activity of this drug combination in patients with HCC with mutant *RAS*.

At least 1 treatment-emergent AE and 1 drug-related AE were experienced by each patient in the study population, most of which were grade 3. The majority of AEs were resolved by temporary dose modifications, concomitant treatments, and supportive care. Liver disorders (i.e., alanine aminotransferase and AST elevation), gastrointestinal disorders (i.e., diarrhea and vomiting), and rash acneiform were more frequently reported than in the two phase III sorafenib monotherapy studies. Notably, grade 2 or 3 diarrhea, one of the most frequently reported AEs related to both study treatments, has recently been identified as a positive predictor of improved OS for patients receiving sorafenib treatment (21). The incidence of grade 3 or 4 drug-related AST elevations was markedly higher in this study (in 38.3% of patients) compared with experience with either monotherapy in patients with HCC, but also compared with combination treatment in phase I. The underlying mechanism is not fully understood, but is likely to be due to the underlying level of liver disease in this patient population.

The actual daily dose of both refametinib and sorafenib received was less than the planned doses, 65% and 62%, respectively, taking into account dose interruptions. Patients initiated sorafenib at a lower dose (600 mg) for cycle 1 and then could escalate to 800 mg for cycle 2. However, only a small percentage of patients were able to escalate. The incidence of dose modifications (reductions or interruptions) was notably higher in this study (95.7%) compared with the ORIENTAL and SHARP studies (30.9% and 26.0%, respectively). This may be largely a result of the high incidence of reported grade 3 AEs in our study. Of note, the incidence of treatment discontinuation due to AEs was lower in our study (27.1%) than in the SHARP study (38.0%), but higher than in the ORIENTAL study (19.5%). There was a relatively high incidence of treatment-related deaths reported in our study; however, the majority of deaths that occurred during treatment and within 30 days after the last study treatment dose were due to HCC-related events, and of those deaths that occurred over 30 days after the last dose of study treatment, the majority were due to PD.

Therefore, the combination of refametinib and sorafenib appeared to be clinically active, although the high incidence of dose modifications may have compromised efficacy. That the majority of patients who responded to this regimen had mutant *KRAS* tumors is an observation that requires future investigation. Because of the high incidence of high-grade AEs experienced by patients in this study, a phase III study investigating the combination therapy of refametinib plus sorafenib in an unselected patient population is not merited. However, the finding that *RAS* mutations were identified in patients with long-lasting PR suggests that this patient subgroup may have a distinct benefit from refametinib treatment. Further clinical trials are currently being conducted to explore this observation and to learn if a similar efficacy benefit can be obtained from refametinib monotherapy or if combination therapy with sorafenib is needed.

Disclosure of Potential Conflicts of Interest

C. Kappeler and H. Krissel have ownership interest (including patents) in Bayer Pharma AG. W.Y. Tak reports receiving a commercial research grant from Samil Pharmaceutical and a speakers bureau honoraria from Bayer HealthCare, and is a consultant/advisory board member for Bayer HealthCare and Gilead Sciences Korea. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

The authors take full responsibility for the scope, direction, and content of the article and have approved the submitted article.

Authors' Contributions

Conception and design: C.-Y. Lin, C. Hsu, K.-M. Rau, R.T.P. Poon, C. Kappeler, P. Rajagopalan, H. Krissel
Development of methodology: H.Y. Lim, W.-S. Hsieh, C. Kappeler, H. Krissel, M. Jeffers, W.Y. Tak
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H.Y. Lim, J. Heo, H.J. Choi, C.-Y. Lin, J.-H. Yoon, C. Hsu, R.T.P. Poon, W. Yeo, J.-W. Park, M.H. Tay, W.-S. Hsieh, M. Jeffers, C.-J. Yen, W.Y. Tak
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H.Y. Lim, J. Heo, C.-Y. Lin, C. Kappeler, P. Rajagopalan, H. Krissel, M. Jeffers, C.-J. Yen, W.Y. Tak
Writing, review, and/or revision of the manuscript: H.Y. Lim, J. Heo, H.J. Choi, C.-Y. Lin, J.-H. Yoon, C. Hsu, R.T.P. Poon, W. Yeo, M.H. Tay, W.-S. Hsieh, C. Kappeler, P. Rajagopalan, H. Krissel, C.-J. Yen, W.Y. Tak
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J. Heo, K.-M. Rau, W.-S. Hsieh, C.-J. Yen
Study supervision: J. Heo, C.-Y. Lin, H. Krissel

Acknowledgments

The authors thank Katherine Wilson, PhD, at Complete HealthVizion for her assistance in the preparation and revision of the draft manuscript, based on detailed discussion and feedback from all the authors.

Grant Support

Editorial assistance was funded by Bayer HealthCare Pharmaceuticals.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received December 20, 2013; revised July 2, 2014; accepted August 22, 2014; published OnlineFirst October 7, 2014.

References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012;62:10–29.
2. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology 2007;132: 2557–76.
3. London WT, McGlynn KA. Liver cancer. In: Schottenfeld D, Fraumeni J.F.Jr, editors. Clear epidemiology and prevention. New York: Oxford University Press; 2006. p. 763–86.
4. Hung H. Treatment modalities for hepatocellular carcinoma. Curr Cancer Drug Targets 2005;5:131–8.
5. Poon RTP, Fan ST, Tsang FHF, Wong J. Locoregional therapies for hepatocellular carcinoma: a critical review from the surgeon's perspective. Ann Surg 2002;235:466–86.
6. Harrison LE, Koneru B, Baramipour P, Fisher A, Barone A, Wilson D, et al. Locoregional recurrences are frequent after radiofrequency ablation for hepatocellular carcinoma. J Am Coll Surg 2003;197: 759–64.
7. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology: Hepatobiliary Cancers (Version 2.2014). Available from: http://www.nccn.org/professionals/physician_gls/pdf/hepatobiliary.pdf.
8. Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol 2009;10:25–34.
9. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359:378–90.
10. Iverson C, Larson G, Lai C, Yeh LT, Dadson C, Weingarten P, et al. RDEA119/BAY 869766: a potent, selective, allosteric inhibitor of MEK1/2 for the treatment of cancer. Cancer Res 2009;69:6839–47.
11. Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene 2007; 26:3291–310.

12. Huynh H, Nguyen TTT, Pierce Chow KH, Tan PH, Soo KC, Tran E. Over-expression of the mitogen-activated protein kinase (MAPK) kinase (MEK)-MAPK in hepatocellular carcinoma: its role in tumor progression and apoptosis. BMC Gastroenterol 2003;3:19.
13. Schmieder R, Puehler F, Neuhaus R, Kissel M, Adjei AA, Miner JN, et al. Allosteric MEK1/2 inhibitor refametinib (BAY 86-9766) in combination with sorafenib exhibits antitumor activity in preclinical murine and rat models of hepatocellular carcinoma. Neoplasia 2013;15:1161–71.
14. Weekes CD, Von Hoff DD, Adjei AA, Leffingwell DP, Eckhardt SG, Gore L, et al. Multicenter phase I trial of the mitogen-activated protein kinase 1/2 inhibitor BAY 86-9766 in patients with advanced cancer. Clin Cancer Res 2013;19:1232–43.
15. Breunig C, Mueller BJ, Umansky L, Wahl K, Hoffmann K, Lehner F, et al. BRaf and MEK inhibitors differentially regulate cell fate and microenvironment in human hepatocellular carcinoma. Clin Cancer Res 2014;20:2410–23.
16. Marrero JA, Lencioni R, Ye SY, Kudo M, Bronowicki JP, Chen XP, et al. Final analysis of GIDEON (Global Investigation of therapeutic DEcisions in hepatocellular carcinoma [HCC] and Of its treatment with sorafeNib [Sor]) in >3000 Sor-treated patients (pts): clinical findings in pts with liver dysfunction [abstract 4126]. J Clin Oncol (Meeting Abstracts) 2013;31.
17. Baselga J, Segalla JG, Roche H, Del Giglio A, Pinczowski H, Ciruelos EM, et al. Sorafenib in combination with capecitabine: an oral regimen for patients with HER2-negative locally advanced or metastatic breast cancer. J Clin Oncol 2012;30:1484–91.
18. Nakano M, Tanaka M, Kuromatsu R, Nagamatsu H, Sakata K, Matsugaki S, et al. Efficacy, safety, and survival factors for sorafenib treatment in Japanese patients with advanced hepatocellular carcinoma. Oncology 2013;84:108–14.
19. Minami H, Kawada K, Ebi H, Kitagawa K, Kim YI, Araki K, et al. Phase I and pharmacokinetic study of sorafenib, an oral multikinase inhibitor, in Japanese patients with advanced refractory solid tumors. Cancer Sci 2008;99:1492–8.
20. Wellcome Trust Sanger Institute. Catalogue of somatic mutations in cancer (COSMIC). Available from: http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/. Accessed October 22, 2014.
21. Higgins MJ, Jelovac D, Barnathan E, Blair B, Slater S, Powers P, et al. Detection of tumor PIK3CA status in metastatic breast cancer using peripheral blood. Clin Cancer Res 2012;18:3462–9.