Clinical Cancer Research

# A Phase II and Biomarker Study of Sorafenib Combined with Modified FOLFOX in Patients with Advanced Hepatocellular Carcinoma

Lipika Goyal[1,2], Hui Zheng[3], Thomas A. Abrams[2,4], Rebecca Miksad[2,5], Andrea J. Bullock[2,5], Jill N. Allen[1,2], Matthew B. Yurgelun[2,4], Jeffrey W. Clark[1,2], Avinash Kambadakone[6], Alona Muzikansky[4], Michelle Knowles[1], Aralee Galway[1], Anthony J. Afflitto[1], Caroline F. Dinicola[1], Eileen Regan[4], Tai Hato[2,7], Emilie Mamessier[2,7], Kohei Shigeta[2,7], Rakesh K. Jain[2,7], Dan G. Duda[2,7], and Andrew X. Zhu[1,2]

## Abstract

**Purpose:** Sorafenib is a standard first-line treatment for advanced hepatocellular carcinoma (HCC). The phase III SHARP trial showed a median time-to-progression (mTTP) of 5.5 months, overall response rate (ORR) of 2%, and median overall survival (mOS) of 10.7 months with sorafenib. FOLFOX4 has shown modest activity in advanced HCC. We evaluated the combination of sorafenib and modified (m)FOLFOX in a single-arm, multicenter phase II study.

**Patients and Methods:** The study included Child–Pugh A patients with advanced HCC and no prior systemic therapies. Patients received sorafenib 400 mg twice a day for 2 weeks, followed by concurrent mFOLFOX [5-fluorouracil (5-FU) 1,200 mg/m$^2$/day for 46 hours, leucovorin 200 mg/m$^2$, and oxaliplatin 85 mg/m$^2$ biweekly]. The primary endpoint was mTTP with an alternative hypothesis of 7 months, and secondary endpoints included ORR, mOS, and circulating biomarkers.

**Results:** The study enrolled 40 patients: HCV/EtOH/HBV, 43%/28%/13%; Child–Pugh A5, 70%. Notable grade 3/4 adverse events (AE) included AST/ALT elevation (28%/15%), diarrhea (13%), hyperbilirubinemia (10%), hand–foot syndrome (8%), and bleeding (8%). mTTP was 7.7 months [95% confidence interval (CI): 4.4–8.9], ORR 18%, and mOS 15.1 months (7.9–16.9). Sorafenib + mFOLFOX increased plasma PlGF, VEGF-D, sVEGFR1, IL12p70, and CAIX and CD4$^+$ and CD8$^+$ effector T lymphocytes and decreased plasma sVEGFR2 and s-c-KIT and regulatory T cells (Tregs). Shorter TTP was associated with high baseline sVEGFR1. Shorter TTP and OS were associated with increases in Tregs and CD56$^{Dim}$ natural killer (NK) cells after sorafenib alone and plasma sMET after combination treatment (all $P < 0.05$).

**Conclusions:** Sorafenib + mFOLFOX met the prespecified endpoint with encouraging efficacy but moderate hepatotoxicity. Thus, this regimen may be effective in select patients with adequate liver reserve. Biomarker evaluations suggested a correlation between time-to-progression (TTP) and angiogenic biomarkers and circulating Tregs.

## Introduction

An estimated 850,000 patients receive a new diagnosis of primary liver cancer annually worldwide, and with its poor prognosis, an alarmingly similar number of patients die from it each year (810,000; ref. 1). Hepatocellular carcinoma (HCC) accounts for the vast majority of cases of primary liver cancer, and its incidence is on the rise both in the United States and globally (2, 3). Most commonly seen in patients with chronic viral hepatitis and alcoholic cirrhosis, nonalcoholic fatty liver disease is increasingly recognized as a risk factor for HCC (4). The comorbid cirrhosis that often accompanies the diagnosis curbs patients' ability to tolerate local and systemic treatment regimens and can lead to hepatic decompensation and cytopenias.

Limited strides have been made in the first-line treatment of advanced HCC since the multi-kinase inhibitor, sorafenib, has become the standard of care in 2007. Sorafenib inhibits multiple targets including VEGFR, PDGFR, and the RAF family kinases and offers an overall response rate (ORR) of 2% to 3%, time-to-progression (TTP) of up to 5.5 months, and a median overall survival (OS) of up to 10.7 months (5, 6). No single agent or combination has significantly improved median OS in randomized phase III trials against sorafenib to date (7–10), although lenvatinib demonstrated noninferiority (11).

In Asia, FOLFOX4 has shown a signal of activity in patients with advanced HCC in the first-line setting. In a randomized phase III trial (EACH), FOLFOX4 was compared with doxorubicin in an Asian population where approximately 75% of patients had no

[1]Department of Medicine, Massachusetts General Hospital Cancer Center, Boston, Massachusetts. [2]Harvard Medical School, Boston, Massachusetts. [3]Biostatistics Center, Massachusetts General Hospital, Boston, Massachusetts. [4]Dana-Farber Cancer Institute, Boston, Massachusetts. [5]Beth Israel Deaconess Medical Center, Boston, Massachusetts. [6]Department of Radiology, Massachusetts General Hospital, Boston, Massachusetts. [7]Steele Laboratories for Tumor Biology, Department of Radiation Oncology, Massachusetts General Hospital Research Institute, Boston, Massachusetts.

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

D.G. Duda and A.X. Zhu are the cosenior authors of this article.

**Corresponding Author:** Lipika Goyal, Massachusetts General Hospital Cancer Center, Harvard Medical School, POB/Lawrence House, 10 North Grove Street, Boston, MA 02114. Phone: 617-726-2000; Fax: 617-724-3166; E-mail: lgoyal@partners.org

**doi:** 10.1158/1078-0432.CCR-18-0847

## Translational Relevance

In a phase II trial in patients with advanced hepatocellular carcinoma (HCC), the combination of sorafenib and mFOLFOX demonstrated encouraging efficacy but given the moderate hepatotoxicity, it may be best suited for select patients with adequate liver reserve. Biomarker analysis showed significant changes in angiogenic and immune biomarkers, such as baseline plasma sVEGFR1, and change in circulating sMET and effector natural killer (NK) and T-cell subsets, as well as associations with outcomes that should be further evaluated for sorafenib alone or in combination with other therapies.

prior systemic therapy for advanced HCC (12). FOLFOX4 consisted of bolus 5-fluorouracil (5-FU) 400 mg/m$^2$, infusional 5-FU 600 mg/m$^2$ over 22 hours on day 1 and 2, bolus leucovorin 100–200 mg/m$^2$, and oxaliplatin 85 mg/m$^2$, repeated every 2 weeks. FOLFOX4 demonstrated an improved ORR (8.2% vs. 2.7%, $P = 0.02$), a median progression-free survival (PFS; 2.93 vs. 1.77 months, $P = 0.0002$), and a trend toward improved median OS (6.40 vs. 4.97 months, $P = 0.07$) compared with doxorubicin. The regimen had no significant increased toxicity over doxorubicin except for more frequent peripheral neuropathy. The FOLFOX4 regimen gained approval for advanced HCC in China and has served as an empiric treatment option by some physicians in the United States before the recent approvals of the multikinase inhibitor, regorafenib, and the anti-PD-1 antibody, nivolumab, in 2017 (13, 14).

Preclinical studies provided rationale for the therapeutic potential of combining sorafenib with traditional chemotherapy for the treatment of HCC. Specifically, the antiangiogenic effects of multikinase inhibitors like sorafenib may alter the structure and function of tumor vasculature and thereby impact drug delivery of cytotoxic agents (15, 16). For example, anti-VEGF agents may transiently "normalize" the tumor vasculature and increase blood perfusion. This may provide a "window of opportunity" during which antiangiogenic agents increase cancer cell exposure to cytotoxic drugs and potentially increase cell killing, as indicated by studies of anti-VEGF therapy with chemotherapy in lung cancer (17). However, high-dose and/or chronic VEGF inhibition can lead to profound vascular pruning and decreased blood perfusion and drug delivery in some tumors, as also seen in lung cancer (18). The interaction of sorafenib with chemotherapy remains largely unexplored in HCC, both in terms of clinical efficacy and impact on tumor angiogenesis and cellular immunity.

This phase II and biomarker study was designed to evaluate the combination of sorafenib with modified (m)FOLFOX in the first-line systemic treatment of patients with advanced HCC. Of note, the safety of sorafenib with FOLFOX has been evaluated in first-line treatment for metastatic colon cancer in the RESPECT trial, a randomized phase II trial of mFOLFOX6 combined with sorafenib versus placebo (19). Toxicity was manageable but rates of grade 3 and 4 neutropenia, diarrhea, and hand–foot syndrome were higher in the sorafenib arm, so the regimen for this study was modified to eliminate the 5-FU 400 mg/m$^2$ bolus. Moreover, the study design included a 2-week lead-in with sorafenib alone to explore pharmacodynamic and response biomarkers for the first time for this combination therapy. We report the final results of this single-arm, multicenter study, including the correlative studies of circulating biomarkers, with focus on antiangiogenic and immune cytokines and cells consistent with mechanism of action of sorafenib (20–22).

## Patients and Methods

### Study population

Patients with histologically proven, measurable, advanced HCC with no prior treatment with systemic therapy were eligible. Patients were required to have either Barcelona Clinic Liver Cancer (BCLC) stage C disease or BCLC stage B disease with inability to tolerate or failed treatment with transarterial chemoembolization (TACE). Other inclusion criteria included Child–Pugh score of A5 or A6, Eastern Cooperative Oncology Group (ECOG) performance status $\leq$1; absolute neutrophil count $\geq$1,500/μL, platelet count $\geq 1 \times 10^5$/μL, hemoglobin $\geq$10 g/dL; serum creatinine $\leq$2.0 mg/dL; total bilirubin <2.0 mg/dL, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) $\leq 6\times$ ULN; age $\geq$18 years; and life expectancy of $\geq$12 weeks. Patients with a prior history of liver-directed therapy for their HCC (chemoembolization, radioembolization, bland embolization, radiotherapy, radiofrequency ablation, and microwave ablation) were eligible if the liver-directed therapy was performed more than 4 weeks prior to their first dose of sorafenib and if measurable disease was present outside of previously treated field or due to progression in the field.

Key exclusion criteria included uncontrolled hypertension defined as BP >150 mm Hg systolic, or >90 mm Hg diastolic despite optimal medical management; ascites refractory to diuretics; grade 3 or 4 bleeding within 4 weeks of enrollment; presence of a nonhealing wound or ulcer, or a bone fracture; history of a bleeding diathesis or coagulopathy; class III or IV congestive heart failure; active coronary artery disease or unstable angina; cardiac arrhythmias requiring antiarrhythmic therapy other than beta blockers or digoxin; QTc >500 milliseconds; history of organ allograft; any malabsorption condition; concurrent malignancy; CLIP score >3; clinically apparent brain metastases or carcinomatous meningitis; major surgery within 30 days; alternative investigational agent within 28 days; known HIV infection; and pregnancy or lactation. Concomitant use of therapeutic anticoagulation with a vitamin K antagonist or with heparin, potent CYP3A4 inducers, and aspirin >100 mg daily were prohibited. Patients were recruited at the Dana Farber/Harvard Cancer Center (Boston, MA), and the protocol was approved by the Partners Institutional Review Board. The trial was conducted in accordance to the ethical guidelines of the Declaration of Helsinki. All patients provided written informed consent before study participation (NCT01775501).

### Study design

Patients received sorafenib 400 mg orally twice a day continuously for a 14-day lead-in period followed by concurrent modified FOLFOX–5-FU continuous infusion 1,200 mg/m$^2$/day for 46 hours, and leucovorin 200 mg/m$^2$ bolus and oxaliplatin 85 mg/m$^2$ initiated on day 1 and 15 of each 28-day cycle. Dose reductions were permitted down to two dose levels of sorafenib, 400 mg orally daily and 200 mg orally daily. Dose reductions of 5-FU and oxaliplatin were allowed up to four times with a 25% dose reduction of the previous each time. The primary endpoint was TTP, and secondary endpoints included ORR, OS, and safety and tolerability, and correlation of circulating cellular and plasma biomarkers with response.

Patients were evaluated for response serologically with AFP levels and radiographically with CT and/or MRI every 8 weeks. Response was determined by an independent radiologic review using RECIST version 1.1 and modified RECIST (mRECIST), and patients continued treatment until disease progression, unacceptable toxicity, withdrawal of consent, or physician's decision to discontinue. Patients were monitored for safety weekly for the first 7 weeks and then every other week thereafter. Safety evaluations included vital signs, physical exam, performance status evaluation, complete blood count, blood chemistries, coagulation studies, amylase, lipase, uric acid, lactate dehydrogenase, urinalyses, and ECG. Adverse events (AE) were assessed according to the NCI Common Toxicity Criteria for Adverse Events (NCI CTCAE) version 4.0. Safety data were monitored on an ongoing basis by an independent Data and Safety Monitoring Board (DSMB). Bayer Pharmaceuticals provided funding support for this trial.

### Correlative studies

***Plasma biomarkers.*** Analyses of potential plasma biomarkers of sorafenib were performed by measuring proteins in the plasma at baseline (on or prior to day 1 of sorafenib) and their changes after 3 days (range 3–6 days) and 15 days (range 15–18) of sorafenib alone, and after 29 days (range, 28–31), and 43 days (range, 43–46) during combination treatment, with ranges available to accommodate weekends and holidays. Fresh blood samples were collected in 15-mL EDTA tubes and centrifuged for 30 minutes at 1,500 rpm at 4°C without brake to separate plasma and buffy coats. Plasma samples were stored at −70°C until analysis.

Plasma analysis was carried out for a panel of circulating proangiogenic and proinflammatory biomarkers: VEGF, placental growth factor (PlGF), soluble (s)VEGFR1/FLT-1, basic fibroblast growth factor (bFGF), VEGF-C, VEGF-D, sTIE-2, IL1β, IL2, IL4, IL6, IL8, IL10, IL12p70, IFNα, and TNFα using multiplex protein array plates from Meso Scale Discovery; and carbonic anhydrase (CA)IX, sVEGFR2, s-c-KIT, and sMET using ELISA kits from R&D Systems and Life Technologies/Invitrogen, respectively. All samples were run in duplicate and included two controls and a proficiency sample, with an acceptable criterion of 75% to 125% recovery. Analysis was performed in the Clinical Laboratory Improvement Amendments–certified core of the Steele Laboratories at Massachusetts General Hospital (Boston, MA).

***Cellular biomarkers.*** After removal of the plasma (see above), the buffy coat was washed in 5% BSA in PBS solution, and centrifuged for 20 minutes at 1,500 rpm. Ammonium-chloride-potassium lysing buffer (Lonza) was used to lyse red blood cells, and the leukocytes were stained using the following antibodies: CD3-PerCP/Cy5.5 (UCHT1), CD56-APC (B159), CD4-FITC (RPA-T4), CD8-APC/Cy7 (RPA-T8), CD25-PE (2A3), and CD127-PE/Cy7 (HIL-7R-M21; all BD Biosciences). Flow cytometric analysis was performed on an LSR-II cytometer (BD Biosciences). The gating strategy is shown in Supplementary Fig. S1.

### Statistical analysis

A sample size of 40 achieved 80% power to detect the difference between the null hypothesis median time-to-progression (mTTP) of 5 months and an alternative hypothesis median TTP of 7 months. This was at a 10% significance level using a one-sided test based on the elapsed time, assuming a study follow-up period of 24 months. All patients who received at least one dose of sorafenib were included for safety and efficacy analysis.

TTP was defined as the time from trial registration to evidence of either radiographic progression as defined by RECIST 1.1 and mRECIST or clinical disease progression as determined by the investigator, whichever occurred first. Patients who discontinued treatment for other reasons were censored at the date of trial discontinuation. OS was defined as the time from trial registration to death from any cause. Patients with incomplete follow-up or without adequate disease evaluations were censored at date last documented to be progression-free. Kaplan–Meier estimates of median TTP and OS were calculated along with their corresponding 95% confidence intervals (CI). For biomarker changes over time, we used the Wilcoxon signed-rank test. Given the exploratory nature of these analyses, we did not correct for multiple comparisons. Cox proportional hazards regression model was used to assess the association of biomarkers with TTP and OS. The point estimates in the Cox regression model with a 95% CI are presented. All analyses were performed using SAS 9.4 (SAS Institute).

### Data availability statement

Full datasets are available on request due to privacy restrictions. The data that support the findings of this study and data not shown are available on request from the corresponding and senior authors L. Goyal, D.G. Duda, and A.X. Zhu. The data are not publicly available due to them containing information that could compromise research participant privacy.

## Results

### Patient characteristics

The study completed the targeted accrual of 40 patients between January 2013 and May 2017, and the median follow-up time was 10.7 months (Fig. 1). One patient met eligibility criteria during screening, but her AST was >6× ULN on day 1 of sorafenib; she was treated with sorafenib off trial and excluded from the safety analysis but was included in the intent-to-treat population for all other analysis. The median age of the patients was 65 years old, and most patients had BCLC stage C HCC (95%) and Child–Pugh score of A5 (70%; Table 1). Risk factors for HCC included chronic hepatitis C (43%), chronic hepatitis B (13%), alcoholic cirrhosis (28%), metabolic syndrome (15%), and hemochromatosis (3%); several patients had overlapping risk factors, especially with alcohol abuse. In terms of prior therapies, 8 (20%) patients had prior surgery and 12 (30%) patients had prior liver-directed therapies. On baseline radiology, 19 (40%) patients had vascular involvement and 30 (75%) patients had distant metastases. In addition, 50% of patients had a baseline AFP >400 ng/mL.

At the time of analysis, 37 patients had discontinued treatment due to radiologic progression ($n = 13$, 35%), clinical progression with stable disease by RECIST criteria ($n = 8$, 22%), withdrawal of patient consent ($n = 7$, 19%), physician's decision ($n = 4$, 11%), AEs ($n = 4$, 11%), and conversion to resectability ($n = 1$, 3%). The patients who came off for clinical progression predominantly came off for disease progression on scans below the +20% cutoff for progressive disease by RECIST v1.1 and/or a significant rise in AFP combined with their clinical status. Following discontinuation on the trial, 8 of 37 patients continued study treatments off trial with either sorafenib combined with mFOLFOX ($n = 4$), mFOLFOX alone ($n = 3$), or sorafenib alone ($n = 1$) due to increased flexibility with dose adjustments off protocol. Several patients maintained sufficient health to proceed onto second-line

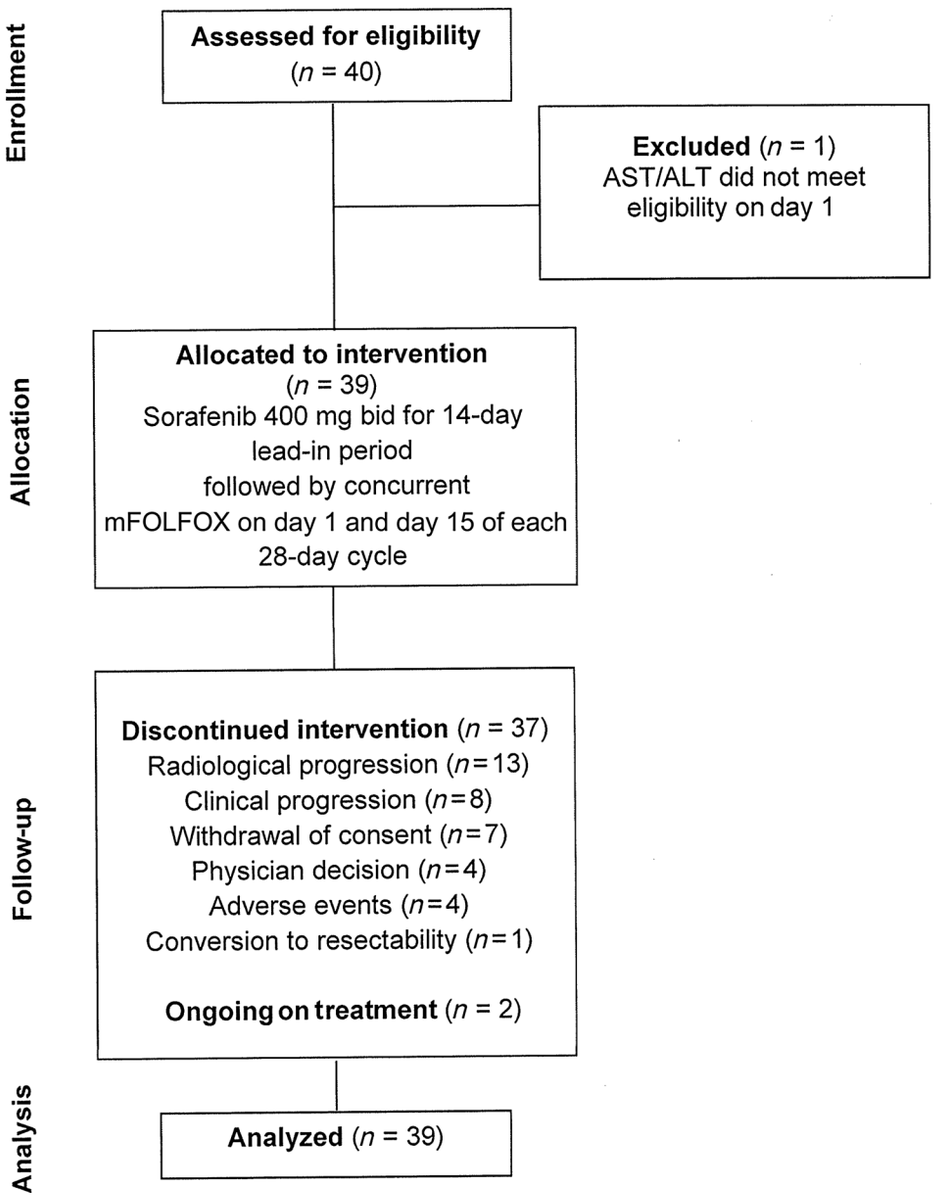


**Figure 1.**
CONSORT diagram showing the flow of participants from enrollment, therapy, and analysis for the sorafenib combined with FOLFOX study.

clinical trials ($n = 8$), liver-directed therapy ($n = 5$), or curative-intent surgery ($n = 1$; Supplementary Table S1).

### Safety

The combination of sorafenib and FOLFOX had moderate toxicity. Patients received a median of 8.0 (range, 0–33) cycles of sorafenib and 4.5 (range, 0–25) cycles of mFOLFOX. Median time to first dose reduction of sorafenib was 28 days (range, 10–182 days). Dose reductions in sorafenib were required in 74% of patients, and of the 35 patients who received at least one dose of mFOLFOX, 26 (74%) and 27 (77%) patients required dose reductions in 5-FU and oxaliplatin, respectively. The mean percentage of projected dose intensity (±SD) was 49% ± 24% for sorafenib and 62% ± 21% for oxaliplatin. Six patients required inpatient oxaliplatin desensitization, and no patients discontinued oxaliplatin due to allergy-related issues.

Treatment-related AEs occurring in >25% of patients and all grade 3/4 AEs are shown in Table 2. The most common drug-related AEs of any grade were fatigue (69%), hypophosphatemia (64%), diarrhea (59%), elevated ALT (56%), nausea (54%), and thrombocytopenia (46%), and most of these were grade 1 or 2. Additional toxicities potentially related to sorafenib and attributed to therapy included hypertension (28%), hand–foot syndrome (31%), mucositis (26%), and bleeding (8%). Grade ≥3 AEs were notable for AST elevation (28%), ALT elevation (15%), diarrhea (13%), bilirubin (10%), anemia (10%), hand–foot syndrome (8%), and bleeding (8%).

The toxicities that led to trial discontinuation in 4 patients were non-ST elevation myocardial infarction, subarachnoid hemorrhage with ischemic stroke, gastrointestinal hemorrhage, and elevated transaminases and total bilirubin. The death that occurred while a subject was on study was recorded within 30 days of study treatment; the patient developed confusion and

**Table 1.** Patient baseline characteristics (ITT population, $n = 40$)

| Characteristic | Total ($N = 40$) N (%) |
|---|---|
| Median age at start of treatment, years (range) | 65 (29–76) |
| Age at start of treatment (*N*, %) | |
| ≤50 | 5 (12.5%) |
| 50–69 | 26 (65%) |
| ≥70 | 9 (22.5%) |
| Sex (*N*, %) | |
| Male | 34 (85%) |
| Female | 6 (15%) |
| Race (*N*, %) | |
| White | 35 (87.5%) |
| Black | 1 (2.5%) |
| Asian | 1 (2.5%) |
| Not reported | 3 (7.5%) |
| ECOG performance status (*N*, %) | |
| 0 | 16 (40%) |
| 1 | 24 (60%) |
| Child–Turcotte–Pugh (*N*, %) | |
| A5 | 28 (70%) |
| A6 | 12 (30%) |
| CLIP score (*N*, %) | |
| 0 | 2 (5%) |
| 1 | 10 (25%) |
| 2 | 15 (37.5%) |
| 3 | 13 (32.5%) |
| OKUDA stage (*N*, %) | |
| I | 26 (65%) |
| II | 14 (35%) |
| BCLC score (*N*, %) | |
| B, Failed TACE | 2 (5%) |
| C | 38 (95%) |
| Etiology[a] (*N*, %) | |
| HCV | 17 (42.5%) |
| Alcohol | 11 (27.5%) |
| Metabolic syndrome | 6 (15%) |
| HBV | 5 (12.5%) |
| Hemochromatosis | 1 (2.5%) |
| Unknown | 6 (15%) |
| Vascular involvement, extrahepatic disease, and baseline AFP (*N*, %) | |
| Macrovascular involvement | 19 (40%) |
| Extrahepatic disease | 30 (75%) |
| Baseline AFP ≥400 ng/mL | 17 (44%) |

[a]Patients may have had multiple etiologies.

somnolence attributed to liver failure in the setting of clinical disease progression, and he passed at home.

## Efficacy

The study met its primary endpoint for efficacy with a median TTP of 7.7 months (95% CI, 4.4–8.9 months; Fig. 2A), and the median overall survival (mOS) was 15.1 months (95% CI: 7.9–16.9; Fig. 2B). The ORR, according to RECIST 1.1, was 18% (95% CI, 7.5%–33.5%), and the stable disease rate was 51% at 18 weeks for a total disease control rate of 69%. However, using mRECIST, 4 patients with stable disease by RECIST v1.1 converted to partial response (PR), bringing the ORR by mRECIST up to 28%.

Patient outcomes were also analyzed by etiology and AFP value. We found no association between TTP and etiology after sorafenib + mFOLFOX treatment. Among the 17 (43.5%) patients with an AFP ≥ 400 ng/mL, the mTTP was 6.5 months, the mOS was 10.6 months, and 14 patients had a drop in AFP of ≥50%. This compared unfavorably to the 22 (56.4%) patients with AFP < 400 ng/mL, who had a mTTP of 8.0 months and a mOS of 16.8 months, although the difference was not statistically significant ($P = 0.13$ for TTP, $P = 0.10$ for OS).

Notably, one patient who was 71 years old at diagnosis of a locally advanced 12.2 cm HCC underwent 23.4 months of treatment on trial with discontinuation of oxaliplatin at 22.9 months for neuropathy, and he had a −43.6% response by RECIST v1.1 criteria. He underwent an R0 partial hepatectomy, and his pathology showed a 5.4 cm pT2aN0 poorly differentiated mixed hepatocellular cholangiocarcinoma with vascular invasion and his background liver showed Ishak 3–4 of 6 fibrosis. The hepatocellular component was positive for glypican-3 and arginase-1 and negative for keratin 19. The cholangiocellular component was focally positive for keratin 19 and negative for glypican-3 and arginase-1. Of note, his pretreatment biopsy < 1 month prior to starting sorafenib showed HCC that was heppar-1 positive. Thus, tumor heterogeneity can lead to a missed diagnosis of hepatocholangiocarcinoma due to sampling of a single small area of the tumor by biopsy. He remains off treatment with no evidence of disease at 48.1 months of follow-up.

## Correlative biomarker studies

During sorafenib treatment alone, there was a significant increase in plasma VEGF, PlGF, VEGF-D, sMET, and TNFα (at days 3 and 15); and sVEGFR1, CAIX, and the fraction of $CD8^+$ T cells (at day 15); decrease in plasma sVEGFR2 and the fraction of $CD56^{Dim}$ natural killer (NK) cells (at day 3); and plasma sVEGFR2 and s-c-KIT (at day 15) compared with baseline (all $P < 0.05$, Table 3). During treatment with sorafenib + mFOLFOX, there was a significant increase in plasma PlGF, sVEGFR1, CAIX, and VEGF-D (at days 29 and 43), in circulating $CD3^+$, $CD3^+CD4^+$, and $CD3^+CD8^+$ lymphocyte fractions at day 29, and in plasma IL12p70 (at day 29) and TNFα (at day 43; all $P < 0.05$, Table 3). Moreover, there was a significant decrease in plasma sVEGFR2 and s-c-KIT, and the fraction of regulatory T cells (Tregs) at day 29 and 43 during combination therapy (Table 3). The rest of the biomarkers measured were not significantly changed (bFGF, VEGF-C, IFNγ, IL2, IL6, IL8, and IL10; Supplementary Table S2) or undetectable at the majority of the time points (IL1β, IL4, and IL13; data not shown).

When evaluated for associations with the primary endpoint, we found that shorter TTP was associated with: (i) higher plasma levels of sVEGFR1 and lower plasma levels of sMET at baseline, (ii) an increase in plasma VEGF, bFGF, circulating $CD3^+$, $CD3^+CD4^+$ Tregs, $CD56^{Bright}$ NK lymphocyte fractions (at day 3), and plasma s-MET (at day 15) after sorafenib alone, and (iii) an increase in circulating $CD56^{Dim}$ NK-cell fraction (at day 29) and plasma IL8 and sMET (at day 43) after sorafenib + mFOLFOX (all $P < 0.05$, Table 4). Examination of biomarkers associated with OS showed direct correlations between changes in plasma IL10 (day 3), IL6, and sMET (day 43) and in ratios of activated CTLs, Tregs, and all the NK populations (at day 3) and $CD56^{Dim}$ NK cells (at day 15; Supplementary Table S2). When dichotomizing the biomarkers using the median values, shorter TTP was associated with: (i) higher plasma IL10 [HR = 3.90 (95% CI: 1.44, 10.56), $P = 0.0074$] and lower $CD56^{Bright}$ NK lymphocyte fraction [HR = 0.294 (95% CI: 0.10, 0.90), $P = 0.031$] at baseline (Supplementary Fig. S2); (ii) higher plasma IL8 [HR = 3.04 (95% CI: 1.06, 8.75), $P = 0.039$], and sMET [HR = 4.55 (95% CI: 1.29, 16.04), $P = 0.019$] at day 29; and (iii) plasma s-MET [HR = 3.28 (95% CI: 1.07, 10.04), $P = 0.037$] at day 43 after sorafenib + mFOLFOX.

**Table 2.** Drug-related AEs in >25% of patients and all grade 3/4 AEs

| Adverse event (AE) | Grade 1/2 *n* (%) | Drug-related AE Grade 1/2 *n* (%) | Grade 3/4 *n* (%) | Drug-related AE Grade 3/4 *n* (%) | All grades *n* (%) | Drug-related AEs All grades *n* (%) |
|---|---|---|---|---|---|---|
| Hematologic | | | | | | |
| Thrombocytopenia | 21 (54%) | 16 (41%) | 2 (5%) | 2 (5%) | 23 (59%) | 18 (46%) |
| Anemia | 16 (41%) | 14 (36%) | 4 (10%) | 3 (8%) | 20 (51%) | 17 (44%) |
| Lymphopenia | 9 (23%) | 8 (21%) | 8 (21%) | 7 (18%) | 17 (44%) | 15 (38%) |
| Neutropenia | 12 (31%) | 11 (28%) | 3 (8%) | 3 (8%) | 15 (38%) | 14 (36%) |
| Non-hematologic | | | | | | |
| Fatigue | 29 (74%) | 24 (62%) | 3 (8%) | 3 (8%) | 32 (82%) | 27 (69%) |
| Diarrhea | 19 (49%) | 19 (49%) | 5 (13%) | 4 (10%) | 24 (62%) | 23 (59%) |
| Nausea | 24 (62%) | 21 (54%) | 0 (0%) | 0 (0%) | 24 (62%) | 21 (54%) |
| Peripheral sensory neuropathy | 21 (54%) | 18 (46%) | 0 (0%) | 0 (0%) | 21 (54%) | 18 (46%) |
| Anorexia | 22 (56%) | 17 (44%) | 0 (0%) | 0 (0%) | 22 (56%) | 17 (44%) |
| Rash | 16 (41%) | 13 (33%) | 1 (3%) | 1 (3%) | 17 (44%) | 14 (36%) |
| Palmar–plantar erythrodysesthesia | 10 (26%) | 9 (23%) | 3 (8%) | 3 (8%) | 13 (33%) | 12 (31%) |
| Hypertension | 11 (28%) | 9 (23%) | 2 (5%) | 2 (5%) | 13 (33%) | 11 (28%) |
| Mouth sores | 10 (26%) | 9 (23%) | 1 (3%) | 1 (3%) | 11 (28%) | 10 (26%) |
| Constipation | 17 (44%) | 9 (23%) | 0 (0%) | 0 (0%) | 17 (44%) | 9 (23%) |
| Pain | 14 (36%) | 4 (10%) | 2 (5%) | 0 (0%) | 16 (41%) | 4 (10%) |
| Abdominal pain | 15 (38%) | 4 (10%) | 1 (3%) | 0 (0%) | 16 (41%) | 4 (10%) |
| Bleeding[a] | 4 (10%) | 2 (5%) | 3 (8%) | 1 (3%) | 7 (18%) | 3 (8%) |
| Cough | 9 (23%) | 2 (5%) | 1 (3%) | 0 (0%) | 10 (26%) | 2 (5%) |
| Fever | 5 (13%) | 0 (0%) | 1 (3%) | 1 (3%) | 6 (15%) | 1 (3%) |
| Insomnia | 10 (26%) | 0 (0%) | 0 (0%) | 0 (0%) | 10 (26%) | 0 (0%) |
| Laboratory abnormalities | | | | | | |
| Hypophosphatemia | 12 (31%) | 12 (31%) | 14 (36%) | 13 (33%) | 26 (67%) | 25 (64%) |
| ALT increased | 18 (46%) | 16 (41%) | 6 (15%) | 6 (15%) | 24 (62%) | 22 (56%) |
| AST increased | 13 (33%) | 10 (26%) | 11 (28%) | 9 (23%) | 24 (62%) | 19 (49%) |
| Alkaline phosphatase increased | 20 (51%) | 15 (38%) | 2 (5%) | 2 (5%) | 22 (56%) | 17 (44%) |
| Hyperbilirubinemia | 11 (28%) | 10 (26%) | 4 (10%) | 4 (10%) | 15 (38%) | 14 (36%) |
| Lipasemia | 4 (10%) | 3 (8%) | 9 (23%) | 9 (23%) | 13 (33%) | 12 (31%) |
| Hyponatremia | 12 (31%) | 9 (23%) | 5 (13%) | 2 (5%) | 17 (44%) | 11 (28%) |
| Hyperglycemia | 19 (49%) | 5 (13%) | 8 (21%) | 1 (3%) | 27 (69%) | 6 (15%) |
| Hypokalemia | 5 (13%) | 4 (10%) | 1 (3%) | 0 (0%) | 6 (15%) | 4 (10%) |
| Hypoalbuminemia | 11 (28%) | 3 (8%) | 1 (3%) | 0 (0%) | 12 (31%) | 3 (8%) |

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.
[a]Bleeding: hematoma, hepatic hemorrhage, rectal hemorrhage.

There were no significant associations with the other biomarkers at any of the timepoints.

## Discussion

Advanced HCC stands as a unique solid tumor where targeted therapy and immunotherapy have both been approved, but no traditional chemotherapy has yet shown a survival benefit. Multiple single-agent regimens such as doxorubicin, cisplatin, 5-FU, mitoxantrone and etoposide, and combinations thereof have been tested and have failed to demonstrate meaningful activity (23–26). The combination of cisplatin, IFNα, doxorubicin, and 5-FU (PIAF) yielded a promising ORR of 26% in a phase II trial but ultimately failed in a randomized phase III trial against doxorubicin (27). Multiple factors likely contribute to HCC's chemoresistance, including its molecular heterogeneity, multiple etiologies, and comorbid liver dysfunction. The high rate of expression of gene products that can lead to drug resistance, such as p-glycoprotein and mutant p53, may also play important roles (28, 29).

With the approval of sorafenib for advanced HCC in 2007, sorafenib and chemotherapy have been tested in combination in an attempt to improve upon the efficacy of either alone. Doxorubicin has been subject to most extensive investigation as it

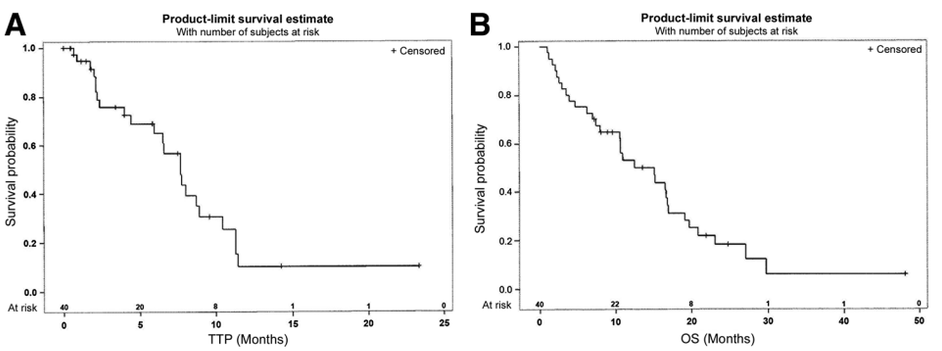


**Figure 2.**
Kaplan–Meier curves for survival for all patients with advanced HCC treated with sorafenib and mFOLFOX. **A,** mTTP; **B,** mOS.

**Table 3.** Baseline and change (%) in plasma and cellular biomarkers (significant changes highlighted in gray)

| Plasma/cellular biomarker (unit) | Baseline Median (IQR), *n* | ΔDay 3 | ΔDay 15 | ΔDay 29 Mean (95% CI); *n* (patients) | ΔDay 43 |
|---|---|---|---|---|---|
| VEGF (pg/mL) | 147 (96–275) | 127.6 (25.8–229.4) | 129.0 (45.8–212.2) | −23.42 (130.1–83.3) | −48.9 (−143.3–45.5) |
| | *n* = 39 | *n* = 31 | *n* = 30 | *n* = 27 | *n* = 27 |
| *P* | N/A | 0.016 | 0.0036 | 0.66 | 0.30 |
| VEGF-D (pg/mL) | 982 (746–1,131) | 177.4 (68.3–286.6) | 420.7 (228.1–613.2) | 473.6 (322.8–624.4) | 354.2 (214.6–493.7) |
| | *n* = 39 | *n* = 31 | *n* = 30 | *n* = 27 | *n* = 27 |
| *P* | N/A | 0.0024 | 0.0001 | <0.0001 | <0.0001 |
| TNFα (pg/mL) | 3.0 (2.2–3.9) | 0.34 (0.11–0.56) | 0.42 (0.01–0.84) | 0.26 (0.07–0.59) | 0.66 (0.20–1.12) |
| | *n* = 39 | *n* = 31 | *n* = 30 | *n* = 27 | *n* = 27 |
| *P* | N/A | 0.0044 | 0.048 | 0.12 | 0.0068 |
| IL12p70 (pg/mL) | 0.13 (0.13–0.19) | −0.04 (−0.10–0.02) | −0.05 (−0.11–0.01) | 0.05 (0.01–0.09) | −0.03 (−0.08–0.02) |
| | *n* = 39 | *n* = 31 | *n* = 29 | *n* = 27 | *n* = 27 |
| *P* | N/A | 0.14 | 0.10 | 0.031 | 0.25 |
| sMET (pg/mL) | 862 (677–1,094) | 57.1 (11.9–102.2) | 131.7 (55.6–207.7) | 23.0 (−45.5–91.6) | −62.1 (−212.4–88.3) |
| | *n* = 39 | *n* = 19 | *n* = 30 | *n* = 27 | *n* = 27 |
| *P* | N/A | 0.015 | 0.0014 | 0.50 | 0.40 |
| PlGF (pg/mL) | 11.4 (8.7–14.0) | 11.89 (7.09–16.69) | 18.84 (12.36–25.33) | 12.54 (6.53–18.55) | 11.89 (7.82–15.97) |
| | *n* = 39 | *n* = 31 | *n* = 30 | *n* = 27 | *n* = 27 |
| *P* | N/A | <0.0001 | <0.0001 | 0.0002 | <0.0001 |
| sVEGFR1/sFLT1 (pg/mL) | 163 (103–219) | −80.2 (−240.8–80.5) | 984.0 (431.1–1,536.9) | 949.7 (608.8–1,290.5) | 1,106.4 (659.0–1,553.7) |
| | *n* = 39 | *n* = 31 | *n* = 30 | *n* = 27 | *n* = 27 |
| *P* | N/A | 0.32 | 0.0011 | <0.0001 | <0.0001 |
| sVEGFR2 (pg/mL) | 7,732 (6,661–8,273) | −330.2 (−629.4 to −31.0) | −2,217.1 (−2,625.0 to −1,809.2) | −1,649.8 (−2048.8 to −1,250.7) | −1,897.3 (−2,427.0 to −1,367.6) |
| | *n* = 39 | *n* = 31 | *n* = 30 | *n* = 27 | *n* = 27 |
| *P* | N/A | 0.032 | <0.0001 | <0.0001 | <0.0001 |
| s-c-KIT (ng/mL) | 11.2 (9.4–12.9) | 0.18 (−0.26–0.62) | −1.34 (−1.83 to −0.85) | −3.43 (−4.20 to −2.66) | −3.21 (−4.14 to −2.28) |
| | *n* = 39 | *n* = 31 | *n* = 30 | *n* = 27 | *n* = 27 |
| *P* | N/A | 0.41 | <0.0001 | <0.0001 | <0.0001 |
| CAIX (pg/mL) | 351 (141–591) | 52.57 (−2.3–107.4) | 102.8 (19.9–185.7) | 170.0 (76.8–263.2) | 184.6 (63.7–305.5) |
| | *n* = 39 | *n* = 31 | *n* = 30 | *n* = 27 | *n* = 27 |
| *P* | N/A | 0.060 | 0.017 | 0.0009 | 0.0042 |
| $CD3^+$ lymphocytes (%) | 49 (39–58) | 2.43 (−3.36–8.23) | 5.63 (2.16–13.42) | 9.37 (2.88–15.856) | 9.62 (−0.90–20.15) |
| | *n* = 30 | *n* = 19 | *n* = 20 | *n* = 11 | *n* = 8 |
| *P* | N/A | 0.39 | 0.15 | 0.0092 | 0.067 |
| $CD3^+$ $CD4^+$ lymphocytes (%) | 30 (24–43) | 1.55 (2.26–5.36) | 1.94 (3.70–7.58) | 6.95 (1.43–12.47) | 7.72 (−0.58–16.02) |
| | *n* = 30 | *n* = 19 | *n* = 20 | *n* = 11 | *n* = 8 |
| *P* | N/A | 0.40 | 0.48 | 0.019 | 0.064 |
| $CD3^+$ $CD4^+$ memory T cells (%) | 27.47 (22.11–38.43) | 3.53 (−2.35–9.41) | 4.04 (−2.86–10.94) | 7.67 (2.39–12.97) | 8.30 (0.01–16.60) |
| | *n* = 30 | *n* = 19 | *n* = 20 | *n* = 11 | *n* = 8 |
| *P* | N/A | 0.060 | 0.24 | 0.0090 | 0.050 |
| Tregs (%) | 0.46 (0.22–1.09) | −0.02 (−0.20–0.15) | −0.13 (−0.35–0.08) | −0.47 (−0.89 to −0.06) | −0.57 (−1.03 to −0.11) |
| | *n* = 30 | *n* = 19 | *n* = 20 | *n* = 11 | *n* = 8 |
| *P* | N/A | 0.77 | 0.21 | 0.030 | 0.022 |
| $CD3^+$ $CD8^+$ lymphocytes (%) | 10 (7–16) | 1.35 (−0.93–3.62) | 4.02 (0.91–7.14) | 2.18 (0.33–4.04) | 1.56 (−1.06–4.18) |
| | *n* = 30 | *n* = 19 | *n* = 20 | *n* = 10 | *n* = 8 |
| *P* | N/A | 0.23 | 0.015 | 0.025 | 0.20 |
| $CD56^{Dim}$ NK cells (%) | 10 (6–17) | −3.65 (−6.01 to −1.30) | −1.45 (−4.50–1.59) | −0.06 (−4.15–4.02) | −4.71 (−9.88–0.47) |
| | *n* = 30 | *n* = 19 | *n* = 20 | *n* = 11 | *n* = 8 |
| *P* | N/A | 0.0044 | 0.33 | 0.97 | 0.069 |

**Table 4.** Correlation between circulating biomarker levels and TTP

| Biomarker/time point | Baseline | Change at day 3 | Change at day 15 | Change at day 29 | Change at day 43 |
|---|---|---|---|---|---|
| VEGF | 1.000 (0.999–1.002) | 1.506 (1.005–2.258) | 1.563 (0.946–2.583) | 1.248 (0.778–2.003) | 1.423 (0.781–2.590) |
| *P* | 0.87 | 0.047 | 0.081 | 0.36 | 0.25 |
| bFGF | 1.004 (0.999–1.008) | 1.644 (1.041–2.597) | 0.853 (0.579–1.256) | 1.002 (0.792–1.267) | 1.259 (0.807–1.967) |
| *P* | 0.11 | 0.033 | 0.42 | 0.99 | 0.31 |
| sVEGFR1 | 1.001 (1.000–1.002) | 0.954 (0.717–1.270) | 0.986 (0.945–1.029) | 0.923 (0.851–1.001) | 1.009 (0.942–1.081) |
| *P* | 0.039 | 0.75 | 0.51 | 0.054 | 0.80 |
| IL8 | 0.999 (0.992–1.006) | 1.030 (0.789–1.345) | 1.056 (0.683–1.632) | 1.804 (0.908–3.582) | 2.657 (1.095–6.444) |
| *P* | 0.82 | 0.83 | 0.81 | 0.092 | 0.031 |
| sMET | 0.999 (0.997–1,000) | 2.588 (0.009–776.602) | 7.593 (1.050–54.906) | 17.414 (0.685–442.891) | 2876 (27–300468) |
| *P* | 0.025 | 0.74 | 0.045 | 0.084 | 0.0008 |
| $CD3^+$ lymphocytes | 0.99 (0.97–1.03) | 27.78 (2.06–374.99) | 1.96 (0.66–5.76) | 0.99 (0.32–3.11) | 0.98 (0.43–2.23) |
| *P* | 0.071 | 0.012 | 0.22 | 0.97 | 0.97 |
| $CD3^+CD4^+$ T cells | 1.00 (0.96–1.04) | 22.36 (1.76–284.41) | 1.88 (0.67–5.29) | 0.92 (0.34–2.49) | 0.97 (0.49–1.90) |
| *P* | 0.97 | 0.017 | 0.23 | 0.87 | 0.95 |
| Tregs | 1.11 (0.46–2.69) | 2.82 (1.45–5.47) | 1.40 (0.74–2.67) | 0.24 (0.02–2.62) | 3.78 (0.23–63.34) |
| *P* | 0.82 | 0.0022 | 0.30 | 0.24 | 0.36 |
| $CD56^{Bright}$ NK cells | 0.68 (0.22–2.09) | 3.28 (1.11–9.74) | 0.94 (0.30–2.92) | 1.18 (0.33–4.26) | 4.62 (0.06–331.79) |
| *P* | 0.50 | 0.0022 | 0.91 | 0.80 | 0.48 |
| $CD56^{Dim}$ NK cells | 0.96 (0.89–1.04) | 4.68 (0.72–30.58) | 0.99 (0.35–2.78) | 19.25 (1.18–315.42) | 2.11 (0.30–14.88) |
| *P* | 0.33 | 0.11 | 0.98 | 0.038 | 0.45 |

NOTE: *P* values from Wald test. HR was calculated as the increase/decrease in the time-to-progression (TTP) per increase of one unit in the biomarker tested. Data are shown as hazard ratios (HR), with 95% CI (significant changes highlighted in gray).
Abbreviations: bFGF, basic fibroblast growth factor; NK cell, natural killer cells; sMET, soluble MET; sVEGFR, soluble VEGF receptor; Tregs, T regulatory cells.

advanced to phase III testing in CALGB 80802, which evaluated sorafenib + doxorubicin versus sorafenib alone in the first-line systemic treatment of advanced HCC. The study's DSMB halted the study early for futility, however, and sorafenib + doxorubicin failed to show a survival benefit but did show significantly greater grade 3/4 hematologic toxicity (37.8% vs. 8.1%; ref. 30). This study of sorafenib+mFOLFOX was a parallel effort initiated in 2013 based on data from the phase III EACH trial showing a significantly better median PFS and ORR with FOLFOX4 compared with doxorubicin. In this single-arm, phase II trial, sorafenib + mFOLFOX demonstrated promising activity with a TTP of 7.7 months and ORR of 18% in the first-line systemic treatment of advanced HCC. No unexpected toxicities emerged, but multiple patients discontinued treatment or withdrew consent due to toxicity and continued with either sorafenib or mFOLFOX alone off trial.

Notably, sorafenib combined with capecitabine and oxaliplatin (SECOX) demonstrated a similar ORR of 16% in the preliminary report of a phase II trial in the first-line treatment of advanced HCC, but the safety profile was deemed sufficiently manageable to proceed with a randomized phase II trial against sorafenib (NCT02716766). This in part may be due to stricter eligibility criteria in the SECOX trial on three accounts: (i) AST and ALT had to be $\leq$2.5 times the ULN as opposed to $\leq$6.0 times the ULN; (ii) total bilirubin had to be $\leq$1.5 times the ULN as opposed to $\leq$2.0 times the ULN; and (iii) patients with main portal vein thrombosis were excluded. In addition, 84% of the patients in SECOX had chronic hepatitis B compared with 13% in this study, and this may have impacted the underlying hepatic function and drug tolerability.

In our patient cohort, radiologic evaluation with mRECIST allowed improved characterization of treatment response. While RECIST 1.1 and mRECIST were similar in the recognition of PD, mRECIST showed a higher PR rate along with earlier recognition of partial response in these patients. As mRECIST relies on measuring changes in tumor viability–based enhancement characteristics of HCC on multiphasic CT/MRI, they allow earlier and improved characterization of therapeutic effect to sorafenib compared with RECIST 1.1, which measures changes in overall tumor size. Because the morphologic changes can lag behind the changes in tumor vascularity/viability in response to antiangiogenic therapy, mRECIST more closely reflects the biological changes of tumor necrosis or devascularization.

Hypothesis-generating correlative studies from this study confirmed some pharmacodynamic changes for VEGF inhibitors, but also uncovered unexpected biomarker kinetics. Interestingly, while sorafenib alone increased plasma CAIX, PlGF, sVEGFR1, VEGF-D, and VEGF and decreased sVEGFR2 and s-c-KIT [as previously seen with multitargeted tyrosine kinase inhibitors (TKI), data summarized in refs. 31, 32], plasma VEGF levels dropped during sorafenib + FOLFOX. This differential change in plasma VEGF despite the increase in the hypoxia marker, CAIX, suggest a differential mechanism of action for sorafenib when combined with chemotherapy. Of the immune biomarkers evaluated, plasma TNFα increased throughout sorafenib treatment. Among circulating the lymphocyte fractions, sorafenib treatment transiently led to a decrease in $CD56^{Dim}$ NK-cell subset and an increase in $CD3^+CD8^+$ cytotoxic lymphocytes; combination treatment led to a sustained drop in Tregs and transient increases in $CD3^+$, $CD3^+CD4^+$, and $CD3^+CD8^+$, but not $CD8^+CD25^+$ CTL fractions. These exploratory studies provide candidate biomarkers for response for sorafenib + mFOLFOX, which may be relevant for combinations of sorafenib or other TKIs with immunotherapy (e.g., anti-PD1 or anti-CTLA4 antibodies), a strategy that is being actively investigated in HCC (32). In line with data reported on sorafenib alone in advanced HCC (33), a rapid decrease in circulating Tregs after sorafenib alone (at day 3) was associated with superior TTP and OS in this study. Moreover, we found that increases in multiple lymphocyte populations were associated with a shorter TTP and OS after sorafenib + mFOLFOX. These correlations should be further examined in patients treated with sorafenib, where it will be critical to examine the intratumoral infiltration and activation of NK, CTL, and Treg lymphocyte subsets in HCC after treatment. Interestingly, plasma levels of

sMET (a putative endogenous blocker of HGF pathway) were associated with longer TTP when evaluated at baseline, but their increase after treatment was associated with shorter TTP and OS (including when analyzed as binary form variables). Furthermore, high plasma IL10 (a putative biomarker of liver disease and HCC) and an increase in plasma IL8 (a potential mediator of anti-VEGF treatment resistance) posttreatment was associated with shorter TTP, consistent with previous reports (34, 35). Finally, higher baseline levels of sVEGFR1 (an endogenous blocker of VEGF pathway) were associated with outcome of combination therapy. sVEGFR1 is an endogenous inhibitor of VEGF pathway but also a potential surrogate biomarker of liver damage (36). However, changes in biomarkers of hypoxia (plasma CAIX) or the pharmacodynamic biomarkers evaluated by us after sorafenib treatment did not show a significant correlation with outcomes. Future studies using serial biopsies or imaging should determine whether pretreatment vascular parameters of their changes after sorafenib mediate response or resistance to chemotherapy or other treatments (16).

While sorafenib + mFOLFOX showed promising efficacy in patients with advanced HCC in this single-arm phase II study, the role of these therapies will clearly depend on the evolving role of immune checkpoint blockade in this disease. Nivolumab recently gained accelerated FDA approval for the treatment of advanced refractory HCC (14) and is currently being tested in the first-line setting. Given the toxicity profile of sorafenib and the disappointing results of previous trials of sorafenib combinations, immune checkpoint inhibitors are increasingly being used as the backbone for combination regimens. Chemotherapy may potentially lead to release of tumor antigens and thus increase tumor immunogenicity and response to PD-1 blockade, a hypothesis supported by the efficacy of this combination strategy in non–small cell lung cancer. However, with the unclear role of chemotherapy in HCC, the utility of this approach remains untested in HCC. On the other hand, PD-1 blockade is currently being tested in combination with several multikinase inhibitors that have demonstrated efficacy in HCC, such as sorafenib (NCT03211416), lenvatinib (NCT03006926), regorafenib (NCT03347292), and cabozantinib (NCT03299946), and also liver-directed therapies that have demonstrated activity in HCC, such as TACE (NCT03143270), SIRT (NCT03033446), and radiation (NCT03316872).

### Conclusion

Sorafenib + mFOLFOX met its primary endpoint in the first-line management of patients with advanced HCC with encouraging efficacy. However, this combination regimen had moderate toxicity, including hepatotoxicity, and thus may be effective in carefully selected patients with a robust performance status and adequate liver reserve. Furthermore, to aid in the selection of patients for this regimen and others, it will be critical to the field to validate predictive biomarkers of response in HCC.

## Disclosure of Potential Conflicts of Interest

T.A. Abrams is a consultant/advisory board member for Bayer. R. Miksad is an employee of and has ownership interests (including patents) at Flatiron Health, and is a consultant/advisory board member for Ipsen. R.J. Jain is an employee of Tekla Healthcare Investors, Tekla Healthcare Opportunities Fund, Tekla Life Sciences, Tekla World Healthcare Fund, has ownership interests (including patents) at Enlight Biosciences, Opthotech and SynDevRx, and is a consultant/advisory board member for Merck, Opthotech, Pfizer, SPARC, SynDevRx, and XTuit, and reports receiving speakers bureau honoraria from Amgen. D.G. Duda is a consultant/advisory board member for Bayer, Tilos and twoXAR, and reports receiving commercial research grants from Bayer, Bristol-Myers Squibb, Exelixis, and Leap Tx. A.X. Zhu is a consultant/advisory board member for Bayer. No potential conflicts of interest were disclosed by other authors.

## Authors' Contributions

**Conception and design:** L. Goyal, R.K. Jain, D.G. Duda, A.X. Zhu
**Development of methodology:** L. Goyal, R. Miksad, D.G. Duda, A.X. Zhu
**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** L. Goyal, T.A. Abrams, R. Miksad, A.J. Bullock, J.N. Allen, M.B. Yurgelun, J.W. Clark, A. Kambadakone, M. Knowles, E. Regan, T. Hato, E. Mamessier, K. Shigeta, D.G. Duda, A.X. Zhu
**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** L. Goyal, H. Zheng, R. Miksad, A. Kambadakone, A. Muzikansky, A.J. Afflitto, C.F. Dinicola, E. Mamessier, D.G. Duda, A.X. Zhu
**Writing, review, and/or revision of the manuscript:** L. Goyal, H. Zheng, T.A. Abrams, R. Miksad, A.J. Bullock, M.B. Yurgelun, J.W. Clark, A. Kambadakone, M. Knowles, E. Mamessier, K. Shigeta, D.G. Duda, A.X. Zhu
**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** L. Goyal, A.J. Afflitto, C.F. Dinicola, D.G. Duda, A.X. Zhu
**Study supervision:** L. Goyal, R. Miksad, R.K. Jain, D.G. Duda, A.X. Zhu
**Other (nurse practitioner):** A. Galway

## Acknowledgments

We thank Mrs. Anna Khachatryan (Steele Laboratories, Department of Radiation Oncology, Massachusetts General Hospital) for technical support for biomarker studies. This study was funded by Bayer. L. Goyal received the NIH Loan Repayment Program Award and the NIH GI SPORE Award. R.K. Jain and D.G. Duda were supported by NIH P01-CA080124. K. Shigeta was supported by a fellowship from Uehara Foundation. E. Mamessier received a grant from the Philippe Foundation and Canceropole PACA.

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Received March 19, 2018; revised June 14, 2018; accepted August 31, 2018; published first September 6, 2018.



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