FGFR Genetic Alterations Predict for Sensitivity to NVP-BGJ398, a Selective Pan-FGFR Inhibitor

Vito Guagnano, Audrey Kauffmann, Simon Wöhrle, Christelle Stamm, Moriko Ito, Louise Barys, Astrid Pornon, Yao Yao, Fang Li, Yun Zhang, Zhi Chen, Christopher J. Wilson, Vincent Bordas, Mickael Le Douget, L. Alex Gaither, Jason Borawski, John E. Monahan, Kavitha Venkatesan, Thomas Brümmendorf, David M. Thomas, Carlos Garcia-Echeverria, Francesco Hofmann, William R. Sellers, and Diana Graus-Porta

RESEARCH ARTICLE
ABSTRACT

Patient stratification biomarkers that enable the translation of cancer genetic knowledge into clinical use are essential for the successful and rapid development of emerging targeted anticancer therapeutics. Here, we describe the identification of patient stratification biomarkers for NVP-BGJ398, a novel and selective fibroblast growth factor receptor (FGFR) inhibitor. By intersecting genome-wide gene expression and genomic alteration data with cell line–sensitivity data across an annotated collection of cancer cell lines called the Cancer Cell Line Encyclopedia, we show that genetic alterations for FGFR family members predict for sensitivity to NVP-BGJ398. For the first time, we report oncogenic FGFR1 amplification in osteosarcoma as a potential patient selection biomarker. Furthermore, we show that cancer cell lines harboring FGFR1 copy number gain at the 11q13 amplicon are sensitive to NVP-BGJ398 only when concomitant expression of β-klotho occurs. Thus, our findings provide the rationale for the clinical development of FGFR inhibitors in selected patients with cancer harboring tumors with the identified predictors of sensitivity.

SIGNIFICANCE: The success of a personalized medicine approach using targeted therapies ultimately depends on being able to identify the patients who will benefit the most from any given drug. To this end, we have integrated the molecular profiles for more than 500 cancer cell lines with sensitivity data for the novel anticancer drug NVP-BGJ398 and showed that FGFR genetic alterations are the most significant predictors for sensitivity. This work has ultimately endorsed the incorporation of specific patient selection biomarkers in the clinical trials for NVP-BGJ398. Cancer Discov; 2(12); 1118–33. ©2012 AACR.

INTRODUCTION

The fibroblast growth factor (FGF) receptor tyrosine kinase (RTK) family, which consists of fibroblast growth factor receptor 1 (FGFR1), FGFR2, FGFR3, and FGFR4, encompasses the high-affinity receptors for 18 different FGF ligands. These ligand–receptor combinations regulate a broad spectrum of signaling and endocrinologic activities during development and in adult tissue homeostasis (1). In keeping with the importance of FGFR in normal growth control, deregulated FGF signaling has been linked to diseases, most prominently in the pathogenesis of multiple cancers. Epidemiologic and molecular studies have revealed a variety of genetic alterations in components of the FGF/FGFR signaling system, resulting in aberrant receptor activation and thus enhanced downstream signaling.

The underlying genetic alterations are largely tissue specific and include gene amplifications, translocations, and point mutations. Evidence for gene copy number changes has been reported in several studies. In particular, Beroukhim and colleagues (2) analyzed somatic copy number alterations in 3,131 cancer specimens and found that FGFR1 was significantly focally amplified across the entire dataset with a GISTIC (genomic identification of significant targets in cancer) q-value of 9.05E-47 and was located within a region of focal amplification containing only FGFR1, LETM2, and WHSC1L1. In breast cancer, FGFR1 is preferentially amplified in estrogen receptor–positive tumors as shown by chromosome in situ hybridization, and survival analysis indicates that it may also be an independent prognostic factor for poor outcome (3). Furthermore, high-resolution gene copy number analysis in lung cancer revealed FGFR1 amplification preferentially in the squamous subtype (4, 5). FGFR2 copy number gains, albeit with a low incidence, were reported in breast tumors (6, 7) and in gastric cancer in particular in poorly differentiated adenocarcinomas (8–11). Among the ligands, FGF19, located in the common 11q13 amplicon, was recently identified to be a driver gene in liver cancer in cooperation with its neighboring gene, cyclin D1 (12).

Germline mutations in FGFR1, FGFR2, and FGFR3 were first discovered as causative lesions in skeletal dysplasias (13). Kinome exon sequencing in search of human cancer somatic mutations identified FGF signaling components as the most frequently mutated coding regions among protein kinases (14). Somatic mutations of FGFR1 have been found in gliomas and lung tumors (15, 16), of FGFR2 in gastric...
and endometrial carcinomas (17–19), of FGFR3 in bladder carcinomas and multiple myeloma (20, 21), and of FGFR4 in primary rhabdomyosarcomas (22).

In addition, studies of hematologic malignancies have led to the characterization of chromosomal translocations involving FGFR genes. In particular, FGFR1 intragenic translocations between the N-terminus of a transcription factor and the FGFR1 kinase domain leading to constitutive kinase activation by oligomerization are responsible for 8p11 myeloproliferative disorder (23). Similar translocations of FGFR3 are associated with peripheral T-cell lymphoma (24), whereas in multiple myeloma, recurrent chromosomal translocations of 14q32 into the immunoglobulin G (IgG) heavy chain switch region result in deregulated ectopic expression of FGFR3 and the adjacent multiple myeloma SET domain-containing (MMSET) gene (21).

On the basis of the evidence of broad genetic alteration of the FGF/FGFR system in cancer, we hypothesized that the targeted inhibition of FGFRs would be an attractive modality for therapeutic intervention across multiple indications bearing such specific underlying genetic alterations. To this end, we have developed NVP-BGJ398, a potent, orally bioavailable, small-molecule pan-FGFR kinase inhibitor, which is currently in a clinical phase I trial (25). To preclinically identify and validate patient stratification biomarkers to enrich for patients likely to respond to NVP-BGJ398, the Cancer Cell Line Encyclopedia (CCLE) was interrogated. The CCLE is a collection of almost 1,000 cancer cell lines representing multiple tumor types that, in a collaborative effort between The Novartis Institutes for BioMedical Research and the Broad Institute (Cambridge, MA), has been comprehensively annotated in terms of genome-scale mRNA expression, gene copy number alterations, and gene mutations (26). In addition, more than half of these cell lines were subjected to high-throughput cell viability assays upon exposure to hundreds of compounds representing a variety of mechanisms of action, including the FGFR inhibitor NVP-BGJ398. Analysis of these cell lines’ sensitivity profiles revealed that NVP-BGJ398 significantly inhibits proliferation of cancer cell lines bearing FGF/FGFR genetic alterations across various cancer types, thus preclinically validating the hypothesis that a defined patient selection strategy based on tumors harboring FGF/FGFR genetic alterations is likely to enrich for responses to NVP-BGJ398.

RESULTS

NVP-BGJ398 Is a Potent and Selective FGFR Kinase Inhibitor

NVP-BGJ398 is an N-aryl-N’-pyrimidin-4-yl urea derivative that was designed by applying a new and nonconventional strategy to mimic the pyrido[2,3-d]pyrimidin-7-one core structure of a well-known class of protein kinase inhibitors (Fig. 1A; refs. 25, 27). The proposed binding mode of NVP-BGJ398 was elucidated by solving the 3-dimensional structure of the FGFR1 kinase domain in complex with NVP-BGJ398 (Fig. 1B). As shown in Fig. 1B, the 4-(4-ethyl-piperazin-1-yl)-phenylamine NH and the adjacent pyrimidine nitrogen are involved in critical H-bonds with the carbonyl and the amino group of alanine 564 (an amino acid residue located in the hinge region of the ATP-binding pocket), respectively. The urea carbonyl group is engaged in a water-mediated H-bond with the side chain amino group of lysine 514, whereas the aryl ring of the 4-(4-ethyl-piperazin-1-yl)-phenylamine is in contact with the hydrophobic side chains of 2 amino acid residues, glycine 567 and leucine 484 (the former not represented for clarity) in a sandwich-like manner. The 2,6-dichloro-3,5 dimethoxy-aniline fills optimally the complementary cavity in the kinase. Indeed, the perpendicular orientation of the tetrasubstituted benzene ring with respect to the plane of the pseudo-bicyclic system enforced by the 2 chlorine atoms allows productive hydrophobic interactions with several amino acid residues. In addition, this same ring is responsible for an H-bond between the methoxy oxygen and the NH of aspartate 641.

NVP-BGJ398 was tested against the 4 FGFRs and a panel of additional kinases in biochemical and cellular assays. NVP-BGJ398 inhibited FGFR1, FGFR2, and FGFR3 with single digit nmol/L IC50 in biochemical and cellular auto-phosphorylation assays and FGFR4 with 38- to 60-fold lower potency (Fig. 1C and D; Supplementary Table S1). In cellular assays, the most potently inhibited kinase, in addition to the FGFRs, was found to be VEGFR2, displaying 70- to 100-fold reduced potency as compared with FGFR1, FGFR2, and FGFR3. Therefore, NVP-BGJ398 is a selective, pan-FGFR kinase inhibitor, with predominant activity on FGFR1, FGFR2, and FGFR3.

Predicting Responses to NVP-BGJ398 by Means of the CCLE

Activation of the FGFR pathway is a common feature in human cancers with underlying genetic abnormalities in the FGF/FGFR system (28). To test whether tumors presenting these abnormalities depend on FGFR kinase activity, and hence would be sensitive to NVP-BGJ398, and to eventually elucidate predictive patient selection biomarkers for clinical trials with NVP-BGJ398, the antiproliferative activity of NVP-BGJ398 was assayed in a panel of 541 cell lines from the CCLE. From 2 independent high-throughput cell viability screens encompassing 435 and 424 cell lines respectively, with about 80% overlap, cell viability data in triplicate met the quality criteria for NVP-BGJ398 across a total of 517 cell lines (Supplementary Data File S1). Analysis of cell line distribution with respect to the Amax and inflection point values for NVP-BGJ398 across the entire cell viability dataset indicates that a subgroup of cell lines is highly sensitive (n = 35) to the compound, whereas a large majority of cell lines (n = 482) are insensitive (Supplementary Fig. S1A). Sensitive and nonsensitive groups were defined according to specific cutoff values for Amax and inflection point. To mitigate the risk of missing sensitive cell lines because of the accuracy limitations of a high-throughput screening mode, the thresholds for sensitivity in a first filtering step were set at relatively low stringency with Amax ~40 and lower and inflection point 1 µmol/L or higher. To validate the sensitive response calls to NVP-BGJ398, the 35 cell lines fulfilling the above selection criteria (lower left quadrant in Supplementary Fig. S1A) were tested in subsequent viability assays manually.
Predictive Modeling of FGFR Inhibitor Sensitivity

Figure 1. A, structure of NVP-BGJ398. B, the crystal structure of NVP-BGJ398 in complex with the tyrosine kinase domain of FGFR1 at 2.8 Å resolution. C, biochemical FGFR kinase assays; all assays were conducted with purified recombinant enzymes under optimized conditions using peptidic substrates and a microfluidic mobility shift readout using the KinaseGlo Luminescent Kinase Assay. The concentrations for ATP were adjusted to the respective $K_m$ values of the kinase. D, cellular FGFR autophosphorylation assays; HEK293 cells expressing the indicated FGFR were incubated with NVP-BGJ398 for 40 minutes at the indicated concentrations, and inhibition of FGFR Tyr-phosphorylation was measured by ELISA using a capturing FGFR-specific antibody and the antiphospho-tyrosine antibody PY20 coupled to HRP. In C and D, the percentage of phospho-tyrosine inhibition versus dose curves is shown.

To define the range of on-target FGFR-dependent inhibition of cell proliferation, the IC$_{50}$ values obtained in cell viability assays for BaF3 cells rendered dependent on the specific FGFRs were used as a reference. On this basis, only cancer cell lines in which proliferation was inhibited with an IC$_{50}$ less than 500 nmol/L were classified as sensitive. Among the 35 cell lines selected from the high-throughput assays, 28 were confirmed as sensitive to NVP-BGJ398 with IC$_{50}$s ranging from 0.001 to 500 nmol/L (Supplementary Fig. S1B). In addition, 24 selected lines from the CCLE for which high-throughput cell line profiling data were not available were also tested in manual cell proliferation assays, and 4 of them were found to be sensitive to the FGFR inhibitor (Supplementary Fig. S1B). Collectively, among the 541 (517 + 24) cell lines from the CCLE subjected to viability testing, 5.9% (encompassing 21 different cancer types) were found to be sensitive to
NVP-BGJ398 when an IC_{50} cutoff of 500 nmol/L for on-target inhibition of cell proliferation was applied (Fig. 2).

To derive molecular correlates of drug sensitivity, we used a predictive categorical model approach as described (26). The feature matrix examined in this approach encompassed all CCLE genomic data as single genomic features (expression, copy number, COSMIC mutation data), 25 lineage features, 1,777 “GeneSet” features (expression signatures each of them consisting of multiple genes), and a composite “FGFR genetic alteration” feature consisting of 9 distinct types of FGFR genetic alterations: FGFR1, FGFR2, FGFR3, and FGFR4 copy number gains, activating mutations in FGFR1, FGFR2, and FGFR3, as well as the chromosomal translocations for either FGFR1 or FGFR3 previously reported in the literature (21, 29).

From more than 50,000 input features, this analysis identified the “FGFR genetic alteration” feature as the top predictor for response to NVP-BGJ398 followed by 2 mutation features and 2 “GeneSet” features (Fig. 3A and B). As shown in Fig. 2, within the 541 cell lines used in the analysis, only 37 were found to bear genetic abnormalities for either of the FGFRs (total of 6.8%), in line with the general low incidence of these genetic lesions in tumors. Among those, 17 cell lines were sensitive to NVP-BGJ398, representing 53% of all cell lines testing sensitive to the drug (17/32, Supplementary Table S2). In contrast, in the insensitive group, only 3.9% of the cell lines harbored FGFR genetic alterations. This indicates that NVP-BGJ398-sensitive tumor cells are strongly enriched among the panel of lines scoring positive for the “FGFR genetic alteration” feature. The other 2 features revealed by the model among the top 3 predictors are “FGFR2 mutation” and “FGFR3 mutation,” indicating that genetic alterations in individual FGFRs can
Predictive Modeling of FGFR Inhibitor Sensitivity

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**Figure 3.** Predictive modeling of NVP-BGJ398 sensitivity using the CCLE features. Top features of drug response identified by categorical-based predictive modeling. Wilcoxon test or Fisher exact test were conducted for continuous or discrete features, respectively. A, fold change or OR as well as P values and Benjamini–Hochberg corrected P values are reported. The number between parentheses for the GeneSets corresponds to the number of genes in the set. B, heatmap for the top 5 features in the model. NVP-BGJ398 response is shown in dark green for the sensitive cell lines and light green for the insensitive cell lines; dark purple is used for discrete features. For GeneSet expression signatures, continuous Z scores are used. P90 and P10 refer to the 90th and 10th percentiles of the GeneSet scores.

be identified by the predictive categorical model despite their relatively low frequency in the test set. Indeed, all of the FGFR2- and FGFR3-mutated cell lines, except 2 and 1, respectively, were growth inhibited by NVP-BGJ398, in line with its ability to effectively block FGFR downstream signaling in the 6 FGFR-mutated cell lines tested (Supplementary Fig. S2). Of note, all FGFR2- and FGFR3-mutant lines belong to the endometrial and multiple myeloma cancer types, respectively. Additional predictors of sensitivity included the 2 GeneSet expression signatures, “Development FGF-family signaling” and “Inhibition of Hedgehog signaling in medulloblastoma stem cells,” in which protein product components comprise multiple members of the FGF signaling cascade (Supplementary Table S3). Interestingly, the GeneSet expression signature-positive cell lines comprised most of the NVP-BGJ398-sensitive cell lines with FGFR genetic alterations as well as most of the sensitive ones for which no FGFR genetic abnormalities were identified (Supplementary Fig. S3A). Conversely, many of the insensitive cell lines harboring FGFR genetic alterations were GeneSet signature negative or had a low z-score (Supplementary Fig. S3B).

**FGFR1 Amplification Is Associated with Response to NVP-BGJ398**

Because NVP-BGJ398-sensitive FGFR-amplified cell lines were captured by the “FGFR genetic alteration” and “GeneSets” features, we examined further these genomic features across the CCLE. FGFR1 copy number gain defined as log2 ratio ≥1 (equal to ≥4 normalized DNA copies) was observed in cell lines of breast, lung, and osteosarcoma lineages (Fig. 4A). Further analysis of the cell lines within these lineages (n = 145) showed that 5 of the FGFR1-amplified lines were sensitive to NVP-BGJ398 in proliferation assays and displayed constitutive FGFR pathway activation, as measured by the presence of Tyr-phosphorylated FGFR substrate 2 (FRS2), whereas treatment with NVP-BGJ398 led to pathway inhibition (Fig. 4B and C and Supplementary Fig. S1B). The scatter plot of copy number versus transcript expression revealed that the 5 sensitive cell lines were among the highest expressers of...
Figure 4. FGFR1 amplification in breast, lung, and osteosarcoma cancer cells is associated with response to NVP-BGJ398. **A**, box-plot showing FGFR1 copy number expressed as log$_2$ ratio for the 541 cell lines clustered according to cancer type. **B**, scatter plot of breast, lung, and osteosarcoma cancer cell lines showing the correlation between DNA copy number and transcript expression of FGFR1. Cell lines are colored according to response to NVP-BGJ398. **C**, effect of NVP-BGJ398 on FGFR downstream signaling as measured by FRS2 Tyr-phosphorylation and Erk1/2 activation. α-Actinin and total Erk1/2 protein levels are shown as a loading control. **D**, stable G292 cell lines expressing short hairpin RNA (shRNA) under the control of a doxycycline (Dox)-inducible promoter were generated via lentiviral infection and puromycin selection. Western blot analysis shows efficient FGFR1 knockdown, p-FRS2 and p-Erk inhibition with shRNA1237 and shRNA1425, as compared with 2 nontargeting shRNAs (NT sh1 and NT sh2). β-Tubulin Western blot analysis is shown as a loading control. **E** and **F**, effect of FGFR1-targeting as compared with nontargeting shRNAs on monolayer cell proliferation (**E**) and anchorage-independent cell growth assays (**F**) of G292 cells. For monolayer cell proliferation assay, cell growth was monitored at the indicated days after cell seeding, whereas endpoint measurements are given for the soft agar assay (day 15 after cell seeding). **G**, FGFR1 copy number in a panel of 17 primary human osteosarcoma samples was analyzed by quantitative real-time PCR. Data are shown as average with SEM (n ≥ 2). Br, breast; Ch, chondrosarcoma; Co, colorectal; En, endometrial; Es, esophagus; Ew, Ewing sarcoma; Ga, gastric; Gi, glioma; HL, hematopoietic and lymphoid tissue; HN, head and neck; Ki, kidney; Li, liver; Lu, lung; Me, melanoma; Ms, mesothelioma; Nb, neuroblastoma; Os, osteosarcoma; Ov, ovarian; Pa, pancreas; Th, thyroid; UT, urinary tract.
FGFR1 mRNA within the breast, lung, and osteosarcoma lineages (Fig. 4B). Statistical analysis using the Fisher exact test showed that FGFR1 amplification was significantly associated with response to NVP-BGJ398 when all the cell lines were considered (P = 4.8 x 10^-4) and in particular in the breast, lung, and osteosarcoma subsets (P = 1.5 x 10^-3). A requirement of FGFR1 activity for proliferation has been previously shown for FGFR1-amplified breast and lung cancer cell lines (5, 6). Because FGFR1 amplification in osteosarcoma associated with sensitivity to an FGFR inhibitor has not previously been reported, we sought to further assess the role of FGFR1 as a cancer driver in this indication with an independent approach. To this end, lentivirus expressing doxycycline-inducible short hairpin RNAs (shRNA) targeting FGFR1 was introduced into G292 cells. Although infection with viruses expressing 2 nontargeting shRNAs had no effect on the protein expression levels and cell growth, the viruses directing the expression of 2 shRNAs targeting FGFR1 led to a significant decrease in FGFR1 protein expression, FRS2 tyrosine phosphorylation, and extracellular signal-regulated kinase (ERK) phosphorylation. In parallel, the FGFR1 shRNA-containing viruses suppressed G292 cell proliferation in both monolayer and anchorage-independent conditions (Fig. 4D–F). The functional relevance of FGFR1 amplification in the osteosarcoma cell line led us to investigate FGFR1 copy number levels in a panel of primary human osteosarcoma samples. Consistent with FGFR1 amplification in 1 of 7 osteosarcoma cell lines within the CCLE, we identified 1 of 17 primary osteosarcoma samples as FGFR1 amplified (Fig. 4G). Interestingly, both the G292 cell line and the primary tumor sample showed similar levels of amplification, with about 5 copies of the FGFR1 gene in both cases.

These results reveal for the first time that FGFR1 amplification occurs in osteosarcoma, confirm its prevalence in breast and lung cancer cells, and show that FGFR1 is required for cancer cell growth in these settings. Hence, FGFR1 amplification is a predictor of sensitivity to an FGFR inhibitor in these 3 lineages.

FGFR2 Amplification Is Associated with Response to NVP-BGJ398 in Cell Lines and Primary Human Tumors

FGFR2-amplified cell lines were also enriched in the “FGFR genetic alteration” and “GeneSets” positive clusters. Analysis of single-nucleotide polymorphism (SNP) 6.0 array data across the CCLE revealed a high level of FGFR2 amplification (log, ratio ≥1) in cell lines of gastric lineage, as previously shown (8), but also in a colon cancer line (Fig. 5A). Gene amplification in these cell lines was correlated with striking FGFR2 transcript overexpression when specific Affymetrix probesets (211401_s_at) that detect FGFR2 C-terminal splice variants in addition to the canonical FGFR2 form were used (Fig. 5B). These data are consistent with previously published results showing that breast and gastric cancer cells with FGFR2 amplification overexpress the more oncopgenic FGFR2-c3 variant (30). In addition, by means of quantitative real-time PCR (qRT-PCR), we confirmed that NCI-H716 colon cancer cells also overexpress this specific C-terminal–truncated FGFR2 isoform and that only cell lines with FGFR2 amplification showed significant FGFR2-c3 expression (Supplementary Fig. S4A).

In keeping with the high levels of FGFR2 gene expression, FGFR2-amplified gastric (KATOIII and SNU16) and colon (NCI-H716) cancer cell lines showed strong baseline activity of the FGFR pathway, which was modulated upon NVP-BGJ398 treatment (Fig. 5C), and were dependent on FGFR signaling for proliferation, as evident from the low nanomolar IC50 for NVP-BGJ398 (Supplementary Table S2).

In agreement with inhibition of in vitro proliferation, NVP-BGJ398 also effectively inhibited growth of SNU16 tumor xenografts in a dose-dependent manner when administered orally to rats on a daily schedule (Fig. 5D). Tumor growth inhibition was correlated with inhibition of FGFR2 tyrosine phosphorylation in tumor tissue (Fig. 5E), which was almost completely abolished 3 hours after dosing and recovered at 24 hours after dosing, in line with the pharmacokinetic profile of the compound (25).

A statistical analysis by Fisher exact test showed a significant association between FGFR2 amplification and response to NVP-BGJ398 across the CCLE (P = 1.9 x 10^-4), as well as when restricted to the gastric and colon cancer lineages (P = 1.6 x 10^-4). To further test the predictive value of FGFR2 genomic amplification, we interrogated a collection of 49 human primary gastric tumors for which SNP6.0 copy number and Affymetrix expression data had been generated. Two primary tumors showing FGFR2 copy number 4 or more and FGFR2 transcript overexpression were selected for in vivo antitumor efficacy testing in mice (Fig. 6A). Oral treatment with NVP-BGJ398 on a daily schedule led to substantial tumor growth inhibition leading to tumor stasis and regression at daily doses of 15 mg/kg or more (Fig. 6B and C). Pharmacodynamic effects were evaluated in the GAM033 tumor model; at the 15-mg/kg dose, NVP-BGJ398 completely suppressed FGFR2 tyrosine phosphorylation 3 hours after dosing (Fig. 6D), in line with the pharmacokinetic profile of the compound (25).

Thus, FGFR2-amplified cell lines are sensitive to NVP-BGJ398 in vitro as well as when grown in vivo as human tumor xenografts. Hence, we envision that human gastric tumors harboring FGFR2 amplification will be responsive to NVP-BGJ398 in the clinic. Interestingly, in addition to confirming the incidence of this genetic alteration in gastric cancer, we also found FGFR2 amplification in 1 of 22 esophageal tumors, which offers a novel potential clinical opportunity for an FGFR inhibitor (Supplementary Fig. S4B).

FGF19 Amplification in Liver Cancer Correlates with Response to NVP-BGJ398

Approximately 47% of the cell lines responsive to NVP-BGJ398 did not harbor FGFR genetic alterations. Among those, the gene encoding for the FGF19 ligand was found to be amplified (log2 ratio ≥1) in the liver cancer cell lines HUH7, HEPC3, and JHH7 (Fig. 7A), as previously reported (12). The analysis of the CCLE SNP6.0 data revealed 49 additional cell lines with FGF19 copy number gain across various cancer types (Supplementary Fig. S5A, top). However, among the FGF19-amplified cell lines, only 3 liver cell lines showing concomitant expression of β-Klotho were sensitive to NVP-BGJ398, with the exception of a breast and a lung cancer cell line (MDAMB134 and DMS114) that harbor
**Figure 5.** FGFR2 amplification in gastric and colon cancer cell lines is associated with response to NVP-BGJ398.  
**A,** box-plot showing FGFR2 copy number expressed as log2 ratio for the 541 cell lines grouped according to cancer type.  
**B,** scatter plot of gastric and large intestine cancer cell lines showing the correlation between FGFR2 DNA copy number and transcript expression.  
**C,** effect of NVP-BGJ398 on FGFR downstream signaling as measured by FRS2 Tyr-phosphorylation and Erk1/2 activation. α-Actinin and total Erk1/2 protein levels are shown as a loading control.  
**D,** SNU16 tumor xenograft–bearing nude rats received NVP-BGJ398 at the indicated doses or vehicle for 14 consecutive days (n = 6 per group). The changes over time in tumor volume are shown. Statistical analysis was conducted by 1-way ANOVA–Dunnett versus vehicle control (*, P < 0.001).  
**E,** tumor tissues were recovered 3 and 24 hours after dose treatment and analyzed for FGFR2 Tyr-phosphorylation by Western blot analysis. Total FGFR2 Western blot analysis was conducted to monitor equal loading. Pharmacodynamic analysis of tumors treated with 15 mg/kg NVP-BGJ398 was not feasible due to insufficient material. Br, breast; Ch, chondrosarcoma; Co, colorectal; En, endometrial; Es, esophagus; Ew, Ewing sarcoma; Ga, gastric; Gl, glioma; HL, hematopoietic and lymphoid tissue; HN, head and neck; Ki, kidney; Li, liver; Lu, lung; Me, melanoma; Ms, mesothelioma; Nb, neuroblastoma; Os, osteosarcoma; Ov, ovarian; Pa, pancreas; Th, thyroid; UT, urinary tract.
Figure 6. FGFR2 amplification in primary human gastric tumors predicts for response to NVP-BGJ398. A, scatter plot of primary human gastric tumors showing the relationship between FGFR2 copy number and transcript expression (n = 49). The gastric tumors CHGA10 and GAM033 with FGFR2 gene amplification (B) and (C), respectively, were grown subcutaneously in mice. Treatment with NVP-BGJ398 at the indicated doses started when average tumor volume was 150 to 180 mm³ and proceeded for up to 14 or 25 days. Tumor volume changes over the course of treatment are shown. Statistical analysis was conducted by 1-way ANOVA–Dunnett versus vehicle control (⁎, P < 0.001).

The 3 sensitive liver cancer cell lines showed constitutive FRS2 Tyr-phosphorylation, which was abolished upon treatment with NVP-BGJ398 at doses of 50 nmol/L (Fig. 7B). In hepatocytes and liver cancer cells, FGF19 has been shown to signal through FGFR4 (31). In line with these findings, we found that the 3 cell lines expressed significantly high levels of FGFR4 mRNA (Fig. 7A), and conditional silencing of FGFR4 with 3 different shRNAs in the JHH7 cell line, previously shown to require FGF19 for survival, led to significant inhibition of cell proliferation (Fig. 7C and D).

Thus, these results suggest that whereas most cancers with 11q13 amplification may not respond to FGF19/FGFR4 inhibitors, the subset of FGF19-amplified liver cancer with concomitant expression of β-Klotho may provide a suitable niche indication for this therapeutic modality.

DISCUSSION

In this study, we have identified patient selection strategies for NVP-BGJ398, a novel selective pan-FGFR kinase inhibitor
Figure 7. FGF19 amplification in liver cancer cell lines is associated with response to NVP-BGJ398. 

A, scatter plot showing the correlation between FGF19 copy number and transcript expression of FGF19, FGFR4, and β-Klotho (KLB) in liver cancer cell lines. 

B, effect of NVP-BGJ398 on FGFR downstream signaling as measured by FRS2 Tyr-phosphorylation and Erk1/2 activation by Western blot analysis after 40 minutes of FGFR inhibitor treatment. Expression of α-actinin indicates equal loading.

C, effect of 3 different shRNAs targeting FGFR4 in JHH7 cells upon induction with doxycycline (Dox), compared with a nontargeting shRNA. FGFR4 expression and FRS2 Tyr-phosphorylation are shown in doxycycline-induced and noninduced cell lines. Western blot analysis shows α-actin as a loading control.

D, effect of FGFR4 downregulation on monolayer cell proliferation assays at day 7 after cell seeding.
Predictive Modeling of FGFR Inhibitor Sensitivity

Currently, in phase I clinical trials in patients with cancer. To guide patient selection and to maximize the likelihood of patient benefit and successful clinical proof-of-concept for this novel targeted anticancer modality, we have analyzed the sensitivity of more than 500 cell lines from the CCLE to NVP-BGJ398 in cell viability assays and intersected response data with information on gene expression and genomic alterations. We show that NVP-BGJ398 inhibits proliferation of about 6% of the cancer cell lines tested at concentrations that are consistent with its mechanism of action and in line with its highly selective nature. Furthermore, the integrative analysis of the CCLE has revealed “FGFR genetic alteration” as the top predictor for response to NVP-BGJ398 among more than 50,000 input features containing genomic, lineage, and gene set features.

Indeed, among the 541 cell lines in the CCLE with pharmacologic drug sensitivity data, 37 harbored an FGFR genetic alteration and 17 of them were sensitive to NVP-BGJ398; this represents 53% of the total cell lines responding to the drug (17/32). Gene amplifications were most prevalent (10/17) and involved FGFR1, FGFR2, and, surprisingly, also FGFR3, followed by sequence variations in FGFR2 and FGFR3 (6/17) and chromosomal translocations affecting FGFR1 and FGFR3 (3/17). High-resolution SNP6.0 array data across the CCLE subjected to analysis with the GISTIC algorithm revealed that the FGFR1 locus lies in a focal peak region of amplification, whereas FGFR2 was found in a GISTIC peak when the analysis was restricted to the gastric cancer cell lines (32). In this setting, NVP-BGJ398 response was associated in a statistically significant manner with both FGFR1 amplification and FGFR2 amplification. These data confirmed the finding of FGFR1 and FGFR2 copy number alterations in breast, lung, and gastric cancer cell lines as previously reported (5, 6, 9), but it also revealed the occurrence of these genetic lesions in additional cancer types, such as osteosarcoma and colon, respectively. In this context, among the 7 osteosarcoma lines in the CCLE, the one harboring FGFR1 amplification (G292) was significantly growth suppressed under both monolayer and soft-agar conditions upon inducible knockdown of FGFR1 by 2 distinct shRNAs, consistent with the notion that amplified FGFR1 confers cancer dependence. In addition, and for the first time, we report FGFR2 amplification in 1 of 17 primary osteosarcomas, suggesting that this may be another potential indication for an FGFR inhibitor. Similarly, NCI-H710, the only colon cancer cell line with high level FGFR2 amplification, was sensitive to NVP-BGJ398. In line with the notion that FGFR2 is a driver oncogene when its locus is aberrantly amplified, we selected human primary gastric tumors for the presence of FGFR2 copy number alterations and confirmed them to be exquisitely responsive to the selective FGFR inhibitor NVP-BGJ398, whereas models with normal FGFR2 DNA copy number were insensitive to the drug (data not shown). In agreement with previous analyses of FGFR2 copy number alterations conducted by FISH (8, 9) or Southern blot (11), we have found high level amplifications (copy number > 10) of FGFR2 by means of PCR in 5% of gastric tumors among a total of 147 specimens as well as in 1 of 22 esophageal tumors, which has not previously been reported, thus providing additional new opportunities for the therapeutic application of an FGFR inhibitor. Interestingly, we also identified FGFR3 copy number gains in 3 of the bladder cancer cell lines that were inhibited by NVP-BGJ398 (log2 ratio 1 for RT112 and RT112/84 and log2 ratio 0.94 for RT4), which may account for the significantly higher FGFR3 transcript expression in these cell lines (Supplementary Fig. S5B). Taken together, these data support the evaluation of NVP-BGJ398 in cancer types selected upon the presence of FGFR gene amplification.

Genomic predictors of drug sensitivity also revealed FGFR2 and FGFR3 mutation among the top 3 most significant features. The viability of 6 of 9 FGFR-mutated cell lines was pharmacologically inhibited by NVP-BGJ398; they belong to the endometrial and multiple myeloma lineages and showed constitutive FGFR pathway activation (Supplementary Fig. S2), in line with the notion that these mutations result in receptor kinase activation (17, 19, 21). Notably, most endometrial FGFR2-mutated cell lines also carried mutations affecting either PTEN or PIK3CA (Supplementary Table S4), suggesting that the activation of this pathway does not confer resistance to an FGFR-inhibitory therapy in this cancer type. Of note, we observed constitutive AKT phosphorylation in the endometrial cancer cell lines, which was not affected by NVP-BGJ398 treatment (Supplementary Fig. S2). Therefore, phosphoinositide 3-kinase inhibitors may provide opportunities for combination therapy with NVP-BGJ398 in these specific settings.

Interestingly, 54% (n = 20) of the FGFR genetically altered cell lines were not NVP-BGJ398-sensitive. It is likely that at least in some of these cell lines, additional genetic alterations bypass FGFR dependency. For instance, 1 cell line (A375) had a BRAFV600E mutation, and 10% (n = 2) of the cell lines showed amplification of other oncogenes (JMRI JHE2 amplification and NCI-H1703 PDGFRα amplification), whereas 20% (n = 4) harbored KRAS mutations (Supplementary Table S4), and KRAS mutation was revealed by the predictive model as one of the genomic predictors for NVP-BGJ398 insensitivity (data not shown). Thus, we are currently exploring whether hypothesis-driven concomitant targeting of other genetically altered molecular pathways will synergize with NVP-BGJ398 in these settings. Alternatively, and in the case of the breast and lung FGFR1-amplified cell lines that did not respond to NVP-BGJ398, it is plausible that one of the other genes found in the GISTIC peak (LETM2, WHSCII) may have become the driver gene. It is also noticeable that none of the FGFR4-amplified cell lines in our data set responded to the FGFR inhibitor, thus indicating that FGFR4 is not a driver oncogene in those settings.

Conversely, several cell lines that displayed sensitivity to NVP-BGJ398 did not harbor FGFR genetic lesions. Three of them, belonging to the liver cancer type, showed copy number gain for the FGFR4 ligand, FGFR19, and FGFI9 amplification was statistically significantly associated with response to NVP-BGJ398 when the analysis was restricted to liver cancer cell lines. Furthermore, by conditional knockdown of FGFR4, we showed dependency on this RTK in the JHH7 cells, thus supporting the concept of an FGFR9/FGFR4 autocrine loop as the oncogenic driver in liver cancer with FGFI9 amplification. In line with the notion that this autocrine loop is only functional in the
presence of the coreceptor β-Klotho, which is essential for high affinity interactions of FGF19 with FGFR4 (33), we showed that only 3 liver cancer cells with FGF19 amplification and concomitant β-Klotho expression responded to NVP-BGJ398. This suggests that β-Klotho depicts another critical determinant for patient selection, which has not been analyzed previously. Consequently, FGF19 amplification was not associated with NVP-BGJ398 response in other cancer types most likely due to the lack of or low β-Klotho expression. This is in line with a recent study (12) showing that FGF19 amplification correlated with increased expression and with sensitivity to FGF19 blockade only in liver cancer cell lines.

Taken together, we have not detected FGF/FGFR genetic abnormalities in 37.5% (n = 12) of NVP-BGJ398-sensitive cell lines. Most of these cell lines were GeneSet signature-positive or expressed high levels of either of the FGFRs and FGF ligands and generally showed constitutive activation of the FGFR pathway and modulation upon NVP-BGJ398 treatment (Supplementary Fig. S2), suggesting that FGFR dependency could be the result of intrinsic upregulation of components of the FGF signaling system leading to constitutive pathway activation. It will be interesting in future studies to address the underlying mechanisms resulting in FGF/FGFR induction in the absence of gene copy number gain or activating mutations of the receptors. It is plausible that epigenetic modulations or genetic alterations on other pathways ultimately leading to FGF/FGFR elevated expression and/or activation may have occurred. For example, it has recently been discovered that FGFR4 overexpression occurs in alveolar rhabdomyosarcomas with PAX3/7–FKHR translocation and/or activation may have occurred. For example, it has recently been discovered that FGFR4 overexpression occurs in alveolar rhabdomyosarcomas with PAX3/7–FKHR translocation and that FGFR4 is a downstream target of the oncogenic fusion protein (34).

In summary, by leveraging the integration of the CCLE annotation and compound sensitivity data, we have identified genetic alterations in various members of the FGF/FGFR system that confer cancer dependence and thus represent suitable predictive biomarkers to guide patient selection for treatment with selective FGFRs targeting agents, such as the novel pan-FGFR kinase inhibitor NVP-BGJ398. Based on these data, a phase I clinical trial with NVP-BGJ398 is being conducted in patients with cancer bearing FGFR genetic alterations (35).

METHODS

Compound and Antibodies

NVP-BGJ398 has been identified and synthesized in the Global Discovery Chemistry Department at NIBR (Novartis) as described (25). For in vitro studies, 10 mmol/L stock solutions were prepared in 100% dimethyl sulfoxide (DMSO). For in vivo studies in rodents, NVP-BGJ398 was formulated in acetic acid/acetate buffer pH 4.6/PEG300 1:1.

Antibodies used for Western blot analysis were anti-S473P-Akt (#9271), anti-Akt (#9271), anti-T202/P204-Perk1/2 (#9101), anti-Erk1/2 (#1628), anti-V633/654P-FGFR (55H2; #3476) from Cell Signaling; anti-Flg (M2F12) FGFR1 (ssc-57132), anti-Bek (C-17) FGFR2 (ssc-122), anti-FGFR4 (C-16; #sc-124), anti-FRS2 (H-91; #sc-8318) from Santa Cruz; anti-FGFR2 (α-isoforms; MAB6841) from R&D Systems; anti-FRS2/SNT-1 (#05-502), anti-phospho-Tyrosine, clone 4G10 (05-321), anti-α-actinin (#05-384) from Millipore; and anti-β-tubulin (# T4062) from Sigma.

In Vitro Compound Profiling

Biochemical in vitro kinase assays, cellular FGFR autophosphorylation assays, and BaF3 cell proliferation assays were conducted as described (25).

High-Throughput Cell Line Profiling and Manual Cell Proliferation Assays

Cell lines were obtained from American Type Culture Collection (ATCC), Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) and Health Science Research Resources Bank (HSRRB) and cultured in RPMI or Dulbecco’s modified Eagle’s medium plus 10% FBS (Invitrogen) at 37°C 5% CO2 using automated processing. Cell line identities were confirmed using a 48-variant SNP panel and comparing them with previous cell line tests. A detailed description of the high-throughput cell viability assays can be found in the report of Barretina and colleagues (26). In brief, assays were automated and conducted with an ultra-high-throughput screening system. Cell lines were dispensed into tissue culture-treated 1,536-well plates in a final volume of 5 μL and a concentration of 250 cells per well, were allowed to adhere, and were cultured for 12 to 24 hours. Prediluted compounds were transferred to the cells, resulting in a final concentration range of 8 μmol/L to 2.5 μmol/L in more than 8 steps and a uniform DMSO concentration of 0.4%. The cell–compound mixture was incubated for 72 to 84 hours, and cell growth was analyzed by determination of the cellular ATP content (Cell Titer Glo; Promega) using a luminescence plate reader (ViewLux; PerkinElmer). On all plates, wells containing vehicle only and the positive control MG132, a proteasome inhibitor, at 1 μmol/L were included. Raw values were percentage normalized on a plate-by-plate basis such that 0% was equivalent to the median of vehicle wells and ~100% equivalent to the median of the positive control. Quality of cell response to the positive control (MG132) was measured using a standard Z′ factor (36). In general, nearly all responses were more than 0.5, indicating a robust assay window. All dose–response data were reduced to a fitted model using a proprietry decision tree method that is based on the NIH Chemical Genomics Center assay guidelines (26). Fitted models were assessed with a standard Z′ test that was also used to determine which model to use. All data were manually reviewed as well. Parameters derived from the models include IP, the inflection point of the curve; cross-point (CP), the concentration in which the fitted curve crosses ~50%; and Amax, the maximal activity value reached within a model.

For manual cell proliferation assays, cells were seeded in 96-well plates at a density of 101 to 104 cells per well in a volume of 100 μL. Media containing compound dilutions or DMSO was added 24 hours thereafter. After 72 hours or 7 days, Cell Titer Glo was added as done earlier. The concentration of compound providing 50% of proliferation inhibition (IC50) was determined using XLfit (idbs).

Generation of Stable Cell Lines with Hairpin shRNAs

Hairpin shRNAs were cloned in pLKO-Tet-On vector to produce replication-incompetent lentiviruses as described previously (37). Upon lentiviral infection, stable cell lines were generated by selection with puromycin (1.5 μg/mL) for 5 days. For monolayer cell proliferation assays, cells were seeded in 96-well plates and shRNAs were induced with doxycycline. Cell proliferation was evaluated by standard x assays that was also used to determine which model to use. All data were manually reviewed as well. Parameters derived from the models include IP, the inflection point of the curve; cross-point (CP), the concentration in which the fitted curve crosses ~50%; and Amax, the maximal activity value reached within a model.

For manual cell proliferation assays, cells were seeded in 96-well plates at a density of 101 to 104 cells per well in a volume of 100 μL. Media containing compound dilutions or DMSO was added 24 hours thereafter. After 72 hours or 7 days, Cell Titer Glo was added as done earlier. The concentration of compound providing 50% of proliferation inhibition (IC50) was determined using XLfit (idbs).
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Genomic Analysis of Cell Lines and Primary Tumors

A detailed description can be found in the report of Barretina and colleagues (26); see also (32). In brief, DNA copy number was measured using high-density SNP arrays (Affymetrix SNP6.0) and normalized to copy number estimates (log; ratios; with log; ratio 0 being equal to 2N normalized copies) using a Gene- Pattern pipeline (38) and bg18 Affymetrix probe annotations. Sample-specific and recurrent copy number changes were identified using the GISTIC algorithm (39). mRNA expression levels were obtained using Affymetrix U133 plus 2.0 arrays according to the manufacturer’s instructions. The microarray data accession number in GEO is GSE36139.

FGFR2-c3 Isomor mRNA Expression

The primers to specifically monitor expression of the FGFR2-c3 isoform were designed for TaqMan assay: FGFR2-F: CTGGATCT-GAATTTCCTACCTTCCA, FGFR2-R: CCTGACCAACTTTTC-CCAGTTTCT, probe: CCAATGAGATCTGAAAGTTT. For internal control, β-actin primers and probe (ABI catalog number: 4326315E) were mixed with those of FGFR2. The qRT-PCR thermal cycles were run at 95°C for 15 seconds, 56°C for 25 seconds, and 68°C for 45 seconds using TaqMan Universal PCR Master Mix (ABI catalog number 4304437).

FGFR1 PCR from Human Primary Osteosarcoma DNA Samples

Seventeen genomic DNA samples from osteosarcomas were acquired from the Peter MacCallum Cancer Institute, (Melbourne, Australia; ref. 40). The primers used for FGFR1 copy number determination by SYBR green PCR assays were: FGFR1-F: GCATCAATA-GAACCTCTGTGTTG, FGFR1-R: GTTGTGATGCTCGGCTAAC. LINE1 was used as the reference: LINE-F: AAAGGCCGCTCAACTACATG, LINE-R: TGCTTTGAATGCGTCCCAGAG. The assay was carried out using ABI SYBR Green PCR Master Mix (ABI catalog number 4309155) as described previously (40).

FGFR2 PCR from Human Primary Tumor DNA Samples

One hundred and thirteen genomic DNA samples from gastric or esophageal cancer specimens were acquired from Asterand, Indi- vuned, Cytomix, and BioServe. Fifty-six samples from gastric tumors were from PrognoGen Biotechnology Co., LTD, who conducted the copy number analysis by PCR according to Novartis protocols. The primers to quantify FGFR2 locus copy number were designed for SYBR green assays: FGFR2-F: GTCGTGCTGCTGACCACTCAGT, FGFR2-R: AGAGCTCGGCTTCCGCTAG. Quantification using LINE1 as a reference was conducted as described earlier for FGFR1.

Xenograft Rodent Models and Antitumor Efficacy Studies

The experimental procedures involving animal studies strictly adhered to the Association for Assessment and Accreditation of Laboratory Animal Care International guidelines as published in the Guide for the Care and Use of Laboratory Animals, and to Novartis Corporate Animal Welfare policies. The study with the human primary gastric model GM033 was conducted at CrownBio International R&D center. The studies with the human primary gastric model CHGA010 and the cell line–derived xenograft SNU16 were conducted at Novartis facilities. All primary tumor samples were obtained from patients at the time of surgery, with informed written patient consent, and the study was approved by the local ethical committee. Tumor cell, or tumor fragments in the case of primary tumors, were implanted subcutaneously in rodents. Treatment with NVP-BGJ398 or vehicle control started when average tumor size was at least 100 mm³ and tumor volumes were monitored at the indicated times over the course of treatment. Data are presented as mean ± SEM. Comparisons between groups and the vehicle control group were done using 1-way ANOVA followed by Dunnett tests. The level of significance is indicated for each experiment. At the end of treatment, tumors were excised and snap-frozen in liquid nitrogen. Frozen tissues were pulverized using a swing mill (RETSCCH MM200), and tumor powder was lysed in standard protein lysis buffer for further Western blot analysis.

Disclosure of Potential Conflicts of Interest

V. Guagnano, A. Kauffmann, S. Wohrle, C. Stamm, M. Ito, L. Barys, A. Pornon, Y. Yao, F. Li, Y. Zhang, Z. Chen, C.J. Wilson, Y. Bords, M. Le Douget, L.A. Gaither, J. Borawski, J.E. Monahan, K. Venkatesan, T. Brümmendorf, F. Hofmann, W.R. Sellers, and D. Graus-Porta are employees of Novartis Institutes for BioMedical Research. W.R. Sellers holds the position of VP/Global Head of Oncology in Novartis Institutes for BioMedical Research and has ownership interest (including patents) in Novartis Pharmaceuticals. C. Garcia-Echeverria was an employee of Novartis Institutes for BioMedical Research and is now an employee of Sanofi and has ownership interest (including patents) in Sanofi. No potential conflicts of interest were disclosed by D.M. Thomas.

Authors’ Contributions

Conception and design: Y. Yao, Y. Zhang, L.A. Gaither, F. Hofmann, W.R. Sellers, D. Graus-Porta


Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S. Wohrle, C. Stamm, M. Ito, L. Barys, A. Pornon, Y. Yao, Y. Zhang, Z. Chen, C.J. Wilson, L.A. Gaither, J. Borawski, J.E. Monahan, D.M. Thomas, D. Graus-Porta


Writing, review, and/or revision of the manuscript: V. Guagnano, A. Kauffmann, M. Ito, Y. Zhang, Z. Chen, L.A. Gaither, T. Brümmendorf, C. Garcia-Echeverria, F. Hofmann, W.R. Sellers, D. Graus-Porta

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Ito, A. Pornon, Y. Zhang, C.J. Wilson, Y. Bords, M. Le Douget, L.A. Gaither, D.M. Thomas

Study supervision: Y. Zhang, Z. Chen, F. Hofmann, D. Graus-Porta

Scientific and managerial supervision and mentorship: C. Garcia-Echeverria

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Vito Guagnano, Audrey Kauffmann, Simon Wöhrle, et al.


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