First Selective Small Molecule Inhibitor of FGFR4 for the Treatment of Hepatocellular Carcinomas with an Activated FGFR4 Signaling Pathway

Margit Hagel, Chandra Miduturu, Michael Sheets, Nooreen Rubin, Weifan Weng, Nicolas Stranksy, Neil Bifulco, Joseph L. Kim, Brian Hodous, Natasja Brooijmans, Adam Shutes, Christoph Winter, Christoph Lengauer, Nancy E. Kohl, and Timothy Guzi
ABSTRACT

Aberrant signaling through the fibroblast growth factor 19 (FGF19)/fibroblast growth factor receptor 4 (FGFR4) signaling complex has been shown to cause hepatocellular carcinoma (HCC) in mice and has been implicated to play a similar role in humans. We have developed BLU9931, a potent and irreversible small-molecule inhibitor of FGFR4, as a targeted therapy to treat patients with HCC whose tumors have an activated FGFR4 signaling pathway. BLU9931 is exquisitely selective for FGFR4 versus other FGFR family members and all other kinases. BLU9931 shows remarkable antitumor activity in mice bearing an HCC tumor xenograft that overexpresses FGF19 due to amplification as well as a liver tumor xenograft that overexpresses FGF19 mRNA but lacks FGF19 amplification. Approximately one third of patients with HCC whose tumors express FGF19 together with FGFR4 and its coreceptor klotho β (KLB) could potentially respond to treatment with an FGFR4 inhibitor. These findings are the first demonstration of a therapeutic strategy that targets a subset of patients with HCC.

SIGNIFICANCE: This article documents the discovery of BLU9931, a novel irreversible kinase inhibitor that specifically targets FGFR4 while sparing all other FGFR paralogs and demonstrates exquisite kinome selectivity. BLU9931 is efficacious in tumors with an intact FGFR4 signaling pathway that includes FGF19, FGFR4, and KLB. BLU9931 is the first FGFR4-selective molecule for the treatment of patients with HCC with aberrant FGFR4 signaling. Cancer Discov; 5(4); 1–14. © 2015 AACR.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a complex and heterogeneous tumor type with multiple genetic and epigenetic alterations (1). Sorafenib, standard of care for patients with advanced HCC in many countries, slows the growth of advanced liver cancers and increases patient survival by an average of 3 months (2, 3). With the minimal survival benefit provided by sorafenib and no second-line or third-line treatment options available, there is a pressing need for more effective HCC therapies. Recent genomic analyses of HCC (4–8) have provided insights into the recurrent genomic aberrations of this cancer type. Unfortunately, they have yielded few opportunities for novel therapeutic strategies, and for the most part newly identified driver genes or pathways have remained technically intractable for drug discovery. However, a focal amplification on chromosome 11q13.3 has been described in a subset of HCCs (4–8). Only three genes (9) reside within the peak region of amplification, FGF19, CCND1, and OROAV1.

Fibroblast growth factor 19 (FGF19) is a tightly controlled hormone that regulates bile acid synthesis and hepatocyte proliferation in the normal liver via activation of its receptor, fibroblast growth factor receptor 4 (FGFR4; ref. 10). Bile acid induces FGF19 expression in the intestine, and the increased circulating levels of the hormone result in hepatic repression of cholesterol 7α-hydroxylase (CYP7A1), which mediates the key regulatory step in hepatic bile acid synthesis. In addition, FGF19 has been shown to induce expression of proliferative markers, such as EGR1, c-FOS, and AFP (11). In the subset of HCCs harboring FGF19 amplification, the resulting overexpression of FGF19 in the hepatocyte itself leads to the activation of this pathway and to a switch from intestine-driven endocrine to autocrine hepatocellular signaling control. It has been demonstrated that overexpression of FGF19 in transgenic mice produces liver tumors that are sensitive to treatment with FGFR4 or FGF19 antagonist antibodies (12, 13). In addition, the genetic knockout of Fgfr4 prevented transgenic mice with exogenous expression of Fgf19 from developing tumors. These studies provide a strong rationale for targeting FGFR4 to treat a genetically defined subgroup of patients with HCC with an activated FGFR4 signaling pathway (6, 10, 12, 13).

To date, potent and selective FGFR4 inhibitors are not available to patients. A number of FGFR inhibitors are currently in clinical trials to treat cancers with FGFR1, 2, or 3 aberrations. However, most of these agents are either pan-FGFR inhibitors with promiscuous kinome activity (see Supplementary Fig. S1), such as LY-2874455 (14) and ponatinib (15), or selective FGFR1–3 inhibitors with moderate to weak potency against FGFR4, such as BGJ398 (16) and AZD4547 (17). The lack of kinome selectivity invariably results in toxicity related to off-target activity, whereas inhibition of FGFR1 and 3 causes soft-tissue mineralization and hyperphosphatemia. These off-target, dose-limiting toxicities have been well described in both animals and patients (18) and would preferably be avoided to achieve potent FGFR4 inhibition.

Efforts to discover selective, ATP-competitive, reversible inhibitors against FGFR4 have been challenging, given the high sequence similarity among the four FGFR paralogs...
and typical potency bias toward paralogs 1 to 3. However, a cysteine located near the ATP-binding site in FGFR4 is unique among FGFR family members, and rare among other kinases. We therefore postulated that targeting this cysteine could provide an effective strategy for the discovery of selective FGFR4 inhibitors that spare FGFR1, 2, and 3 as well as other kinases. Covalent inhibitors directed against a cysteine have been developed for a number of kinases, and two such drugs, ibrutinib (targeting BTK) and afatinib (targeting EGFR), were recently approved for various cancer indications (19, 20).

This study describes the identification of BLU9931, a highly selective, covalent, small-molecule inhibitor of FGFR4 that spares the other FGFR paralogs and demonstrates exquisite kinase selectivity. BLU9931 exhibits robust and dose-dependent inhibition of FGFR4 activity in vitro and significant antitumor activity—including complete responses—in HCC xenograft models with an intact FGFR4 signaling pathway.

RESULTS

BLU9931 Is a Potent, Paralog-Selective, and Irreversible Inhibitor of FGFR4

FGFR4 contains two cysteines proximal to its ATP-binding pocket (19). One, Cys478, located at the tip of the P-loop, is conserved among all FGFR paralogs, and has been successfully targeted by a covalent FGFR pan-inhibitor (21). A second cysteine, Cys552, in the hinge region of FGFR4, is unique among the four paralogs (Fig. 1A) and thereby provides a selectivity handle to specifically target FGFR4. Notably, only six other kinases possess a cysteine at the equivalent position, all with overall low sequence similarity to FGFR4 (Supplementary Fig. S2). Thus, we hypothesized that a small-molecule inhibitor of FGFR4 that covalently binds to Cys552 would exhibit selectivity for FGFR4 versus other FGFR family members and all other kinases.

We utilized structure-based design principles to develop a potent and selective FGFR4 inhibitor starting from known FGFR inhibitor templates. Specifically, we focused on 6,6-hetero bicyclic templates as exemplified by PD173074, an inhibitor in the pyrido[2,3-d]pyrimidine heterocyclic core class with nanomolar cellular potency against FGFR1–3 (22, 23). The high potency and good kinase selectivity of PD173074 stems from the dimethoxyphenyl substituent (22, 23). The high potency and good kinase selectivity of PD173074 stems from the dimethoxyphenyl substituent occupying the hydrophobic pocket, providing FGFR-family selectivity. Distinctly, the aniline phenyl ring adopts a dihedral rotation of approximately 60°, stabilized in part by the methyl substituent at the C3 position. As hypothesized, this rotation directs the ortho-substituted acrylamide toward the Cys552 sulfur, and in a direction that should clash with the tyrosine side chains of FGFR1–3 at the same position in the kinase hinge. To achieve covalency, the reactive acrylamide moiety adopts a trans-amide conformation, which positions the terminal carbon proximal to the Cys552 sulfur.

Figure 1. BLU9931 is a selective FGFR4 inhibitor. A, the unique cysteine in the FGFR4 hinge region (Cys552, hinge-1 residue) provides an opportunity for selective inhibition through covalent modification. The corresponding residue in FGFR1, 2, and 3 is a tyrosine. GK, gatekeeper residue; B, chemical structure of BLU9931 N-((6-(2,6-dichloro-3,5-dimethoxyphenyl)quinazolin-2-yl)amino)-3-methylphenylacrylamide. C, crystal structure of BLU9931 in complex with FGFR4. Protein carbon atoms are colored green and inhibitor carbons orange. The Cys552 sulfur is colored yellow and drawn as a covalent adduct with BLU9931. Hydrogen bonds from the aminoquinazoline portion of BLU9931 to the FGFR4 kinase hinge region are depicted with dotted lines.
BLU9931, a Selective FGFR4 Inhibitor for the Treatment of HCC

Bond angles and bond distances about the covalent adduct are near optimum values, and the quinazoline core superimposes well with the pyridopyrimidine core of PD173074, suggesting that the covalent engagement requires minimal distortion of the optimally bound inhibitor.

Mass spectrometry was performed to confirm that BLU9931 covalently modified the FGFR4 kinase. Incubation of BLU9931 with recombinant FGFR4 resulted in a mass shift consistent with the formation of a covalent complex between BLU9931 and FGFR4 protein. This mass shift was not observed when a reduced analogue of BLU9931 was used (Supplementary Fig. S3). To confirm targeting of Cys552 by BLU9931, covalent complexes were digested with chymotrypsin and subjected to electrospray ionization–tandem mass spectrometry (ESI–MS/MS). This digestion resulted in 95% proteolytic coverage producing 22 peptides containing Cys552, and 14 of these peptides showed a mass consistent with BLU9931 adduct formation (data not shown), confirming that the nucleophile of interest, Cys552, was successfully targeted.

When assessing a covalent inhibitor such as BLU9931, potency is expressed by using the ratio (k_{inact}/K_i) of the inactivation rate constant (k_{inact}) with respect to the binding constant (K_i) as the amount of active FGFR4 enzyme changes over time. BLU9931 is a potent inhibitor of FGFR4 [k_{inact}/K_i = (0.6) × 10^6 (mol/L)^{-1} s^{-1}] (Supplementary Table S1). We further determined that BLU9931 does not elicit general reactivity toward thiols, such as glutathione, by demonstrating a negligible IC_{50} shift of BLU9931 inhibition in FGFR4 enzyme activity assays performed in the presence of 1 mM glutathione (data not shown).

Consistent with structural predictions, the covalent bond formation with Cys552 in FGFR4 afforded BLU9931 exquisite potency and paralog selectivity. BLU9931 showed potent inhibition of FGFR4 enzyme activity (IC_{50} = 3 nmol/L), but weak inhibition of FGFR1 (IC_{50} = 591 nmol/L), FGFR2 (IC_{50} = 493 nmol/L), and FGFR3 (IC_{50} = 150 nmol/L) activity. The ability of BLU9931 to form a covalent bond with FGFR4 affords significant potency, as a noncovalent analogue with a reduced acrylamide moiety exhibits much less potent inhibition of FGFR4 enzyme activity (IC_{50} = 938 nmol/L).

Kinome-wide selectivity was demonstrated by profiling BLU9931 at a 3 μmol/L concentration across a panel of 456 kinases and disease-relevant kinase mutants using the KINOMEscan methodology (24). This analysis revealed that BLU9931 displayed significant binding to only two of the 398 wild-type kinases, FGFR4 (99.7% inhibition relative to DMSO control; K_d = 6 nmol/L) and CSF1R (90.1% inhibition relative to DMSO control; K_d = 2716 nmol/L). The kinome-wide selectivity profile can be described using a selectivity score [S(10)] that reflects the number of kinases bound by BLU9931 with a percentage of DMSO control <10 over the total number of wild-type kinases (24). The S(10) at 3 μmol/L for BLU9931 is 0.005. Of note, BLU9931 showed only weak activity against other kinases possessing a cysteine at the same position as Cys552 (9% and 0% inhibition at 3 μmol/L relative to DMSO control against MAPKAPK2 and TTK, respectively, for kinases available in the KINOMEscan panel). Activity was also measured against p70S6Kb (IC_{50} > 10 μmol/L).

BLU9931 Potently and Selectively Inhibits FGFR4 Signaling and Inhibits Proliferation in HCC Cell Lines with an Activated FGFR4 Signaling Pathway

Next, we sought to demonstrate that BLU9931 potently and selectively inhibited FGFR4 signaling in cells using FGFR-driven cancer cell lines. MDA-MB-453 cells predominantly express a mutated form of FGFR4 due to genomic amplification (26). In DMS114 cells, BLU9931 showed minimal reduction of phosphorylation in any of the tested pathway components downstream of FGFR1. In contrast, LY-2874455 demonstrated potent, dose-dependent reduction of phosphorylation of FGFR4 signaling pathway components, including fibroblast growth factor receptor substrate 2 (FRS2), MAPK, and AKT. BGJ398 also showed dose-dependent inhibition of the FGFR4 signaling cascade in MDA-MB-453 cells, albeit with lowered potency (Fig. 2A).

To determine the selectivity of BLU9931 in cells, we evaluated the effect of the compound on signaling in DMS114 cells. In these cells, the pathway downstream of FGFR1 is activated through overexpression of FGFR1 as a consequence of gene amplification (26). In DMS114 cells, BLU9931 showed minimal reduction of phosphorylation in any of the tested pathway components downstream of FGFR1. Consistent with structural predictions, the covalent bond formation with Cys552 in FGFR4 afforded BLU9931 exquisite potency and paralog selectivity. BLU9931 showed potent inhibition of FGFR4 enzyme activity (IC_{50} = 3 nmol/L), but weak inhibition of FGFR1 (IC_{50} = 591 nmol/L), FGFR2 (IC_{50} = 493 nmol/L), and FGFR3 (IC_{50} = 150 nmol/L) activity. The ability of BLU9931 to form a covalent bond with FGFR4 affords significant potency, as a noncovalent analogue with a reduced acrylamide moiety exhibits much less potent inhibition of FGFR4 enzyme activity (IC_{50} = 938 nmol/L).

Kinome-wide selectivity was demonstrated by profiling BLU9931 at a 3 μmol/L concentration across a panel of 456 kinases and disease-relevant kinase mutants using the KINOMEscan methodology (24). This analysis revealed that BLU9931 displayed significant binding to only two of the 398 wild-type kinases, FGFR4 (99.7% inhibition relative to DMSO control; K_d = 6 nmol/L) and CSF1R (90.1% inhibition relative to DMSO control; K_d = 2716 nmol/L). The kinome-wide selectivity profile can be described using a selectivity score [S(10)] that reflects the number of kinases bound by BLU9931 with a percentage of DMSO control <10 over the total number of wild-type kinases (24). The S(10) at 3 μmol/L for BLU9931 is 0.005. Of note, BLU9931 showed only weak activity against other kinases possessing a cysteine at the same position as Cys552 (9% and 0% inhibition at 3 μmol/L relative to DMSO control against MAPKAPK2 and TTK, respectively, for kinases available in the KINOMEscan panel). Activity was also measured against p70S6Kb (IC_{50} > 10 μmol/L).

Next, we sought to demonstrate that BLU9931 potently and selectively inhibited FGFR4 signaling in cells using FGFR-driven cancer cell lines. MDA-MB-453 cells predominantly express a mutated form of FGFR4, FGFR4<sup>Y367C</sup>, which leads to ligand-independent activation of kinase activity and activation of signaling downstream of FGFR4 (25). We compared BLU9931 activity in this cell line with two pan-FGFR inhibitors, BGJ398 and LY-2874455. BGJ398 is a potent and selective inhibitor of FGFR1–3 kinases that is approximately 10-fold less potent versus FGFR4 kinase (Supplementary Fig. S1). LY-2874455 potently inhibits all FGFRs and possesses poor selectivity across the kinome (Supplementary Fig. S1).

In MDA-MB-453 cells, BLU9931 and LY-2874455 demonstrated potent, dose-dependent reduction of phosphorylation of FGFR4 signaling pathway components, including fibroblast growth factor receptor substrate 2 (FRS2), MAPK, and AKT. BGJ398 also showed dose-dependent inhibition of the FGFR4 signaling cascade in MDA-MB-453 cells, albeit with lowered potency (Fig. 2A).

To determine the selectivity of BLU9931 in cells, we evaluated the effect of the compound on signaling in DMS114 cells. In these cells, the pathway downstream of FGFR1 is activated through overexpression of FGFR1 as a consequence of gene amplification (26). In DMS114 cells, BLU9931 showed minimal reduction of phosphorylation in any of the tested pathway components downstream of FGFR1. In contrast, LY-2874455 and BGJ398 demonstrated very potent dose-dependent inhibition of phosphorylated FGFR (pFGFR), pFRS2, pMAPK, and pAKT (Fig. 2B).

We also sought to test the potency of BLU9931 in an HCC model that represents the patient population for which a selective FGFR4 inhibitor could be beneficial. For this purpose, we chose the Hep 3B cell line, in which FGFR19 is amplified. BLU9931 and the two pan-FGFR inhibitors showed dose-dependent inhibition of the signaling cascade downstream of FGFR4. Similar to what was observed in the MDA-MB-453 cells, BLU9931 and LY-2874455 exhibited potent inhibition of phosphorylation of the FGFR4 pathway components, whereas BGJ398 showed less potent inhibition (Fig. 2C). It is notable that in Hep 3B cells, little to no inhibition of pAKT was observed with any of the tested compounds. This result is notable as treatment with BLU9931 led to induction of caspase-3/7 activity, indicative of induction of apoptosis, at concentrations of BLU9931 that resulted in inhibition of signaling downstream of FGFR4 (Supplementary Fig. S4).

It has previously been demonstrated that HCC cell lines that overexpress FGFR19 due to genomic amplification are sensitive to an FGFR4 inhibitor only when a fully functional receptor/ligand complex consisting of FGFR19, FGFR4, and the coreceptor KLB is present (27). To assess the antiproliferative activity of BLU9931 in HCC cell lines, we interrogated a selected panel of 11 HCC cell lines with varying levels of expression of FGFR19, FGFR4, and KLB (28; Table 1). Consistent with the previous finding, HCC cell lines that express a fully functional receptor complex as well as ligand were sensitive to BLU9931, with EC_{50}...
**Table 1. BLU9931 inhibits proliferation of HCC cell lines that express an intact FGFR4 signaling complex**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>FGF19 mRNA expression (FPKM)</th>
<th>FGF19 DNA copy number</th>
<th>FGFR4 mRNA expression (FPKM)</th>
<th>FGFR4 DNA copy number</th>
<th>KLB mRNA expression (FPKM)</th>
<th>KLB DNA copy number</th>
<th>BLU9931 EC50 (μmol/L)</th>
<th>BGJ398 EC50 (μmol/L)</th>
<th>Proliferation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hep 3B</td>
<td>13.291</td>
<td>4.727</td>
<td>121.607</td>
<td>1.464</td>
<td>12.170</td>
<td>2.945</td>
<td>0.07</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>HUH-7</td>
<td>20.898</td>
<td>4.167</td>
<td>133.175</td>
<td>2.277</td>
<td>32.138</td>
<td>1.662</td>
<td>0.11</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>JHH-7</td>
<td>44.191</td>
<td>9.453</td>
<td>88.595</td>
<td>1.959</td>
<td>12.897</td>
<td>2.580</td>
<td>0.02</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>PLC/PRF/5</td>
<td>0.027</td>
<td>2.368</td>
<td>64.001</td>
<td>1.824</td>
<td>13.263</td>
<td>2.209</td>
<td>&gt;10</td>
<td>8.17</td>
<td></td>
</tr>
<tr>
<td>SK-HEP-1</td>
<td>0.023</td>
<td>2.118</td>
<td>12.047</td>
<td>2.159</td>
<td>0.037</td>
<td>1.371</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td></td>
</tr>
<tr>
<td>SNU-182</td>
<td>0.020</td>
<td>2.030</td>
<td>1.993</td>
<td>2.854</td>
<td>0.014</td>
<td>1.958</td>
<td>6.41</td>
<td>6.00</td>
<td></td>
</tr>
<tr>
<td>SNU-387</td>
<td>0.024</td>
<td>8.828</td>
<td>0.057</td>
<td>2.212</td>
<td>0.055</td>
<td>1.466</td>
<td>6.92</td>
<td>1.95</td>
<td>0.02</td>
</tr>
<tr>
<td>SNU-398</td>
<td>0.030</td>
<td>2.267</td>
<td>14.046</td>
<td>2.250</td>
<td>0.147</td>
<td>2.357</td>
<td>1.95</td>
<td>8.31</td>
<td></td>
</tr>
<tr>
<td>SNU-423</td>
<td>0.000</td>
<td>2.251</td>
<td>0.443</td>
<td>2.382</td>
<td>0.034</td>
<td>2.077</td>
<td>&gt;10</td>
<td>5.10</td>
<td></td>
</tr>
<tr>
<td>SNU-449</td>
<td>0.014</td>
<td>1.727</td>
<td>45.177</td>
<td>1.718</td>
<td>0.369</td>
<td>1.734</td>
<td>&gt;10</td>
<td>5.10</td>
<td></td>
</tr>
<tr>
<td>SNU-878</td>
<td>12.189</td>
<td>13.563</td>
<td>36.324</td>
<td>2.107</td>
<td>2.438</td>
<td>1.576</td>
<td>5.15</td>
<td>1.22</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** Highlighted in bold: expression FPKM > 1, copy number ≥ 4, sensitivity to BLU9931 ≤ 1 μmol/L.
Shaded rows: cell lines exhibiting EC50 ≤ 1 μmol/L in the BLU9931 proliferation assay.
Data represent average of N=2.
Abbreviation: FPKM, fragments per kilobase of exon per million mapped reads.
values of <1 μmol/L (Table 1). Notably, the three most sensitive cell lines—Hep 3B, HuH7, and JHH7—also harbor a copy-number gain of FGF19. All other tested HCC lines, with the exception of SNU-878, lack expression of one of the required genes (Table 1) and were not sensitive to BLU9931. Interestingly, BGJ398 showed a similar pattern of sensitivity across the panel of HCC cell lines, with the exception of the SNU-398 cells that were sensitive to BGJ398 but not to BLU9931. Notably, the three cell lines that were sensitive to growth inhibition by BLU9931 were also sensitive to BGJ398 (Table 1; ref. 27).

**BLU9931 Inhibits Signaling in Hep 3B Cells and Demonstrates Prolonged Duration of Action**

Consistent with effects on proliferation, we also saw modulation of proximal and distal biomarkers of the FGFR4 signaling pathway in the FGF19-amplified HCC cell line Hep 3B. As demonstrated previously, covalent inhibitors can produce a persistent and sustained inhibition of their targets following a brief exposure to the inhibitor; resynthesis of the target protein in the absence of inhibitor results in a return of target-driven activity (29, 30). In Hep 3B cells, brief exposure to BLU9931 led to decreased levels of pFRS2 and pMAPK, and this level of inhibition was maintained for at least 8 hours after removal of the compound (Fig. 3A), which is in agreement with the FGFR4 half-life in these cells (Supplementary Fig. S5). In contrast, BGJ398 inhibited signaling downstream of FGFR4 for 1 to 2 hours in the same assay (data not shown).

BLU9931 exhibited the same prolonged effect on more distal biomarkers of FGF19/FGFR4 signaling in liver cells, namely CYP7A1 and early growth response 1 (EGR1). In a physiological setting, a decrease in signaling through FGFR4 leads to an increase of CYP7A1 mRNA expression due to reversal of a negative feedback loop. Consistent with this model, we observed an induction of CYP7A1 expression for at least 8 hours following the removal of BLU9931 (Fig. 3B). In addition, the expression of the proliferative marker EGR1 was inhibited for a comparable time period (Fig. 3C).

**BLU9931 Demonstrates Antitumor Activity in HCC Xenograft Models**

Having observed that BLU9931 inhibited proliferation of Hep 3B cells in vitro, we next evaluated the ability of BLU9931 to inhibit growth of HCC tumors in vivo using a Hep 3B xenograft model. The initial pharmacokinetic properties of BLU9931 were evaluated in mice following intravenous and oral delivery. BLU9931 displayed moderate bioavailability (18%) and a half-life of 2.3 hours when administered orally at a dose of 10 mg/kg (Supplementary Table S2). We then sought to establish a pharmacokinetic-pharmacodynamic (PK–PD) relationship between BLU9931 and the biomarker CYP7A1 in vivo. Hep 3B tumor-bearing mice were dosed with 10, 30, or 100 mg/kg of BLU9931 every 12 hours for a total of 4 doses. Tumor and plasma samples were collected 0, 8, 12, 20, and 24 hours after the last dose. Significant induction of CYP7A1 expression was
Furthermore, 2 of the 9 mice in the 100-mg/kg twice-daily dose, BLU9931 caused tumor growth inhibition of Hep 3B tumors (Fig. 4B). At dosing with BLU9931 for 21 days resulted in dose-dependent plasma concentration of BLU9931 (Fig. 4A).

Consistent with pharmacodynamic observations, oral dosing with BLU9931 for 21 days resulted in dose-dependent growth inhibition of Hep 3B tumors (Fig. 4B). At the 100-mg/kg twice-daily dose, BLU9931 caused tumor regression. Furthermore, 2 of the 9 mice in the 100-mg/kg treatment group showed no signs of tumor 30 days after cessation of treatment (data not shown). The antitumor efficacy of sorafenib, the only approved systemic treatment for HCC, was also evaluated in this study. Sorafenib dosed once daily at 30 mg/kg had only a modest effect on tumor growth. Hep 3B tumors induced significant loss of body weight, as evidenced by the weight loss observed in the vehicle-treated mice. The reason for the weight loss is unclear, but it was consistently observed in this model in our experiments. BLU9931 prevented this weight loss in a dose-dependent manner, demonstrating that the highest doses of BLU9931 were not only efficacious but also well tolerated (Fig. 4C). In contrast, mice treated with sorafenib exhibited body weight loss similar to that seen in vehicle-treated mice, a result that is consistent with the modest antitumor efficacy of the agent at the tested dose.

To examine the dependency on FGFR4 signaling in samples overexpressing FGF19 but without FGF19 amplification, we examined available cell lines from HCC patient-derived xenografts (PDX; ref. 31). According to our findings that concurrent expression of FGF19, FGFR4, and KLB is necessary to convey sensitivity to BLU9931 in the HCC cell line panel (Table 1), we first confirmed that the PDX-derived cell lines expressed FGF19 and FGFR4 at levels similar to those of the parental tumors (Fig. 5A–C). We also examined expression levels of KLB (Fig. 5D). This analysis identified LIXC012 and LIXC011 as the only two PDX cell lines robustly expressing all three genes, in a similar fashion to Hep 3B cells. LIXC011 expressed similar levels of KLB as compared with LIXC012 and low but detectable levels of FGF19 and FGFR4 (Fig. 5B–D).

In line with our expectations, treatment with BLU9931 led to robust induction of CYP7A1 expression only in the cell lines that express FGF19, FGFR4, and KLB (LIXC011, LIXC012, and Hep 3B; Fig. 5E). Furthermore, induction of CYP7A1 by BLU9931 correlated with sensitivity to the inhibitor in proliferation assays (Fig. 5F). Treatment with BLU9931 led to growth inhibition in LIXC011, LIXC012, and Hep 3B cells, at EC50 of 1.2 μmol/L, 0.14 μmol/L, and 0.04 μmol/L, respectively. Notably, and consistent with our hypothesis, no PDX-derived cell line without detectable FGF19 expression responded to treatment with BLU9931 as measured by CYP7A1 induction or inhibition of proliferation.

We next asked whether an HCC model overexpressing FGF19 independent of gene amplification would respond to BLU9931 in vivo. We performed a PK–PD study in LIXC012 tumor–bearing mice with BLU9931 at doses of 30, 100, or 300 mg/kg (Fig. 6A), as described above for the Hep 3B xenograft model. In the 100- and 300-mg/kg BLU9931 groups, CYP7A1 mRNA expression was induced, and, as observed with Hep 3B xenografts, it returned to baseline 12 to 20 hours following the last dose (Fig. 6A). Having observed potent inhibition of proliferation with BLU9931 in LIXC012 cells in vitro, we examined whether treatment of LIXC012 xenografts would also lead to inhibition of proliferation. Tumor samples were taken 8, 12, 20, and 24 hours after the last dose and stained for the proliferative marker Ki-67. We observed a dose-dependent reduction of Ki-67–positive cells following treatment with BLU9931 (Fig. 6B). Together, these data from the
Figure 5. BLU9931 is active in vitro in the FGF19-overexpressing PDX-derived cell lines. 

A, 28 PDX tumors were analyzed for FGF19 copy number and expression. Three of the PDXs (11%) had focal FGF19 amplifications with copy number ≥3 and expressed detectable levels of FGF19. An additional 6 PDXs (21%) had levels of FGF19 expression comparable to the FGF19-amplified samples but nearly diploid FGF19 copy numbers. 

B–D, PDX-derived cell lines were analyzed for expression of FGF19 (B), FGFR4 (C), and KLB (D), respectively, by qRT-PCR. E, CYP7A1 expression is induced after 8 hours of BLU9931 (100 nmol/L) treatment in cell lines with an intact FGFR4 signaling pathway. CYP7A1 mRNA levels were determined by qRT-PCR and are expressed as mean (n = 2) ± SEM. F, BLU9931 inhibits proliferation in PDX-derived cell lines with an intact FGFR4 signaling pathway. Proliferation was determined after 72 to 120 hours of compound treatment and measured using CellTiter-Glo (n = 3). Data, mean ± SEM. RPKM, reads per kilobase of exon per million mapped reads.

<table>
<thead>
<tr>
<th>FGFR4 RPKM &gt; 0.5</th>
<th>FGFR4 RPKM &lt; 0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circle size:</td>
<td>Circle color:</td>
</tr>
<tr>
<td>KLB RPKM &lt; 0.5</td>
<td>KLB RPKM &gt; 10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FGF19 copy number (log₂ ratio)</th>
<th>FGF19 mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.005</td>
</tr>
<tr>
<td>1</td>
<td>0.010</td>
</tr>
<tr>
<td>2</td>
<td>0.015</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>KLB mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>0.005</td>
</tr>
<tr>
<td>0.010</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fold induction of CYP7A1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BLU98931 IC₅₀ (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>15</td>
</tr>
</tbody>
</table>

LIX001 LIX002 LIX003 LIX004 LIX005 LIX006 LIX007 LIX008 LIX009 LIX010 LIX011 LIX012

Hep 3B
pharmacodynamic analyses suggested that treatment of mice bearing LIXC012 tumors with BLU9931 should have an antitumor effect.

We therefore treated LIXC012 tumor–bearing mice with BLU9931 and observed a robust tumor growth inhibition in a dose-dependent fashion (Fig. 6C). Significant tumor growth inhibition was observed at the 100-mg/kg and 300-mg/kg twice-daily doses of BLU9931, with stable disease observed in mice treated with BLU9931 at 300 mg/kg. Similar to what was observed in the Hep 3B model, LIXC012 tumors caused significant body-weight loss in the mice. Treatment with BLU9931 at efficacious doses reversed this body-weight loss (Fig. 6D). It is notable that daily treatment with sorafenib at 40 mg/kg also resulted in tumor growth inhibition in this model, possibly reflecting the high vascularity of these tumors. However, this antitumor efficacy was accompanied...
by severe body weight loss comparable to that observed in the vehicle-treated mice.

**DISCUSSION**

HCC is a devastating disease with limited treatment options. Here, we present data suggesting that a potent and selective FGFR4 inhibitor, such as BLU9931, might be an effective therapy for the subset of patients with HCC whose tumors are driven by aberrant FGFR4 signaling in response to autocrine FGF19 stimulation.

To achieve the goal of developing a highly selective FGFR4 inhibitor, we designed a compound that forms a covalent bond with Cys552 of FGFR4. This covalent strategy resulted in BLU9931, which exhibits ≥50-fold greater inhibition of FGFR4 as compared with FGFR1–3. A potential advantage of this exquisite FGFR paralog selectivity is the ability to circumvent concerns about toxicities due to inhibition of FGFR1 and FGFR3. It has been well documented that pan-FGFR inhibitors suffer dose-limiting toxicities in the clinic due to potent inhibition of FGFR1 and FGFR3 resulting in hyperphosphatemia (18). In addition to being paralog-sparing, BLU9931 also has excellent kinase selectivity, exhibiting >90% inhibition of binding to only one other kinase, CSF1R, when tested at a concentration of 3 μmol/L against a panel of 398 kinases. In view of the kinase selectivity of BLU9931, it is not surprising that the compound is well tolerated at doses that result in tumor stasis and tumor regression in *in vivo* tumor xenograft models. Similar tolerability at efficacious doses might also be achieved in patients with HCC, a hypothesis that will be tested in clinical trials with a potent and selective FGFR4 inhibitor.

Consideration has to be given to potential on-target toxicity due to the role the FGF19–FGFR4 axis plays in regulating *de novo* bile acid synthesis. It has been demonstrated (32) that administration of an antibody against FGF19 to cynomolgous monkeys leads to dose-related liver toxicities accompanied by severe diarrhea and low food consumption. A potential cause for the symptoms could be the observed rise in *CYP7A1* expression and concomitant increase in bile acid synthesis. It has been demonstrated that *CYP7A1* expression and concomitant increase in bile acid synthesis. It has been demonstrated that high levels of FGF19 mRNA are associated with severe diarrhea and low food consumption. A potential cause for the symptoms could be the observed rise in *CYP7A1* expression and concomitant increase in bile acid synthesis. It has been demonstrated that high levels of FGF19 mRNA are associated with severe diarrhea and low food consumption. A potential cause for the symptoms could be the observed rise in *CYP7A1* expression and concomitant increase in bile acid synthesis. It has been demonstrated that high levels of FGF19 mRNA are associated with severe diarrhea and low food consumption. A potential cause for the symptoms could be the observed rise in *CYP7A1* expression and concomitant increase in bile acid synthesis. It has been demonstrated that high levels of FGF19 mRNA are associated with severe diarrhea and low food consumption. A potential cause for the symptoms could be the observed rise in *CYP7A1* expression and concomitant increase in bile acid synthesis. It has been demonstrated that high levels of FGF19 mRNA are associated with severe diarrhea and low food consumption. A potential cause for the symptoms could be the observed rise in *CYP7A1* expression and concomitant increase in bile acid synthesis. It has been demonstrated that high levels of FGF19 mRNA are associated with severe diarrhea and low food consumption.

Our data have several implications for identifying the optimal responder population in the clinic. First, liver cancer cells of patients who might benefit from treatment with BLU9931 must constitutively express the ligand FGF19. Such overexpression in liver cancer cells can be the consequence of a focal amplification of the FGF19 gene itself (e.g., in Hep 3B cells). However, it can also be achieved by other mechanisms, as exemplified by LIXC012, a BLU9931-sensitive cell line with diploid FGF19 copy number but high levels of FGF19 mRNA. The underlying genetic or biologic mechanism responsible for FGF19 overexpression in the absence of FGF19 gene amplification has not yet been elucidated.

Second, expression of FGF19 alone is insufficient to result in antiproliferative activity of liver cancer cells when treated with an FGFR4 selective inhibitor. Evaluation of the antiproliferative activity of BLU9931 in a panel of established and PDX-derived HCC cell lines demonstrates that sensitive liver tumors express all key components of an intact FGFR4 signaling pathway, including not only FGF19 but also its receptor FGFR4 and the coreceptor KLB. Indeed, three of four established HCC cell lines expressing high levels of FGF19, FGFR4, and KLB mRNA showed growth inhibition by BLU9931 with EC₅₀ values less than 1 μmol/L. The fourth cell line, SNU878, showed robust expression of FGF19 and FGFR4 and low-level expression of KLB and exhibited an EC₅₀ of 5.2 μmol/L in the proliferation assay. It remains unclear whether a minimal level of expression of any of the signaling complex components is required for response to FGFR4 inhibition. In this regard, it is notable that the PDX-derived cell line LIXC012, which expresses high levels of FGF19, FGFR4, and KLB mRNA, showed an EC₅₀ of 0.14 μmol/L in the proliferation assay, whereas LIXC011, which expresses high levels of KLB and low but detectable levels of FGF19 and FGFR4 mRNA, was almost 10-fold less sensitive, exhibiting an EC₅₀ of 1.2 μmol/L.

Third, high-level, focal amplification of 11q13 is not necessarily indicative of FGF19 being a driver oncogene to which the tumor cell is addicted, as has been previously suggested (6). For example, one of the tested HCC cell lines, SNU387, exhibits FGF19 copy-number gains >8 but is not sensitive to treatment with BLU9931 in the proliferation assay (EC₅₀ > 6.9 μmol/L). We speculate that in these rare cases, FGF19 is simply a passenger gene that has been coamplified in the context of *CCND1* amplification for which such tumors might have been genetically selected. This hypothesis is further substantiated by the fact that FGF19 expression levels remain low in this HCC cell line. It is notable that expression of FGFR4 and KLB is also low in the SNU387 cell line (Table 1).

Finally, BLU9931 demonstrates a robust antiproliferative effect in 83% of cell lines (3 of 4 established HCC lines and 2 of 2 PDX-derived lines) with an intact FGFR4 signaling pathway measured by detectable expression of FGF19, FGFR4, and KLB. A meta-analysis of publicly available HCC genomic data (4–7) and The Cancer Genome Atlas (TCGA) provisional HCC data (Supplementary Fig. S6) suggests that FGF19 genomic amplifications are present in about 6% to 12% of HCC samples. Data from the TCGA analysis and our limited HCC cell line panel suggest that most, but not all, of these tumors will express FGF19 together with FGFR4 and KLB. In addition, approximately one fourth of HCC tumors (21% in PDX and 34% in TCGA) overexpress FGF19 in the absence of focal gene amplification, for reasons that remain to be elucidated, and most of them also express FGFR4 and KLB (Fig. 5A; Supplementary Fig. S6). As seen in our...
analysis of the antiproliferative effects of BLU9931 in HCC cell lines, most, but not all, liver tumors with a fully functional FGFR4 signaling pathway are sensitive to FGFR4 inhibition (5 of 6). Thus, a potent and selective FGFR4 inhibitor with suitable pharmaceutical properties, such as BLU9931, might represent an effective treatment for up to one third of patients with HCC. Additional preclinical studies, particularly in models in which FGFR19 expression is achieved in the absence of amplification, are required to confirm this estimate. Most importantly, the detection of FGFR19 expression in liver cancer cells constitutes a valuable and practical method for the identification of patients with HCC who will likely respond to therapy.

In summary, BLU9931 represents a first-in-class, paralog-selective, irreversible FGFR4 inhibitor that demonstrates remarkable antitumor activity in FGFR19-expressing HCC xenograft models with an intact FGFR4 signaling pathway at doses that are well tolerated. Thus, BLU9931 might be an effective therapy for the subset of patients with HCC whose tumors are driven by autocrine signaling through FGFR4. Based on the data presented here, we believe that a potent and selective FGFR4 inhibitor, such as BLU9931, is worthy of clinical investigation with the hope to as many as one third of patients with HCC.

**METHODS**

**BLU9931**

Synthesis of BLU9931 is recently described as “compound 25” on pages 43 to 44 of the patent application WO2014019090. Detailed structure-activity relationship studies leading to the discovery of BLU9931 will be described in a future publication.

**FGFR4 Crystallization and Structure Determination**

A construct comprising residues 450 to 751 of human FGFR4 absent a small flexible loop (residues 570–578) and bearing a C-terminal histidine tag was expressed in Sf21 insect cells and purified following a previously published protocol for FGFR2 (33). Crystals with BLU9931 were obtained by hanging drop methods against a reservoir containing 0.10 mol/L Bis–Tris, pH 4.5, 0.20 mol/L Li-sulfate and 16% (w/v) PEG 3350 at 4°C. Well-differacting crystals grew to full size over 4 days. A complete diffraction dataset to 1.7 Å was collected at the European Synchrotron Radiation Facility (ESRF; Grenoble, France), beamline ID14-4 with crystals frozen to 100 K. Crystals belonged to space group P2_1 with unit cell dimensions a = 39.1, b = 58.1, c = 58.7, \( \alpha = \gamma = 90^\circ \), and \( \beta = 94.4^\circ \). Molecular replacement was accomplished using 2FGI (FGFR2) as a search model. Iterative rounds of manual rebuilding (COOT; ref. 34) and refinement (REFMAC5; ref. 35) were used for all curve fitting.

**Kinase Selectivity Profiling**

BLU9931 and BGJ398 were screened at a single concentration of 3 μmol/L and LY-2874455 was screened at a single concentration of 1 μmol/L using the KINOMEscan Assay Platform (DiscoverRx; ref. 24).

**Cell Culture**

Hep 3B, PLC/PRF/5, SK-HEP-1, SNU-1R2, SNU-387, SNU-398, SNU-423, and SNU-449 cell lines were obtained from the ATCC; JHH-7 from the Japanese Collection of Research Bioresources Cell Bank; HUH-7 from the RIKEN Bioresource Center Cell Bank; and SNU-878 from the Korean Cell Line Bank, and were grown in the media recommended by the vendor. MDA-MB-453 and DMS114
cells (ATCC) were grown in RPMI-1640 Medium (Life Technologies) supplemented with 2 mmol/L L-glutamine, nonessential amino acids, 100 U of penicillin/mL, 100 µg of streptomycin/mL, and 10% FBS. The cell lines were authentication either using a panel of 88 SNPs and assessing the genotypes by Fluidigm and comparing with the cell line genotypes in the Cancer Cell Line Encyclopedia (28) or by short tandem repeat (STR) analysis using up to 22 loci and comparing to available published STR profiles. Authentication of the cell lines was performed within the last year. Lic0903, Lic002, Lic003, Lic004, Lic006, Lic011, and Lic012 (Shanghai ChemPartner Co., LTD; ref. 31) were grown in RPMI-1640 medium containing 10% FBS, 10 µg/mL insulin (Life Technologies), 2 µmol/L hydrocortisone, and 2.5 mg/mL glucose.

Immunoblotting and Antibodies

After treatment of cells, cells were pelleted and lysed in cell extraction buffer (Life Technologies), containing 1× protease and 1× phosphatase inhibitor cocktail (Sigma). Total protein concentration was determined using a standard Bradford assay. Western blotting was performed on cell lysates normalized to 50 µg total protein in loading buffer (Life Technologies). Normalized lysates were run on SDS-PAGE and transferred to a nitrocellulose membrane (Life Technologies). The membrane was incubated overnight at 4°C with primary antibodies (1:1,000). Antibodies used in these studies were from Cell Signaling Technologies (anti-FGFR4, anti-FGFR1, anti-phospho-FGFR, anti-c-MYC, anti-phospho-FRS2-α (Tyr196), anti-phospho-p44/42 MAPK (ERK1/2) (Thr202/Tyr204), anti-p44/42 MAPK (ERK1/2) (3A7), anti-phospho-AKT, and anti-AKT), and Sigma Aldrich (anti-actin). Membranes were washed, incubated with IRDye secondary antibodies (LI-COR), washed again, and imaged on an Odyssey Fc (LI-COR).

Detection of FGFR4 Pathway Components Using qRT-PCR

Hep 3B and PDX-derived cells were plated in 6-well polycarbonate plates at 50,000 cells per well and allowed to attach. Cells were treated with compound for 1 hour, media were removed, and cells were washed with PBS. In addition, cells were washed 3 times with serum-free media containing 100 ng/mL FGF19 to remove all compounds but keep the FGF19 concentration high despite the washes. Samples were collected at times indicated, and cell pellets were frozen at ~−20°C. RNA extractions were performed according to Qiagen RNAeasy protocol. qRT-PCR was performed in a 384-well format using the ViiA7 Real-time PCR system from Applied Biosystems. Each sample was run in duplicate using 100 ng of cDNA in 20 µL reaction volume. All Taqman reagents were purchased from Life Technologies (see Supplementary Methods).

Proliferation Studies

Established and PDX-derived HCC cell lines were seeded in 96-well plates in respective growth media, allowed to attach overnight, and treated with a dilution series of test compounds for two cell-doubling times. Cell viability was determined by CellTiter-Glo (Promega), and results represented as background-subtracted relative light units normalized to a DMSO-treated control. Relative EC_{50} values were determined at 50% inhibition between the top and bottom plateau of the dose-response curve.

Detection of Activated Caspase-3/7

Hep 3B cells were seeded overnight at 2.5 × 10^4 cells in white 96-well plates in media containing 5% FBS, then treated for 24 hours at indicated compound concentrations, and processed according to the manufacturer’s instructions using the Caspase-3/7 Glo assay (Promega). Data are expressed relative to DMSO control-treated cells.

FGFR4 Turnover Studies

Cells were plated at 4 × 10^4 cells per well of a 6-well plate in media containing 10% serum and allowed to adhere overnight. Cells were treated with 10 µg/mL cycloheximide for indicated times. Samples were subjected to gel electrophoresis followed by immunoblotting as described above.

In Vivo Studies

All procedures relating to animal handling, care, and treatment were performed according to the guidelines approved by the Institutional Animal Care and Use Committee (IACUC) of the companies performing the study following the guidance of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

BLU9931 was formulated in 0.5% carboxymethylcellulose/1% Tween 80 and dosed orally as a suspension twice daily. Sorafenib was dissolved in Cremaphor:EtOH (1:1) and diluted with saline or water to yield the stock solution. Sorafenib was dosed orally once daily. All compound doses are expressed as mg/kg free base.

The Hep 3B xenograft studies were performed at Wuxi AppTec Co., Ltd. Female Balb/c nude mice were inoculated subcutaneously in the right flank with Hep 3B tumor cells (10 × 10^6) in PBS mixed with 10% Matrigel (50:50). Treatment was started when the average tumor size had reached approximately 400 to 600 mm³ for the PK–PD study and approximately 200 mm³ for the efficacy study. The LIXC012 xenograft studies were performed at Shanghai ChemPartner Co., LTD. Female nu/nu mice were inoculated subcutaneously in the right flank with LIXC012 cells (2 × 10^6) in RPMI-1640 medium supplemented with Matrigel (50:50). Treatment was initiated when the average tumor volume was 300 to 400 mm³ for the PK–PD study and 100 to 200 mm³ for the efficacy study.

For efficacy studies, 9 mice were included in each treatment group. Body weight was measured daily. Tumor volume was measured twice weekly in two dimensions using a caliper, and the volume was expressed in mm³ using the following formula: V = 0.5 a × b², where a and b are the long and short diameters of the tumor, respectively. Data are presented as mean ± SEM.

For PK–PD studies, 3 mice were included in each treatment group. Mice received four doses of compound or vehicle. Blood and tumors were collected 8, 12, 20, and 24 hours following the last dose. The concentration of BLU9931 in plasma was determined by LC/MS-MS. A section of each tumor was immediately frozen in liquid nitrogen and stored at −80°C.

At the termination of the LIXC012 PK–PD study, tumor sections were fixed in 4% formalin and embedded in paraffin. Ki-67 protein expression was detected using rabbit monoclonal antibody D2H10 (Cell Signaling Technology) at 1:200 dilution and visualized using the anti-rabbit EnVision+ HRP System (Dako). The percentage of cells positive for Ki-67 was determined by manual counting.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: M. Hagel, C. Miduturu, J.L. Kim, B. Hodous, C. Winter, C. Lengauer, N.E. Kohl, T. Guzi
Development of methodology: M. Hagel, C. Miduturu, N. Rubin, W. Weng, N. Stranksy, J.L. Kim, A. Shutes
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Hagel, C. Miduturu, M. Sheets, N. Rubin, W. Weng, A. Shutes, C. Lengauer
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Hagel, C. Miduturu, N. Stransky, J.L. Kim, N. Brooijmans, A. Shutes, C. Winter

Writing, review, and/or revision of the manuscript: M. Hagel, C. Miduturu, N. Stransky, J.L. Kim, C. Winter, C. Lengauer, N.E. Kohl, T. Guzi

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C. Miduturu, J.L. Kim, C. Lengauer

Study supervision: M. Hagel, C. Miduturu, C. Lengauer, N.E. Kohl

Other (compound design and synthesis): N. Bifulco

Other (structure-based drug design): N. Brooijmans

Acknowledgments

The authors thank Shanghai ChemPartner Co., LTD, and WuXi AppTec Co. for performing the in vivo studies; CRELUX GmbH for performing crystallographic studies; John Leszyk, Associate Director of Proteomics and Mass Spectrometry, University of Massachusetts Medical School, for all mass spectrometry results; and all members of Blueprint Medicines for their critical review and helpful support.

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

Received September 10, 2014; revised January 30, 2015; accepted February 3, 2015; published OnlineFirst March 16, 2015.

REFERENCES


First Selective Small Molecule Inhibitor of FGFR4 for the Treatment of Hepatocellular Carcinomas with an Activated FGFR4 Signaling Pathway

Margit Hagel, Chandra Miduturu, Michael Sheets, et al.

Cancer Discovery  Published OnlineFirst March 16, 2015.

Updated version
Access the most recent version of this article at:
doi:10.1158/2159-8290.CD-14-1029

Supplementary Material
Access the most recent supplemental material at:
http://cancerdiscovery.aacrjournals.org/content/suppl/2015/03/21/2159-8290.CD-14-1029.DC1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.