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Phase 1/2 Study of Everolimus in Advanced Hepatocellular Carcinoma

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Abstract

BACKGROUND—The phosphoinositide 3-kinase/Akt/mammalian target of rapamycin pathway plays a critical role in the pathogenesis of hepatocellular carcinoma (HCC). We performed a single-arm, phase 1/2 study of everolimus in patients with advanced HCC.

METHODS—Patients with histologically confirmed measurable advanced HCC, 0–2 prior regimens, and adequate hematologic, hepatic, and renal functions received everolimus at 5 mg/day or 10 mg/day orally (6 weeks/cycle). The primary end points were determination of a safe dosage of everolimus (phase 1) and progression-free survival (PFS) at 24 weeks (phase 2).

RESULTS—Twenty-eight patients were enrolled and evaluable for efficacy and toxicity. No dose-limiting toxicities were observed at the 5 mg/day (n = 3) or 10 mg/day (n = 6) dosage level in phase 1. Twenty-five patients received everolimus at 10 mg/day. Grade 3–4 adverse events included lymphopenia (n = 3), aspartate transaminase (n = 3), hyponatremia (n = 2), and 1 patient each with anemia, alanine transaminase, hyperglycemia, proteinuria, rash, and hypoxia. One patient (4%) had partial response (95% confidence interval [CI], 0.9%–19.6%). The median PFS and overall survival were 3.8 months (95% CI, 2.1–4.6) and 8.4 months (95% CI, 3.9–21.1), respectively. The estimated PFS rate at 24 weeks was 28.6% (95% CI, 7.9%–49.3%).

CONCLUSION—Everolimus was well tolerated in patients with advanced HCC, and 10 mg/day was defined as the phase 2 dosage. Although the study did not proceed to the second stage of phase 2, preliminary antitumor activity was observed with everolimus in patients with advanced HCC, most of whom had prior systemic treatment.

Keywords

hepatocellular carcinoma; everolimus; mTOR inhibitors; angiogenesis; clinical trial

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CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the third most common cause of cancer-related mortality worldwide.¹ After decades of research, 2 randomized trials demonstrated that sorafenib improved survival in patients with advanced HCC and established sorafenib as a standard first-line treatment for advanced HCC.^{2, 3} Other molecularly targeted agents are under clinical development⁴; however, most of these agents also target angiogenesis pathways. Therefore, novel agents that inhibit other hepatocarcinogenesis pathways are urgently needed.

Increasing evidence supports an important role for the phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway and potential for inhibiting mTOR as a therapeutic strategy in HCC.⁵ Aberrant mTOR signaling as reflected by upstream activation through epidermal growth factor receptor (EGFR) and insulin-like growth factor 1 receptor is well documented, and mutations in p110alpha (PIK3CA) have been reported in HCC.⁶ Hepatitis B virus X-proteins activate the PI3K pathway by modulating phosphorylation of Akt.⁷ The mTOR pathway is involved in production of proangiogenic factors, including vascular endothelial growth factor (VEGF); therefore, inhibition of the mTOR pathway may have antiangiogenic activity in HCC.⁸ Preclinical data have demonstrated that mTOR inhibitors were effective in inhibiting cell growth and tumor vascularity in HCC cell lines and HCC tumor models.^{8, 9} HCC can be induced in a transgenic hepatocyte-specific phosphatase and tensin homolog (PTEN)-deficient mouse model.¹⁰ Phosphorylation of ribosomal protein S6 kinase (p-RPS6) downstream of PI3K/mTOR is present in approximately 50% of HCC cases. Chromosomal gains in the mTOR-associated protein RICTOR and p-RPS6-positive staining correlate with HCC recurrence following surgical resection.¹¹

To evaluate whether inhibition of the PI3K/Akt/mTOR pathway is safe and effective in patients with advanced HCC, we initiated a phase 1/2 study of everolimus, an oral small-molecule serine-threonine kinase inhibitor of mTOR, in patients with advanced HCC.

MATERIALS AND METHODS

Patient Population

The trial was approved by the institutional review board at the Dana-Farber/Harvard Cancer Center (Boston, Mass). All patients provided written informed consent before study participation.

Eligibility criteria were as follows: histologically proven, measurable, locally advanced, or metastatic HCC; no more than 2 prior chemotherapeutic and biologic regimens; prior chemoembolization therapy (only if performed >4 weeks before study entry and measurable disease outside of the chemoembolization field was present); age ≥ 18 years; Eastern Cooperative Oncology Group performance status of 0–2; Cancer of the Liver Italian Program score ≤ 3; and adequate bone marrow, renal, and hepatic function (absolute neutrophil count ≥ 1000/IL, hemoglobin level ≥ 9 g/dL, platelet count ≥ 75,000/IL, serum creatinine level ≤ 2.0 mg/dL, total bilirubin level <3.0 mg/dL, and aspartate aminotransferase and alanine aminotransferase levels ≤ 7 times the upper limit of normal).

Exclusion criteria were as follows: concurrent malignancies; significant medical comorbidities, including severely impaired lung function and uncontrolled diabetes; significant cardiovascular disease, including symptomatic congestive heart failure, myocardial infarction ≤ 6 months before study treatment, serious uncontrolled cardiac arrhythmia, and unstable angina; chronic use of systemic steroids or immunosuppressive therapy; active bleeding; proteinuria at baseline (>2 g/day); pregnancy or lactation; brain

metastases; impairment of gastrointestinal function or gastrointestinal disease that may alter the absorption of everolimus; or prior use of mTOR inhibitors.

Study Treatment

Eligible patients received everolimus continuously daily by mouth until disease progression, unacceptable toxicity, or withdrawal of consent. Six weeks of the study drug were considered 1 cycle of treatment. The phase 1 stage of the study used the conventional 3+3 design. The starting dosage of everolimus was 5 mg/day. The planned escalated dosage level was 10 mg/day and the de-escalated dosage level was 5mg every other day.

Everolimus was interrupted and supportive management instituted for grade 3–4 hematologic or grade 3 nonhematologic toxicities. Everolimus was reinitiated with a 50% dosage reduction if resolution of the toxicity to less than grade 2 occurred within 14 days; otherwise, treatment was discontinued. Treatment was also discontinued for grade 4 nonhematologic toxicity, grade 2 or higher pneumonitis, or continued toxicity after reinstitution of everolimus at 5mg every other day. Dose-limiting toxicity (DLT) likely attributed to everolimus was evaluated during the first cycle (42 days). DLT was defined as any grade 4 neutropenia or thrombocytopenia; any grade 3 or higher nonhematologic toxicity, excluding alopecia, nausea, and vomiting (not controlled by standard antiemetic therapy); grade 3 or higher diarrhea requiring hospitalization or lasting >24 hours despite aggressive loperamide therapy; grade 3 or higher fasting hyperglycemia persisting for >1 week despite treatment with either oral antidiabetic agents or insulin; and grade 3 serum transaminases (aspartate transaminase and alanine transaminase) for patients with grade 0–1 at baseline or 7 times the upper limit of normal for patients with grade 2 at baseline.

On-study evaluations included toxicity assessments, peripheral blood count measurements, and a full chemistry panel every other week. Lipid panel and serum alpha-fetoprotein (AFP) levels were measured monthly. Patients were evaluated with computed tomography or magnetic resonance imaging every 6 weeks; response and progression were evaluated by independent radiologic review using RECIST criteria.¹²

Correlative Analyses

Genotyping was performed on formalin-fixed, paraffin-embedded tissue. Eleven of 25 patient samples from the phase 2 portion of the study were available for evaluation. Total nucleic acids were isolated using a custom platform based on the Agencourt FormaPure system (Beckman Coulter Genomics). Nucleic acids were screened for 120 somatic mutations across 13 essential cancer genes (APC, BRAF, CTNNB1, EGFR, FLT3, JAK2, KIT, KRAS, NOTCH1, NRAS, PIK3CA, PTEN, and TP53) using a high-throughput assay based on the ABI Prism SNaPshot Multiplex system.¹³ Mutations were confirmed by direct sequencing.

Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tissue sections using standard methods and the following polyclonal antibodies and dilutions (Cell Signaling Technology): 1:50 phospho-Akt (Ser473), 1:15 phospho-mTOR (Ser2448) and 1:75 phospho-Ribosomal Protein S6 (Ser235/236). Activation of Akt/mTOR signaling was defined as an IHC score of 2+ to 3+ (moderate to high) across each target protein on a scale of 0 to 3+.

Statistical Methods

The primary endpoint for the phase 1 portion was determination of the safety of everolimus. Two planned dose levels of everolimus were evaluated based on previous studies

demonstrating that the optimum biologic dose is 10 mg. The primary endpoint for the phase 2 portion was the progression-free survival (PFS) rate at 24 weeks.

The trial was prespecified to include patients from the phase 1 portion (no more than 2 dose levels) into the phase 2 portion of the study. The phase 2 portion had a 2-stage design, with a target accrual of 22 evaluable patients for the first stage and a total of 40 patients for the whole study. If at least 3 of the evaluable patients included in the analysis for the first stage of the phase 2 portion were progression-free at 24 weeks, the trial would proceed to the second stage. We hypothesized that everolimus would improve the 24-week PFS rate from 10% to 25%. As designed, the overall study with 40 evaluable patients would have an 80% power at a 0.05 level of significance to detect a 15% difference of 24-week PFS rate from 10% to 25%.

Secondary endpoints included overall response rate, toxicity, time to tumor progression (TTP), median PFS, and overall survival. The Kaplan-Meier method was used to estimate the distribution of PFS and survival. All analyses were performed using SAS version 9.2 software (SAS Institute, Cary, NC).

RESULTS

Patient Characteristics

Between October 2007 and June 2009, 28 patients entered into the trial (9 in phase 1, 19 in phase 2) and all were evaluable for efficacy and toxicity based on intention-to-treat analysis (Figure 1). Baseline patient characteristics are summarized in Table 1. There were 18 men (64%) and 10 women (36%) with a median age of 65 years (range, 33–81 years). Median Eastern Cooperative Oncology Group performance status was 1 (range, 0–2). Twenty-three (82%) patients had underlying Child-Pugh class A cirrhosis and 13 (47%) patients had hepatitis B virus (HBV) or hepatitis C virus (HCV) infection. The majority of patients had Barcelona Clinic Liver Cancer stage C (26 [93%]) and extrahepatic (24 [86%]) disease. Twenty patients (71%) had received prior systemic therapy, including 11 (39%) patients who had received prior sorafenib therapy and 9 (32%) patients who had received other systemic regimens, including bevacizumab alone or in combination with gemcitabine/oxaliplatin and sunitinib or ramucirumab on clinical trials. The median serum bilirubin level was 0.6 mg/dL (range, 0.6–2.9 mg/dL), and 23 (82%) patients had an elevated serum AFP level at the time of study entry.

Toxicity

In the phase 1 portion of the study, dose-finding escalation was performed with 2 planned dosages of 5 mg/day and 10 mg/day. Three patients were initially enrolled at the 5 mg/day dosage level. No DLTs were encountered during cycle 1 of treatment; therefore, an additional 3 patients were enrolled at the 10 mg/day dosage level. One patient developed grade 3 lymphopenia thought to be related to everolimus. An additional 3 patients were enrolled at the 10 mg/day dosage level. No DLTs were encountered, and the phase 2 dosage was determined to be 10 mg/day.

The 2-stage phase 2 portion of the study proceeded with an additional 19 patients enrolled, for a total of 25 patients treated with the 10 mg/day dosage and included in stage 1 analysis of the phase 2 portion of the study. Per protocol specification, 3 additional patients were enrolled to have 22 patients evaluable for efficacy for the first stage of the phase 2 study, as 1 withdrew consent during the first cycle of treatment and 2 patients were removed for unacceptable toxicities before completing the first set of restaging scans. A total of 60 cycles were administered, with a mean dosage of 373 mg per 6-week cycle (6 weeks); 88.8% of the targeted dosage was delivered to the 25 patients included in the phase 2 analysis.

Everolimus was generally well tolerated at the 10 mg/day dosage. The most common all-grades adverse events thought to be at least possibly related to everolimus included fatigue, hyperglycemia, diarrhea, myelosuppression, transient elevation of transaminase levels, hyponatremia, anorexia, stomatitis, and rash (Table 2).

Grade 3 or 4 events were relatively infrequent. Transient elevation of transaminase levels was observed, with 3 (12%) patients experiencing grade 3 elevations of aspartate transaminase and 1 (4%) patient experiencing grade 3 elevations of alanine transaminase. Grade 3 lymphopenia was seen in 3 (12%) patients, and 2 (8%) patients had grade 3 hyponatremia; 3 other grade 3 events (hyperglycemia, rash, and proteinuria), were seen in 1 patient each. One patient had grade 4 anemia secondary to gastrointestinal bleeding. This patient was found to have gastric antral vascular ectasia on endoscopy and was treated with argon plasma photocoagulation; treatment was resumed without recurrent bleeding episodes. Grade 4 hypoxia occurred in 1 patient with underlying chronic obstructive pulmonary disease and pneumonia without evidence of pneumonitis.

Clinical Efficacy

Clinical efficacy was analyzed in all 25 patients treated at the 10 mg/day dosage level. Everolimus induced a partial response in 1 (4%) patient (95% confidence interval [CI], 0.9%–19.6%) and 10 (40%) patients (95% CI, 23%–59%) achieved stable disease (SD) for at least 12 weeks with a disease control rate of 44%. Seven patients (25%) had a >50% decrease in serum AFP level from baseline. A waterfall plot of the percent change in the sum of diameters of tumor lesions from baseline in 22 patients with available repeat scans is shown in Figure 2A.

With a median follow-up time of 7.2 months, the median PFS of this cohort was 3.8 months (95% CI, 2.1–4.6 months) (Figure 2B), the median TTP was 3.9 months (95% CI, 2.1–5.5 months), and the median overall survival was 8.4 months (95% CI, 3.9–21.1 months) (Figure 2C). Because only 2 (8%) patients were progression-free at 24 weeks, the study did not proceed to the second stage of the phase 2 portion of the study. The estimated PFS rates at 24 weeks and 12 weeks, based on the Kaplan-Meier method, were 28.6% (95% CI, 7.9%–49.3%) and 57.1% (95% CI, 35.7%–78.6%), respectively.

Unplanned exploratory analyses of 25 patients treated at the 10 mg/day dosage level revealed that patients who had received prior sorafenib treatment (n = 10) had similar PFS, TTP, and overall survival compared with those who had not received prior sorafenib treatment (n = 15). In addition, patients with no viral etiology (n = 14) had longer TTP compared with those with HBV (n = 6) or HCV infection (n = 5) (data not shown).

Correlative Analyses

Key cancer gene mutations were queried using a previously published testing platform that was established in our institution.¹³ Of 11 available samples tested, a mutation in CTNNB1 (G34E; 101G>A) was identified in 1 patient (Figure 3A and Table 3). This patient experienced SD during the first 2 cycles of everolimus treatment. No other gene mutations were identified.

Activation of the Akt/mTOR pathway was identified through phosphorylation of Akt at Ser473 (p-Akt), phosphorylation of mTOR at Ser2448 (p-mTOR), and phosphorylation of the downstream mTOR complex 1 target, ribosomal protein S6 at Ser235/236 (p-RPS6). Robust pathway activation in tumor tissue was observed in 3 out of 11 patient samples (Table 3). Moderate (2+) to high (3+) levels of p-Akt, p-mTOR, and p-RPS6 were noted in a patient demonstrating a partial response to everolimus as well as the CTNNB1 mutant–positive patient experiencing SD (Figure 3B and Table 3). Strong signaling (3+) was

observed in a patient with steatohepatitis experiencing 18 weeks of SD. Statistical significance was not reached, possibly due to limited sample size.

DISCUSSION

Approval of sorafenib has stimulated drug development research in advanced HCC. However, most current efforts are focused on antiangiogenic agents, and several VEGF receptor–targeted agents are being evaluated in phase 3 clinical trials. For patients who have progressed or cannot tolerate sorafenib, there are currently no standard therapies. Therefore, there is an urgent unmet need to develop additional effective agents, particularly those with novel mechanisms of action that target hepatocarcinogenesis. In this single-arm phase 1/2 study, 10 mg/day everolimus was defined as the phase 2 dosage and demonstrated preliminary activity in advanced HCC patients.

Daily and weekly dosing of everolimus have both been explored in other phase 1 studies. Everolimus given at 10 mg/day has inhibited mTOR activity in peripheral mononuclear cells, skin cells, and tumors, as measured by abrogated phosphorylation of downstream target proteins.^{14–16} In addition, these studies have suggested that daily dosing may result in more profound and persistent inhibition of mTOR activity than weekly administration. Chen et al¹⁷ reported their early experience of a randomized phase 1 and pharmacokinetic study of everolimus in advanced HCC. Two different schedules were tested in this randomized phase 1 study: continuous daily dosing and weekly dosing. DLTs observed included hyperbilirubinemia, ALT elevation, thrombocytopenia, infection, diarrhea, and cardiac ischemia. The MTD for the weekly and daily dosing schedule was determined to be 70 mg and 7.5 mg, respectively. Interestingly, reactivation of HBV was observed in 4 patients, and flaring of HCV was observed in 1 patient.

Given prior extensive experience of everolimus dosing in previous studies in other tumors, we chose a daily schedule and only examined 2 planned dosage levels at 5 mg/day and 10 mg/day instead of using the full conventional dose escalation scheme. In the phase 1 portion of our study, only 1 patient developed grade 3 lymphopenia at the 10 mg/day dosage level. The phase 2 portion of the study, with an additional 19 patients, confirmed the tolerability of the 10 mg/day dosage level. Consistent with the reported toxicity profile of everolimus, the most common adverse events included fatigue, hyperglycemia, diarrhea, myelosuppression, transient elevation of transaminases, hyponatremia, anorexia, stomatitis, and rash. Grade 3 events were relatively infrequent and included elevation of aspartate transaminase levels (12%), lymphopenia (12%), and hyponatremia (8%). Grade 4 events were rare and included 1 patient each with anemia and hypoxia. All patients with HBV in our study received suppressive anti-HBV therapy while on everolimus and no reactivation of HBV or HCV flaring were seen. Given the known immunosuppressive activity of mTOR inhibitors, patients with HBV and HCV infection should have their liver function tests and viral load closely monitored.

Although the first stage of the phase 2 portion of the study did not reach the criteria to proceed with the second stage, it provided initial evidence of antitumor activity of single agent everolimus at 10 mg/day in advanced HCC. Despite inclusion of a heavily pretreated population in our study (71% received prior systemic therapy, including 39% with prior sorafenib use and 32% with 2 prior systemic regimens), we observed a disease control rate, median PFS, and median TTP of 44%, 3.8 months, and 3.9 months, respectively. However, most patients had SD of short duration, which contributed to only 2 patients being progression-free at 24 weeks in the phase 2 portion of the study. Due to the limitations of our study (small sample size, lack of a randomized control arm, potential for patient selection bias, and heterogeneity of the patient population), clinical activity of everolimus

should be further defined in advanced HCC in future trials. Our study also underscores the importance and challenges of optimally designing phase 2 trials in HCC and the value of including a randomized control arm.¹⁸

No common somatic mutations in cancer driver genes known to regulate the PI3K/Akt/mTOR signaling pathway were identified in a small cohort of patients with archived tissue samples available. However, immunohistochemical analysis identified activation of Akt/mTOR signaling in more than 25% of samples evaluated. The complexity of the PI3K/Akt/mTOR pathway has become increasingly recognized, particularly in relation to its potential as a therapeutic target in cancer.^{19, 20} Prior data suggest the presence of a negative feedback loop, whereby increased activation of mTOR leads to a physiologic brake on further stimulation of this pathway.^{21–24} In some tumor types, mTOR inhibitors may interfere with this inhibitory feedback, resulting in a paradoxical increase in signaling by PI3K and increased activation of other Akt target proteins that promote cell survival. Although the loss of this negative feedback from mTOR inhibition may limit the efficacy of single-agent mTOR inhibitors in these tumor types, it supports investigation of treatment regimens that combine mTOR inhibitors with other agents, such as direct inhibitors of PI3K, Akt, or upstream receptor tyrosine kinases.

In conclusion, everolimus given at 10 mg/day as a single agent was well tolerated in patients with advanced HCC. Although the study did not meet the predefined criteria to proceed to the second stage of phase 2, everolimus demonstrated preliminary antitumor activity in patients with advanced HCC, most of whom had received prior systemic therapy. Given substantial data implicating activation of the PI3K/Akt/mTOR pathway in HCC, the unique mechanism of action of mTOR inhibitors, and preliminary clinical experience, additional studies are needed in patients with HCC to further define its efficacy either alone or in combination with sorafenib or other agents that might have additive or synergistic activity by modulating additional components of the PI3K/Akt/mTOR or other signaling pathways.

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Figure 1.

A CONSORT diagram illustrating all patients enrolled in the study is shown.

Figure 2.

(A) A waterfall plot shows the percent change from baseline in the sum of diameters of tumor lesions in 22 patients with repeat scans. (B) A Kaplan-Meier estimate of progression-free survival is shown. (C) A Kaplan-Meier estimate of overall survival is shown.

Figure 3. Genotype and immunohistochemical analysis results are shown. (A) A CTNNB1 101G>A (G34E) somatic mutation was identified in the tumor of a patient exhibiting stable disease to everolimus treatment. The mutation is indicated by an asterisk (*) in the multiplexed SNaPshot panel and is magnified in the box on the right. The genomic DNA peak represents the wild-type allele. (B) Immunohistochemical analysis demonstrated activation of the Akt/mammalian target of rapamycin pathway in the tumor of a patient exhibiting a partial response to everolimus treatment.

Table 1**Patient Characteristics**

Characteristic	Value
No. of patients	28
Men/Women (%)	18/10 (64/36)
Median age, y (range)	65 (33–81)
Eastern Cooperative Oncology Group performance status, no. (%)	
0	11 (39)
1	16 (57)
2	1 (4)
Cancer of the Liver Italian Program score, no. (%)	
0	1 (4)
1	9 (32)
2	9 (32)
3	9 (32)
Child-Pugh class, no. (%)	
A	23 (82)
B	5 (18)
Barcelona Clinic Liver Cancer stage, no. (%)	
B (intermediate)	2 (7)
C (advanced)	26 (93)
Macroscopic vascular invasion, no. (%)	6 (21)
Extrahepatic spread, no. (%)	24 (86)
Hepatocellular carcinoma etiology, no. (%)	
Hepatitis B virus	5 (18)
Hepatitis C virus	8 (29)
Alcohol	7 (25)
Hemachromatosis	2 (7)
Steatohepatitis	1 (4)
Unknown	7 (25)
Baseline laboratory values, median (range)	
Albumin (mg/dL)	4.0 (2.4–4.9)
Aspartate transaminase (U/L)	54.5 (25–176)
Total bilirubin (mg/dL)	0.6 (0.2–2.9)
Alpha-fetoprotein (ng/mL)	1729.5 (2.5–178,200.0)
Previous therapy, no. (%)	
Surgical resection	9 (32)
Chemoembolization	5 (18)
Radiofrequency ablation	3 (11)
Radiation therapy	1 (4)
Systemic therapy	20 (71)
Sorafenib	11 (39)

Characteristic	Value
Prior systemic regimens, no. (%)	
0	8 (29)
1	11 (39)
2	9 (32)

Table 2

Toxicity of Everolimus at 10 mg/day (n=25)

Toxicity	Any Grade	Grade 3	Grade 4
Fatigue	13 (52)	–	–
Hyperglycemia	12 (48)	1 (4)	–
Anemia	11 (44)	–	1 (4)
Diarrhea	11 (44)	–	–
Leukopenia	10 (40)	–	–
Platelets	10 (40)	–	–
Aspartate transaminase	9 (36)	3 (12)	–
Hyponatremia	9 (36)	2 (8)	–
Anorexia	9 (36)	–	–
Lymphopenia	8 (32)	3 (12)	–
Stomatitis	7 (28)	–	–
Rash/Desquamation	7 (28)	1 (4)	–
Alanine transaminase	6 (24)	1 (4)	–
Neutropenia	5 (20)	–	–
Alkaline phosphatase	4 (16)	–	–
Proteinuria	4 (16)	1 (4)	–
Vomiting	4 (16)	–	–
Nausea	4 (16)	–	–
Dyspnea	4 (16)	–	–
Weight loss	3 (12)	–	–
Acne rash	3 (12)	–	–
Edema	3 (12)	–	–
Taste disturbance	3 (12)	–	–
Hypoalbuminemia	2 (8)	–	–
Hypokalemia	2 (8)	–	–
Hypertriglyceridemia	2 (8)	–	–
Fever without neutropenia	2 (8)	–	–
Constipation	2 (8)	–	–
Nose bleeds	2 (8)	–	–
Cough	2 (8)	–	–
Insomnia	2 (8)	–	–
Pruritis/Itching	2 (8)	–	–
Pain	2 (8)	–	–
Total bilirubin	1 (4)	–	–
Hypernatremia	1 (4)	–	–
Hypocalcemia	1 (4)	–	–
Hypercholesterolemia	1 (4)	–	–
Bicarbonate	1 (4)	–	–
Hypoxia	1 (4)	–	1 (4)

Toxicity	Any Grade	Grade 3	Grade 4
Upper airway infection	1 (4)	–	–
Allergic rhinitis	1 (4)	–	–
Ascites	1 (4)	–	–
Distention/Bloating	1 (4)	–	–
Central nervous system cerebrovascular ischemia	1 (4)	–	–
Dehydration	1 (4)	–	–
Dry mouth	1 (4)	–	–
Flatulence	1 (4)	–	–
Photosensitivity	1 (4)	–	–
Pneumonitis/Pulmonary infiltrates	1 (4)	–	–

Data are presented as no. (%).

Table 3

Molecular Profiles

Patient No.	Hepatitis Status	Mutational Profiling	Immunohistochemistry			Best Tumor Response
			p-Akt	p-mTOR	p-RPS6	
5	HCV	NMI	Mod	Low	High	SD
9	HBV	NMI	Mod	Low	Low	PD
11	Negative	NMI	Low	Low	Mod	SD
14	HCV	NMI	Mod	Mod	High	PR
18	Negative	CTNNB1 (G34E)	Mod	Mod	High	SD
22	Negative	NMI	Low	Low	Low	SD
23	HBV	NMI	Mod	Low	Mod	PD
24	Negative	NMI	Low	Low	Mod	PD
25	HCV	NMI	ND	ND	ND	PD
26	Steatohepatitis	NMI	High	High	High	SD
28	Negative	NMI	Mod	Low	Mod	SD

HBV indicates hepatitis B virus; HCV, hepatitis C virus; ND, not determined; NMI, no mutation identified; p-Akt, phosphorylated Akt; p-mTOR, phosphorylated mammalian target of rapamycin; p-RPS6, phosphorylated ribosomal protein S6 kinase; PD, progressive disease; PR, partial response; SD, stable disease.