ASP5878, a Novel Inhibitor of FGFR1, 2, 3, and 4, Inhibits the Growth of FGF19-Expressing Hepatocellular Carcinoma

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Abstract

Hepatocellular carcinoma is an aggressive cancer with poor prognosis. Fibroblast growth factor 19, a member of the fibroblast growth factor family, is a ligand for fibroblast growth factor receptor 4. Moreover, it plays a crucial role in the progression of hepatocellular carcinoma. ASP5878 is a novel inhibitor of fibroblast growth factor receptors 1, 2, 3, and 4 that is under development. It inhibits fibroblast growth factor receptor 4 kinase activity with an IC50 of 3.5 nmol/L. ASP5878 is under development. It inhibits the phosphorylation of fibroblast growth factor receptor 4 and its downstream signaling molecules as well as induced apoptosis. Oral administration of ASP5878 at 3 mg/kg induced sustained tumor regression in a subcutaneous xenograft mouse model using Hep3B2.1-7. In HuH-7, an orthotopic xenograft mouse model, ASP5878 induced complete tumor regression and dramatically extended the survival of the mice. These results suggest that ASP5878 is a potentially effective therapeutic agent for hepatocellular carcinoma patients with tumors expressing fibroblast growth factor 19. Mol Cancer Ther; 16(1); 68–75. ©2016 AACR.

Introduction

Hepatocellular carcinoma (HCC) is an aggressive cancer with poor prognosis and the third most common cause of cancer-related deaths worldwide (1). Surgical resection is the most successful treatment for early-stage HCC; however, 70% of patients have recurrence after 5 years (2, 3). Sorafenib, a broad-spectrum kinase inhibitor, has been approved as a molecular-targeted drug for surgically unresectable HCC and has been shown to improve the duration of survival to 10.7 months in comparison with 7.9 months in patients receiving placebo in a phase 3 trial (4). However, more effective therapeutic approaches are urgently required for unresectable HCC.

The signaling pathway activated by FGFs and their cognate ligands, i.e., fibroblast growth factors (FGF), plays an important role in the course of development from early embryogenesis to the formation of various organs. There are 18 genes encoding FGFs and 4 genes encoding FGFR, which are expressed in various cells. Upon ligand binding to FGFR, the receptor is dimerized for the autophosphorylation and recruitment of adaptor proteins such as FGFR substrate 2 (FRS2), which activates several intracellular signaling pathways involved in cell growth, differentiation, and survival (5). FGF19 is a physiologic ligand for FGFR4, which is expressed in and secreted from the small intestine and gallbladder and controls the catabolism of cholesterol in the liver through FGFR4 and coreceptor, βKlotho (5). Furthermore, in recent years, it has been found that FGFR4 is inhibited by the addition of FGF19-neutralizing antibodies, an RNAi that targets FGFR4, or FGFR4 inhibitors (6, 10, 13, 14). Here, we describe the preclinical profile of ASP5878, which is a novel FGFR-selective inhibitor, targeting FGF19-expressing HCC.

In addition, we demonstrated that ASP5878 suppressed the growth of FGF19-expressing HCC cell lines, accompanied by the inhibition of FGFR4 phosphorylation and its downstream signaling molecules, which led to the induction of apoptosis. Furthermore, we showed that the oral administration of ASP5878 induced sustained tumor regression in FGF19-expressing HCC mouse models. Our data indicate that ASP5878 is a potentially effective therapeutic agent for HCC patients with tumors expressing FGF19.

Materials and Methods

Compounds

ASP5878 (Astellas Pharma Inc.; ref. 15) and sorafenib were synthesized in-house. ASP5878 was dissolved in dimethyl
sulfoxide for in vitro experiments or suspended in 0.5% methyl cellulose (MC) for in vivo experiments. Sorafenib was suspended in 12.5% Cremophor EL/12.5% ethanol for in vivo experiments.

Kinase assay

Inhibitory activities of 128 serine/threonine kinases were measured using the Mobility Shift Assay Kit (Carna Biosciences). IC50 values were determined for kinases that were inhibited by more than 50% by 200 nmol/L of ASP5878.

Cell growth assay

The human HCC cell lines HuH-7 (JCRB0403; ref. 16), HUH-6 Clone 5 (JCRB0401; ref. 17), HLF (JCRB0405; ref. 18), huH-1 (JCRB0199; ref. 19), JHH-1 (JCRB1062; ref. 20), JHH-2 (JCRB1028; ref. 20), JHH-5 (JCRB1029; ref. 20), JHH-6 (JCRB1030; ref. 20), and JHH-7 (JCRB1031; ref. 20) were obtained from the Health Science Research Resources Bank. Hep3B2.1-7 (HB-8064), SNU-423 (CRL-2238; ref. 21), SNU-449 (CRL-2234; ref. 21), SNU-182 (CRL-2235; ref. 21), SNU-387 (CRL-2237; ref. 21), SNU-396 (CRL-2233; ref. 21), HepG2 (HB-8065), and MDA-MB-453 (HTB-131) were obtained from the American Type Culture Collection. Li-7 (RCB1941) was obtained from RIKEN Bioresource Center. SNU-739 and SNU-368 were obtained from the Korean Cell Line Bank. PLC/PRF/5 was obtained from DS Pharma Biomedical. HCC cell lines were cultured according to each manufacturer’s guideline. The cell lines in this study were not authenticated by any method, although they have been authenticated by any cell lines were cultured according to each manufacturer’s guide- line. The cell lines in this study were not authenticated by any text in our laboratory but were purchased from the providers of authenticated cell lines and stored at early passages in a central cell bank at Astellas Pharma Inc. The experiments were conducted using low-passage cultures of these stocks. The cells were used in 96-well plates and incubated overnight. The cells were treated with ASP5878 for 5 days. Cell viability was measured using CellTiter-Glo (Promega).

Western blotting, ELISA, and pharmacodynamics

HCC cell lines were lysed with cell lysis buffer containing phosphatase and protease inhibitors, and the protein levels of FGF19, FGFR4, βKlotho, and actin (Clone AC-74) were determined by immunoblotting. Hep3B2.1-7 cells were treated with ASP5878 for 2 hours, and the protein levels of phospho-FRS2 (Tyr436), FRS2, phospho-extracellular signal-regulated kinase (phospho-ERK), ERK, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and actin (Clone AC-74) were detected by immunoblotting. Phosphorylated FGFR4 and total FGFR4 were measured by immunoprecipitation using FGFR4 antibody (A10) or mouse IgG1 isotype control, followed by immunoblotting using an antibody specific for phospho-FGFR (Tyr653/654) and FGFR4 (C16) or sandwich ELISA assay. PARP cleavage and actin (Clone AC-15) were detected by immunoblotting after 48-hour treatment with ASP5878. FRS2, phospho-ERK, ERK, and GAPDH levels in Hep3B2.1-7 tumors were measured by immunoblotting at 6 hours after single oral administration of ASP5878. The following antibodies were purchased: FGFR4 (A10) (sc-136988), FGFR4 (C16) (sc-124), FRS2 (sc-8318), and GAPDH (sc-20357) from Santa Cruz Biotechnology; βKlotho (AF5889) and mouse IgG1 isotype control (MAB002) from R&D systems; phospho-FGFR (Tyr653/654; #3471), PARP (#9542), ERK (#9102), phospho-ERK (Thr202/Tyr204; #9101), and phospho-FRS2 (Tyr436; #861) from Cell Signaling Technology; and actin (Clone AC-15, A5441), actin (Clone AC-74, A5316), and FGF19 (HPA036802) from Sigma-Aldrich. The following materials were purchased: cell lysis buffer from Cell Signaling Technology, phosphatase inhibitor cocktail from Thermo Scientific, protease inhibitor cocktail from Roche Diagnostics, and human total FGFR4 ELISA and human phospho-FGFR4 ELISA kits from R&D systems.

Xenograft models

All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Astellas Pharma Inc. In addition, Astellas Pharma Inc., Tsukuba Research Center, has accreditation status awarded by the Association for Assessment and Accreditation of Laboratory Animal Care International. Four-week-old male nude mice (CAnN.Cg-Foxn1nu/CrlCrlj [nu/nu]) were obtained from Charles River Japan. Hep3B2.1-7 cells were subcutaneously inoculated into the flank of mice at 3 × 10^6 cells/0.1 mL (Matrigel:PBS = 1:1)/mouse and allowed to grow. ASP5878 was administered as a once-daily oral dose initiated after confirming tumor growth in each experiment. Tumor volume was determined using the formula length × width^2 × 0.5. Matrigel was purchased from Corning Incorporated (Life Sciences).

Xenograft models (sorafenib switch)

Hep3B2.1-7 cells were subcutaneously inoculated into the flank of mice at 3 × 10^6 cells/0.1 mL (Matrigel:PBS = 1:1)/mouse and allowed to grow. After confirming tumor growth, mice were divided into two groups (n = 10 and 20 for vehicle and sorafenib groups, respectively) on day 0 on the basis of tumor volume and body weight. Vehicle (Cremophor EL/ethanol) or sorafenib (10 mg/kg) was administered as a once-daily oral dose from days 0 to 13. On day 14, the vehicle group was further divided into two groups (five per group), and the sorafenib group was further divided into four groups (five per group) on the basis of tumor volume and body weight. Vehicle (Cremophor EL/ethanol or 0.5% MC), sorafenib (10 mg/kg), or ASP5878 (3 mg/kg) was administered as a once-daily oral dose from days 14 to 52. The other two groups were used for a different research purpose. Tumor volume was determined using the formula length × width^2 × 0.5.

HCC orthotopic xenograft model

HuH-7–expressing luciferase (HuH-7-Luc) cells were obtained by infecting HuH-7 cells with lentivirus-expressing luciferase in the presence of blasticidin S (10 μg/mL). HuH-7-Luc cells were inoculated into hepatic parenchyma at 3 × 10^5 cells/0.01 mL (Matrigel 100%)/mouse. One week after inoculation, the mice were divided into three groups (n = 5 per group) on day 0 on the basis of bioluminescent imaging. Vehicle (Cremophor EL/ethanol or 0.5% MC), sorafenib (30 mg/kg), or ASP5878 (3 mg/kg) was administered as a once-daily oral dose for 91 days. Tumor growth was monitored by in vivo bioluminescent imaging of the abdomen after intraperitoneally injecting luciferin using IVIS-Lumina2 (PerkinElmer Inc.). During the study period (181 days), the survival of mice bearing hepatic tumors was recorded. The condition of the mice was monitored daily. The mice were scored as dead if any of the following signs of suffering were observed: cachexia, weakness, and difficulty in moving or eating. Mice that were scored as dead were euthanized.
**Results**

**Kinase inhibition profile of ASP5878**

ASP5878 (Fig. 1A) potently inhibited the tyrosine kinase activities of recombinant FGFR1, 2, 3, and 4 with IC\textsubscript{50} values of 0.47, 0.60, 0.74, and 3.5 nmol/L respectively (Table 1). Kinase selectivity was investigated against a panel of 128 human kinases, and the kinases that showed more than 50% inhibition by ASP5878 (200 nmol/L) were FGFRs, VEGFR2, and FMS (Table 1). In total, 117 kinases showed less than 50% inhibition (Supplementary Table S1).

**Antiproliferative effect of ASP5878 and FGF19 expression in 20 HCC cell lines**

A previous report suggested that an FGF19 antibody has the potential to be effective in FGF19-amplified HCC cell lines (6). To characterize the antiproliferative sensitivity of HCC cell lines to ASP5878, we examined the antiproliferative effect of ASP5878 in 20 human HCC cell lines (Fig. 1B). FGF19 protein expression in these cell lines was detected by Western blotting (Fig. 1C). HuH-7, Hep3B2.1-7, and JHH-7 cell lines exhibited potent sensitivity to ASP5878, with IC\textsubscript{50} values of 27, 8.5, and 21 nmol/L respectively (Table 2). FGF19 expression was observed in HuH-7, Hep3B2.1-7, and JHH-7 but not in other cell lines (Fig. 1C). Furthermore, the expression of FGFR4 and βKlotho was observed in HuH-7, Hep3B2.1-7, and JHH-7 (Fig. 1C). HUH-6 Clone 5, SNU-398, Li-7, and HLF were also sensitive to ASP5878 (Fig. 1B). The growth inhibition rate of HLF was 64% and those of other ASP5878-sensitive cell lines were higher than 95% at 1,000 nmol/L, highest concentration examined (data not shown). The mechanisms of

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**Figure 1.**

Antiproliferative effect of ASP5878 in FGF19-expressing HCC cell lines. **A**, Chemical structure of ASP5878. **B**, In vitro proliferation assay. **C**, Expression of FGF19, FGFR4, βKlotho, and actin across a panel of 20 human HCC cell lines. **D**, Inhibition of activated FGFR4 phosphorylation after ASP5878 treatment for 2 hours in Hep3B2.1-7 cells. **E**, Downstream signals after ASP5878 treatment for 2 hours in Hep3B2.1-7 cells. **F**, PARP cleavage induced by ASP5878. Hep3B2.1-7 cells were incubated with ASP5878 at the indicated concentration for 48 hours. Each protein was detected by immunoblotting using specific antibodies.
inhibition are under investigation. Thus, ASP5878 inhibited the cell proliferation of HCC cell lines expressing FGF19.

**Effect of ASP5878 on FGFR signaling**

To assess the modulation of FGFR4 phosphorylation and the downstream signaling pathway by ASP5878, Hep3B2.1-7 cells were treated with ASP5878 and cell lysates were analyzed by Western blotting. FGFR4 phosphorylation was constitutively observed and inhibited by ASP5878 in a concentration-dependent manner (Fig. 1D). In addition, ASP5878 treatment resulted in the suppression of phosphorylation, mobility shift of FRS2, and suppression of ERK phosphorylation (Fig. 1E). Furthermore, concentration-dependent increases in PARP cleavage, an apoptosis marker, were detected at 48 hours after ASP5878 treatment (Fig. 1F). The antiproliferative effects of Hep3B2.1-7 (IC50 = 8.5 nmol/L) and ASP5878-induced PARP cleavage at 10 nmol/L are comparable and have no gaps (Table 2). A similar effect was confirmed in HuH-7 and JHH-7 cell lines (data not shown). The suppression of phosphorylation and mobility shift of FRS2 were shown by another FGFR inhibitor in several cell lines (22). These experiments suggest that the FRS2 mobility shift reflects the phosphorylation status of FRS2 through FGFR signaling.

**ASPS5878 induces shrinkage of FGFR19-expressing HCC xenograft model**

We next evaluated the antitumor activity of ASP5878 in a Hep3B2.1-7 subcutaneous xenograft mouse model. In this model, a once-daily oral administration of ASP5878 induced tumor regression by 9% and 88% at 1 and 3 mg/kg, respectively (Fig. 2A), without affecting the body weight for 14 days (Fig. 2B). To assess the pharmacodynamic effects in vivo, we evaluated the inhibitory effect of ASP5878 on the constitutively activated FGFR downstream signals in Hep3B2.1-7 tumors. The mobility shift of FRS2 and suppression of ERK phosphorylation in Hep3B2.1-7 tumors were observed following the administration of 1 and 3 mg/kg ASP5878 (Fig. 2C). Because it was difficult to detect FGFR4 phosphorylation in Hep3B2.1-7 tumors under xenograft conditions, we examined the effect of ASP5878 on FGFR4 phosphorylation in an MDA-MB-453 breast cancer xenograft model.

**Survival prolongation of ASP5878 in FGFR9-expressing HCC orthotopic xenograft model**

To further evaluate the effect of ASP5878 in another HCC model, we established an imageable FGF19-expressing HCC orthotopic xenograft model. HuH-7-Luc cells were directly inoculated into the livers of mice, and the implanted cells were monitored by bioluminescent imaging of the upper abdominal area (Fig. 3A). The bioluminescent emissions of vehicle-treated control mice increased during the subsequent 24 days. Moreover, we evaluated the effect of sorafenib in this model. Although sorafenib treatment had a slight effect that reduced the emission, the mean bioluminescent emission in the ASP5878-treated group was lower than that in the vehicle-treated group. These results indicate that the ASP5878-treated mice exhibited a lower tumor burden than vehicle- and sorafenib-treated mice. The emission of ASP5878-treated mice reached background levels on day 31. Continuous dosing of ASP5878 sustained the emissions at the background level, and no increase was observed by the end of the dosing at day 90 (Fig. 3A and B). These data indicate that ASP5878 dosing induced sustained tumor regression without tumor regrowth. To confirm complete tumor regression, tumor recurrence was monitored after the cessation of ASP5878 treatment from day 91. No increase in bioluminescent emissions was observed 90 days after the cessation of administration (day 181) when the measurement was stopped (Fig. 3B). These data indicate that ASP5878 induced complete tumor regression at 90 days. Disease-related mortality was also measured (Fig. 3C). All the vehicle-treated control mice died by day 52, and all the sorafenib-treated mice died by day 90. In contrast, no mice treated with 3 mg/kg ASP5878 died during the 90-day experimental period. The effects on survival were accompanied by changes in the bioluminescent emissions, with vehicle-treated mice showing increased emission, whereas mice treated with 3 mg/kg ASP5878 showing reduced emissions.

**Antitumor activity of ASP5878 in the Hep3B2.1-7 xenograft model after sorafenib treatment**

We investigated the effect of ASP5878 in the Hep3B2.1-7 cell line after sorafenib treatment. According to previous reports, a dose of 10 mg/kg/day sorafenib was orally administered (24, 25). In our experiments, sorafenib administration for 14 days caused 40% tumor growth inhibition in the Hep3B2.1-7 xenograft model. Even after continuous sorafenib treatment, the Hep3B2.1-7 tumor gradually enlarged, and 47% tumor growth inhibition was observed by day 31. In contrast, the switch from sorafenib to ASP5878 on day 14 induced 83% tumor regression on day 52 relative to the tumor size observed on day 14 (Fig. 4). This indicates the therapeutic potential of ASP5878 for FGF19-overexpressing HCC patients who previously received sorafenib treatment.
Discussion

Recent clinical success in molecular-targeted therapies for cancer based on the identification of oncogenic gene alterations and their specific inhibitors is associated with dramatic antitumor effect, reduced side effects, and improved patient survival. The molecular-targeted therapies include Herceptin for HER2/neu receptor amplification and overexpression in breast cancer patients (26), XALKORI for echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase fusion oncogene-positive non–small-cell lung cancer (27), and Tarceva for EGFR-mutated non–small-cell lung cancer (28). Although there have been no driver oncogene-targeted therapies for HCC, recent data suggest that amplified or overexpressed FGF19 is a “driver oncogene” in HCC and that blockage of the FGF19–FGFR4 axis could be a novel therapeutic approach for FGF19-overexpressing HCC (6, 10, 12, 29).

There have been reports on the importance of FGF signaling in the development of various types of cancer, and this explains the interest in the development of FGFR- or FGF-selective inhibitors (30). The most clinically advanced agents targeting HCC are mainly broad-spectrum kinase inhibitors with VEGFR2 inhibitory activity (31). The inhibitory activity of such kinase inhibitors on FGFR4 kinase activity is less effective than the inhibitory activity on VEGFR2 (31). On the other hand, FGFR-selective inhibitors (NPV-BGJ398, AZD4547, and JNJ-42756493) are being developed for the treatment of lung/breast cancer with FGFR1 gene amplification, gastric cancer with FGFR2 gene amplification, cholangiocarcinoma with FGFR2 fusion, and urothelial cancer with FGFR3 gene alternations but not for HCC (31–33). Recently, two FGFR4-specific kinase inhibitors, BLU9931 and FGF401, have been reported and tested in clinical trials including HCC patients (13, 14).

We developed ASP5878, an FGFR tyrosine kinase inhibitor with potent FGFR4 inhibitory activity. Here, we demonstrated the preclinical profile of ASP5878 in the FGF19-overexpressing HCC models. ASP5878 is a potent inhibitor of FGFR tyrosine kinases 1, 2, 3, and 4 and is selective against a number of other kinases. Previous studies have shown that cell growth is inhibited by the suppression of FGFR4 expression in FGF19-amplified HCC cell lines with an FGFR4 RNAi or FGFR4-neutralizing antibody and FGFR-selective inhibitors (6, 10, 12–14, 29). These reports suggest that ASP5878 has the potential to be effective in the treatment of FGFR19-expressing HCC. Of the 20 HCC cell lines, we observed abnormal FGFR19 protein expression in HuH-7, Hep3B2.1-7, and JHH-7 cell lines. The proliferation of these cell lines was sensitive to ASP5878 and effectively inhibited by ASP5878. Furthermore, ASP5878 treatment potently inhibited cellular FGFR4, FRS2, and ERK activation and induced apoptosis in these HCC cell lines. Although the expression of FGFR4 and βKlotho was also observed in HuH-7, Hep3B2.1-7, and JHH-7 cell lines, some other HCC cell lines such as HepG2 and JHH-5 expressing FGFR4 and βKlotho without abnormal FGFR19 expression showed low sensitivity to ASP5878. Thus, using ASP5878, we confirmed that FGFR19 overexpression is an oncogenic driver of HCC and a response marker for FGFR4 inhibition in HCC. Immunohistochemical analysis in previous studies demonstrated that FGFR19 expression is associated with recurrence and poor prognosis in HCC patients, and serum FGFR19 levels in HCC patients are higher than those in healthy individuals (9, 10). These data indicate that the immunohistochemical staining of tumor FGFR19 or measurement of serum FGFR19 could be a stratification method for FGFR19-overexpressing HCC patients. In addition to FGF19-overexpressing HCC, HUH-6 Clone 5, HLF, SNU-398, and Li-7 were sensitive to ASP5878; however, FGFR19 expression was not observed. ASP5878
sensitivity of these cell lines could be based on the genetic alteration of other FGF-signaling genes and not the FGF19–FGFR4 axis. For instance, the amplification of FGF3/FGF4 genes and FGF21 overexpression has recently been observed in an HCC patient (34, 35). A subpopulation of HCC patients with FGFR1 overexpression was thought to be addicted to FGFR1 (36). FGFR2 and FGFR3 overexpression contributed to advanced HCC tumorigenesis (37–39). For instance, the SNU398 cell line was found to be sensitive to ASP5878 and NPV-BGJ398 but not to the FGFR4-selective inhibitor BLU9931 (14). FGFR1, 2, and 3, but not FGFR4, could be oncogenic drivers in the SNU398 cell line. We are presently conducting further studies to validate those hypotheses.

Once-daily oral administration of ASP5878 resulted in tumor regression in the Hep3B2.1-7 xenograft model. The antitumor effect was accompanied by the inhibition of FGFR downstream signaling in Hep3B2.1-7 xenograft tumors. These aspects were supported by the results that oral administration of 3 mg/kg ASP5878 induced tumor regression and downstream signals declined to background levels in the Hep3B2.1-7 xenograft model. Furthermore, the survival was enhanced in association with the potent antitumor effect of ASP5878 in the HuH-7 orthotopic xenograft model. Importantly, no tumor regrowth was observed among the two different models during ASP5878 administration. Furthermore, ASP5878 induced complete tumor regression, which was sustained for at least 90 days after the cessation of ASP5878 treatment in the HuH-7 orthotopic xenograft model. These data indicated that ASP5878 treatment of FGF19-expressing HCC induced sustained complete regression. Several FGFR inhibitors are being developed in clinical trials, but ASP5878 has a different profile than FGFR inhibitors (13). AZD4547 and NPV-BGJ398 show at least 30-fold greater selectivity toward FGFR1–3 than toward FGFR4 (22, 40). These inhibitors are categorized as pan-FGFR1, 2, and 3 inhibitors. It has not been demonstrated that those two inhibitors showed activity in HCC vivo model. In contrast, ASP5878 has potent FGFR4 inhibitory activity. It showed remarkable tumor regression and extended survival in an HCC model in vivo, without weight loss. Similar efficacy and tolerability may be achieved in HCC patients; this
Tumor volume is expressed as the mean ± SEM. Hypothesis is currently being evaluated in clinical trials. BLU9931 showed >50-fold greater inhibition of FGFR4 than of FGFR1, 2, and 3 (14). FGFR4 is reported to be an FGFR4-selective inhibitor, but the kinase selectivity profile remains unknown (13). Similar to ASP5878, these inhibitors are improved to improve the therapeutic outcome of HCC patients with FGFR1 overexpression. The advantage of ASP5878 over FGFR4-selective inhibitors is the ability to provide therapeutic benefit to a subset of patients with HCC whose tumors are driven by FGFR1, 2, or 3. In addition to FGFR4, the overexpression of FGFR1, FGFR2, and FGFR3 contributes to advanced HCC tumorigenesis, metastasis, and poor prognosis (36–38). In particular, Paur and colleagues observed that FGFR3 and/or FGFR4 expression was elevated in 68% of HCC patients (38). Moreover, the amplification of FGFR3/FGFR4 genes and FGFR21 overexpression has recently been observed in an HCC patient (34, 35). These ligands bind to FGFR1, 2, and/or FGFR3. ASP5878 may be beneficial for these HCC subpopulations. In fact, the SU9378 cell line was sensitive to ASP5878 and BGI398 but not to BLU9931; SU9378 may be one of the representatives of these HCC subsets. InJ-42756493 was found to inhibit all members of the FGFR family at the low nanomolar IC50 range (41). This type of compound is categorized as a "pan-FGFR1, 2, 3, and 4 inhibitor," including ASP5878. JNJ-42756493 is currently being evaluated in clinical trials. Hyperphosphatemia has been observed with other FGFR inhibitors (e.g., AZD4547 and INJ-42756493) and is likely based on mechanism of action (32, 33). In line with the findings, increased serum phosphate was observed in rodents repeatedly administered with ASP5878 (unpublished data). ASP5878 is currently being evaluated in phase I clinical trials to evaluate the safety, pharmacokinetics, and pharmacodynamics and defines the recommended phase II dose.

In conclusion, using a panel of HCC cell lines in vitro, we have shown that FGFR19 expression levels predict the antiproliferative sensitivity to the novel and potent FGFR-selective agent ASP5878. ASP5878 induced almost complete tumor regression in association with the pharmacodynamic modulation of FGFR downstream signals in a Hep3B2.1-7 xenograft model. Furthermore, ASP5878 showed complete and sustained tumor regression and extended survival in a HuH-7 orthotopic xenograft model. Our findings demonstrate that ASP5878, a novel FGFR inhibitor with potent FGFR4 inhibitory activity, is expected to improve the therapeutic outcomes of HCC patients with FGFR1 overexpression. ASP5878 is currently being evaluated in phase I clinical trials.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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