Oncogenic Characterization and Pharmacologic Sensitivity of Activating Fibroblast Growth Factor Receptor (FGFR) Genetic Alterations to the Selective FGFR Inhibitor Erdafitinib

Jayaprakash D. Karkera1, Gabriela Martinez Cardona1, Katherine Bell1, Dana Gaffney1, Joseph C. Portale1, Ademi Santiago-Walker1, Christopher H. Moy1, Peter King1, Michael Sharp1, Rastislav Bahleda2, Feng R. Luo3, John D. Alvarez1, Matthew V. Lorenzi1, and Suso J. Platero1

Abstract

Fibroblast growth factor receptor (FGFR) genetic alterations are frequently observed in cancer, suggesting that FGFR inhibition may be a promising therapy in patients harboring these lesions. Identification of predictive and pharmacodynamic biomarkers to select and monitor patients most likely to respond to FGFR inhibition will be the key to clinical development of this class of agents. Sensitivity to FGFR inhibition and correlation with FGFR pathway activation status were determined in molecularly annotated panels of cancer cell lines and xenograft models. Pathway inhibition in response to FGFR inhibitor treatment was assessed in cell lines (both in vitro and in vivo) and in samples from patients treated with the FGFR inhibitor JNJ-42756493 (erdaftinib). Frequency of FGFR aberrations was assessed in a panel of NSCLC, breast, prostate, ovarian, colorectal, and melanoma human tumor tissue samples. FGFR translocations and gene amplifications present in clinical specimens were shown to display potent transforming activity associated with constitutive pathway activation. Tumor cells expressing these FGFR activating mutants displayed sensitivity to the selective FGFR inhibitor erdafitinib and resulted in suppression of FGFR phosphorylation and downstream signal transduction. Clinically, patients receiving erdafitinib showed decreased Erk phosphorylation in tumor biopsies and elevation of serum phosphate. In a phase I study, a heavily pretreated bladder cancer patient with an FGFR3-TACC3 translocation experienced a partial response when treated with erdafitinib. This preclinical study confirmed pharmacodynamics and identified new predictive biomarkers to FGFR inhibition with erdafitinib and supports further clinical evaluation of this compound in patients with FGFR genetic alterations. Mol Cancer Ther; 16(8); 1717–26. ©2017 AACR.

Introduction

The successful development of new oncology therapies most unequivocally requires identifying the patients most likely to respond to a particular targeted intervention. Thus, the discovery and characterization of biomarkers that accurately distinguish those more likely to respond patients from a broader population is critical for successful clinical development. Examples of such biomarkers that have already been identified and approved as predictors of response to therapy include human epidermal growth factor receptor (EGFR)-2 amplification, which predicts positive response to trastuzumab in women with breast cancer (1), and KRAS mutation, which predicts resistance to the EGFR inhibitors panitumumab and cetuximab in individuals with colorectal tumors (2, 3). In lung cancer, EGFR-activating point mutations predict activity to EGFR tyrosine kinase inhibitors erlotinib and gefitinib (4), and ALK translocation has been shown to be a predictor of response to crizotinib, a compound targeting the ALK tyrosine kinase (5). More recently, fibroblast growth factor receptor (FGFR)-mediated signaling has been shown to play a role in tumor progression, and recent evidence suggests that FGFRs are oncogenic drivers in various types of cancer (6), highlighting the need to find predictive biomarkers of FGFR to support clinical development.

Fibroblast growth factors (FGF) are a family of secreted factors involved in signaling pathways responsible for embryonic development, cell proliferation, survival, and migration (7). Twenty-two unique FGFR family members have been identified, and are differentially expressed in many, if not all, tissues and organs, albeit with different patterns and timings (7). FGFR activity is mediated by four transmembrane receptor tyrosine kinases (FGFRs 1–4; ref. 8). FGFR binding induces FGFR dimerization, leading to phosphorylation of the intracellular tyrosine kinase domain (9, 10). The dimerization-mediated phosphorylation leads to the activation of downstream signaling transduction pathways, including FRS2, RAS, RAF, mitogen-activated...
protein kinase [MAPK], PI3K/AKT, signal transducer and activator of transcription and Phospholipase-C-γ (9, 11), which culminate in transcriptional regulation that mediates the functional outcomes of FGFR activation.

The normal regulation of FGFR signaling is often genetically subverted to constitutively activate the pathway in a variety of malignancies. FGFR activation is attributed to gene amplification, chromosomal translocation, alternative splicing, or point mutations. Dramatic gene amplification of FGFR2 was originally observed in gastric tumor cell lines, highlighting the oncogenic potential of the receptor family (12). Subsequently, FGFR family alterations have been observed in several other malignancies, including breast cancer (FGFR1 amplification; ref. 13), bladder (FGFR3 mutations; refs. 14–16), colorectal (FGFR1 amplification, FGFR2 and FGFR3 mutations; ref. 17), and hematologic cancers (FGFR3 overexpression; refs. 18, 19). More recently, FGFR1 gene amplifications have also been observed in 10% of squamous cell carcinoma (SCC) lung cancer patient samples, but not in lung adenocarcinoma (20). Notably, this aberration has been observed only in tumor samples from patients with a history of smoking. Approximately 24% of all lung cancers are of the SCC subtype, which is most common in patients with smoking histories (20–22). Unlike other forms of non–small cell lung cancer (NSCLC), for which there are molecularly targeted therapies available, SCC treatment options are generally limited to conventional chemotherapy (23). FGFR1 is one of the first actionable genetic targets to be identified as having therapeutic potential in SCC lung cancer, as evidenced in preclinical studies of FGFR1 inhibition showing regression of FGFR1 amplified tumors in a xenograft model (20, 22). FGFR1 may also be a potential therapeutic target in other smoking-related malignancies (22, 24).

FGFR translocations have also been recently observed in a broad range of malignant cell lines, including glioblastoma, NSCLC, breast cancer, bladder cancer, and cholangiocarcinoma (6, 25, 26); however, they largely remain functionally uncharacterized. FGFR2 was the first family member found to be activated by chromosomal rearrangement (FGFR2–FRAG1) in a functional screen to identify oncogenes in osteosarcoma (27). Recently, an FGFR3–TACC3 translocation has been reported in lung cancer (28, 29). In general, FGFR translocations result in constitutive activation of FGFR, due to fusion of the FGFR kinase domain with the N-terminal portion of an unrelated gene harboring domain that promote constitutive receptor dimerization. This dimerization results in receptor phosphorylation and downstream signaling leading to oncogenic transformation (6, 25, 27).

The identification of genetic alterations in multiple FGFR family members in human cancers highlights pan-FGFR inhibition as a promising therapeutic approach in a variety of malignancies. Several small molecule FGFR inhibitors with differing selectivity and potency profiles, such as brivanib, dovitinib, AZD-4547, NVP-BGJ398, and erdafitinib, are in various stages of clinical development. Here we determined the sensitivity of cell lines harboring FGFR alterations were dependent on the active pathway for survival and therefore sensitive to treatment with erdafitinib. We also extended this characterization to FGFR2 and FGFR3 chromosomal rearrangements recently identified in human tumors (26), highlighting the oncogenic potency of these fusions and their sensitivity to FGFR inhibition. Collectively, these findings further inform the oncogenic potency and frequency of FGFR genetic alterations in human cancers and point to the use of specific FGFR inhibitors as a rational approach to treating patients harboring these genetic aberrations.

Materials and Methods

Experimental gastric tumor models

All animal experiments were performed under approved protocols of the Institutional Animal Use and Care Committee of Janssen Pharmaceuticals, Inc. Human gastric cancer xenografts were established by injection of 10^6 SNU-16 cells (American Type Culture Collection) from culture into male nude Nuf/Nu rats (nude rate—Crl: NIH-Foxn1(mu) from Charles River Laboratories). Tumors were grown for 18 days, followed by 10 days of dosing with JNJ-42883919 (precursor of the clinical candidate erdafitinib) or vehicle. The rats were subsequently sacrificed, and tumors were harvested and prepared into formalin-fixed paraffin-embedded (FFPE) blocks. SNU-16 human gastric carcinoma (G10) cells (10^6 cells) were fixed in 10% neutral-buffered formalin and also processed for FFPE blocks. Tumor sections and cell blocks were cut at a thickness of 4 μm and placed on silanized glass slides. Antigen retrieval was carried out in AR10 citrate buffer (Biogenez) for 20 minutes at 120°C in a Model 2100 Retriever (Prestige Medical). Tumor sections were incubated with rabbit monoclonal anti-human pMAPK antibody or rabbit monoclonal anti-human p-S6 antibody (Cell Signaling Technology) at room temperature for 45 minutes. Cell block sections were incubated with rabbit polyclonal anti-human pFGFR3 (Santa Cruz Biotechnology, Inc.) at room temperature for 45 minutes. The Dako EnVision + DAB rabbit detection system (Dako North America, Inc.) was used as described by the manufacturer, and sections were counterstained in hematoxylin. All staining was carried out on a Biogenex i6000 Automated Staining System. Slides were scanned with an Aperio ScanScope (Leica Biosystems), and images were analyzed for antigen content by positive pixel quantitation using ImageScope software.

Fluorescence in situ hybridization (FISH)

The tissue microarrays (TMA) for human NSCLC, prostate, colorectal, and breast (ER/PR-positive) cancer were constructed and manufactured at ORDIS Biomed using tissues from the Biobank of the Medical University of Graz, the Lung Biobank of the Institute of Pathology, and the Medical University of Innsbruck. Tissue blocks and the TMAs for malignant melanoma, ovarian, and breast cancer were obtained from Asterand Biocodes. The TMAs were constructed with triplicate 0.6-mm diameter cores extracted from FFPE patient tumor samples, and tissue cores derived from canis lupus kidney were used as orientation markers. Collection of tumor specimens was approved by local Institutional Review Boards of Innsbruck and Graz, Austria.

Dual-color FISH probes for estimation of FGFR gene locus amplification were designed, produced, and obtained from Kreatech Diagnostics (acquired by Leica Biosystems). The FGFR1 probe was specific for locus 8p12 and SE8; the FGFR2 probe was specific for locus 10q26 and SE10; and the FGFR3 probe...
was specific for locus 4p16 and the chromosome-4 centromere (SE4). For estimation of FGFR4 gene locus amplification, a probe specific for NSD1 (5q35) was used as a surrogate for the FGFR4 gene locus (5q33) and an hTERT (5p15) probe was used as a hybridization control.

Probe hybridization was executed per standard procedures with optimized pretreatment conditions for the individual tissue types. In brief, 1.5–5 μm sections of TMA s were deparaffinized at 65°C for 30 minutes and washed with Hemo-De solvent. Tissue pretreatment was executed with 0.2 N hydrochloric acid (20–45 minutes, room temperature), followed by microwave heating (600 W, 20 minutes) and a final protease (Abbott) treatment (15–75 minutes, 37°C). For codenaturation, mixed probes (i.e., FGFR1/SE8 and FGFR2/SE10) were applied on each section and incubated at 80°C for 5 minutes. Subsequent hybridization was performed at 38°C–42°C overnight (16–18 hours). Post-hybridization washings were performed with 2× saline-sodium citrate/0.3% NP-40 detergent (72°C, 5 minutes). Finally, slides were dehydrated, stained with 4’,6-diamidino-2-phenylindole, and covered with Vectashield (Vector Laboratories).

FISH signals in tumor tissue were enumerated in nonoverlapping nuclei with complete and intact contours. Target signals (red; i.e., FGFR1 and FGFR2) and control signals (green; i.e., SE8 and SE10) were recorded for each individual core. Digital FISH images were captured using a Zeiss Axiohot microscope with an Axio-.cam MRm camera (Zeiss) at a magnification of 1,000×. Images were processed using Adobe Photoshop 7.0 and exported at a resolution of 150 pixels/inch.

Gene amplification was estimated by the gene ratio (number of target signals vs. control). A gene ratio ≥ 2 was defined as amplification. When gene locus amplification was restricted to <20%–50% of the tumor, an average gene ratio of <2 was computed (i.e., down to 1.4). These cases were classified as amplified but presenting with substantial intratumor heterogeneity. The gene dose of either target or control was computed by the ratio of number of target signals versus nuclei evaluated. A gene dose ≥ 3 was defined as gene dose elevation (i.e., FGFR1, FGFR2, FGFR3, or FGFR4) or polysomy of the probed chromosome (i.e., SE4, SE8, or SE10). Student’s t-test was used to determine statistical differences between FGFR genetic profiles in different types of prostate cancer and between SCC and adenocarcinoma in NSCLC.

**FGFR fusion overexpressing cell lines**

RK3E cells (American Type Culture Collection), from rat kidney epithelial cells, were cultured in DMEM supplemented with 10% FBS and 1% antibiotics (Invitrogen). FGFR fusion gene constructs were designed and cloned into the pReceiver expression vector (Genecopoeia). Clones were transfected into RK3E cells using the Amaxa Cell Line Nucleofector (Lonza) following the manufacturer’s protocol. The stably transfected cells were selected in complete medium with 800 μg/mL of G418 (Invitrogen). Overexpression of the fusions in the stably transfected cells was confirmed by real-time PCR and immunoblotting.

**Colony formation assay**

Anchorage-independent growth of the fusion gene-overexpressing cells was tested. One mL culture medium with 0.8% low melting point agarose was first plated into each of three wells of a six-well plate. After the agar solidified, each well received another 1 mL of 0.4% agar in culture medium containing 100 cells. After 14 days, colonies were fixed and stained with 0.1% cresyl crystal violet (Sigma-Aldrich). The number of colonies was determined microscopically by manual counting from triplicate wells for each cell line.

**Immunoblotting analysis**

Fusion-overexpressing RK3E cells were plated in complete growth medium, serum starved overnight, then re-fed with 0.5% FBS growth media. Cells were treated with 1 μmol/L of erdafitinib and comparator compounds in the presence of ligands for 1 hour. For immunoblotting, whole-cell lysates were collected in RIPA buffer (Thermo Fisher Scientific), and sample protein concentration was assayed using BCA Protein Assay (Thermo Fisher Scientific). Equal amounts of protein (30 μg per lane) were loaded onto 4–12% Bis-Tris gels (Invitrogen) followed by SDS-page. Proteins were transferred to nitrocellulose membranes and probed with antibodies against p-FGFR (#3476), total-FGFR2 (#11835), p-MAPK (#4370), total-MAPK (#4695), p-S6 (#2406), S6 (#2317), B-actin (#3700 or #8457) (Cell Signaling Technology), and total-FGFR3 (Santa Cruz Biotechnology). The membranes were blocked with Odyssey blocking buffer (Li-Cor Biosciences) for 1 hour at room temperature, and then incubated overnight at 4°C in a primary antibody solution (1:1,000) diluted in Odyssey blocking buffer. After three washes in tris-buffered saline plus 0.1% Tween 20 (TBST), the membranes were probed with goat anti-mouse or donkey anti-rabbit JRDye 680RD or 800CW (Li-Cor Biosciences) labeled secondary antiserum (1:10,000) in Odyssey blocking buffer for 1 hour at room temperature. Washes were repeated after secondary labeling and the membranes were imaged using an Odyssey scanner and the Odyssey 3.0 analytical software (Li-Cor Biosciences). Effects of erdafitinib were compared with AZD4547 and NVP-BGJ398.

**Drug response testing for FGFR fusion-overexpressing cell lines**

Fusion-overexpressing RK3E cells were seeded into Cultur-Plate-96 (Perkin Elmer) well plates (1,000 cells/well) in triplicate in complete growth medium plus the ligands FGF-1 and FGF-2 (R&D Systems Inc.). After 24 hours, cells were serum starved overnight then re-fed with 0.5% FBS growth media plus FGF-1 and FGF-2. Seventy-two hours after plating, cells were treated with an 18-point 1:3 dilution series, starting at 10 μmol/L of erdafitinib, AZD4547, and NVP-BGJ398. The Microtiter plates were then incubated for 72 hours and assayed for adenosine triphosphate content using the Cell Titer-Glo Luminescent Cell Viability assay (Promega Corp.) following the manufacturer’s instructions with modifications. Briefly, the cells were allowed to equilibrate to room temperature, at which time a 1:1 mixture of Cell Titer-Glo reagent was added. The cells were then placed on an orbital shaker for 2 minutes and incubated for 10 minutes at room temperature to stabilize the luminescent signal. The luminescence was quantified and the measurements were then conducted using an Envision Multi-label plate reader (Perkin Elmer). IC50 values were calculated using GraphPad Prism 5.0 (GraphPad Software Inc.).

**Test and authentication of cell lines**

The RK3E and SNU-16 cell lines were purchased directly from American Type Culture Collection on September 4, 2014. The cell lines were tested for Mycoplasma using the MycoAlert kit (Lonza) before they were used in any experiments. After four passages, the cell lines were aliquoted and frozen in vials as
stocks. The transformed stable cell lines were last tested in July 2015. No independent cell line authentication was performed on these cell lines.

Phase I clinical trial
Erdaﬁtinib, or INJ-42756493 (Supplementary Fig. S1), discovered in collaboration with Astex Pharmaceuticals, is an investigational agent currently in clinical development. The dose escalation and expansion phase I study has been previously described (30).

Immunohistochemical analysis of tumor pharmacodynamic biomarkers
Tumor biopsies for pharmacodynamic analyses were optional in the dose escalation phase but mandatory in the dose conﬁrmation phase. Tumor biopsy samples were collected at pretreatment (within 28 days prior to the ﬁrst dose of study drug) and posttreatment (day 1 of cycle 2 ± 7 days). Immunohistochemical analysis was performed on the Ventana Benchmark platform using a standard protocol. The phosho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) rabbit mAb #4370 (R647) from Cell Signaling Technology was used as the detecting antibody.

Results
Predictive and pharmacodynamic biomarkers
SNU-16 cells, which carry a high-level FGFR2 amplification, were sensitive to the FGFR inhibitor JNJ-42883919, and FGFR phosphoprotein levels decreased upon treatment with the study drug, as measured by immunohistochemical staining of FFPE tumor cells (Fig. 1A and B). Quantification of the pan-FGFR signal in SNU-16 cells treated with the vehicle versus with the FGFR inhibitor showed a shift from strong labeling to more moderate labeling (Fig. 1B). In athymic rats carrying SNU-16 gastric tumor xenografts, phosphorylated S6 and MAPK (pS6 and pMAPK) levels markedly decreased upon treatment with JNJ-42883919 (Fig. 1C–F), thus conﬁrming downstream signal transduction endpoint modulation that could be explored as pharmacodynamics marker of response in a clinical trial.

To determine the frequency of FGFR ampliﬁcations in human cancers, tumor microarrays were analyzed by FISH for

Figure 1.
Pharmacodynamic markers of FGFR inhibition. SNU-16 cells stained with anti-pan-FGFR. A, Vehicle; B, after treatment with JNJ-42883919. Tumors harvested from rats carrying SNU-16 xenografts were stained with anti-pS6 (C and D) and anti-pMAPK (E and F). Tumors of vehicle control-treated animals are shown in C and E; xenograft tumor of animals treated with JNJ-42883919 is shown in D and F.
the presence and abundance of the four FGFR genes (Fig. 2). In breast tumor samples FGFR1, FGFR2, FGFR3, and FGFR4 were amplified at a frequency of 11.7% (29/248), 6.6% (13/198), 3% (1/29), and 8.6% (15/175), respectively. In NSCLC tumor samples FGFR1, FGFR2, FGFR3, and FGFR4 were amplified at a frequency of 21.6% (45/208), 5.6% (11/195), 5.3% (1/19), and 5.1% (5/99), respectively (Supplementary Table S1). In prostate tumor samples only FGFR1 and FGFR2 amplifications were measured and the frequencies were 14.6% (14/96) and 1.1% (1/94), respectively. While in ovarian cancer FGFR1 and FGFR2 were amplified at a frequency of 2.9% (1/35) and 5.6% (2/36), respectively. No FGFR family gene amplifications were detected in either colon cancer or malignant melanoma tumor samples (Supplementary Table S1).

Characterization of FGFR fusion genes
One tumor cell line, RT-4, in the selected tumor cell line panel analyzed carries an FGFR3 C-terminal translocation and was sensitive to both JNJ-42541707 (IC50 = 27 nmol/L) and erdafitinib (IC50 = 0.19 nmol/L; Supplementary Table S2). Based on this finding, we assessed the capability of other clinically relevant FGFR translocations to transform normal RK3E cells and confer sensitivity to erdafitinib (26). FGFR3:TACC3v1, FGFR3:TACC3v3, FGFR3:BAIP2L1, FGFR2:BICC1, FGFR2:AFF3, FGFR2:CASP7,
FGFR2:CCDC6, and FGFR2:OFD1 translocations were cloned into vectors and transfected into untransformed RK3E recipient cells to determine the oncogenic potential of the FGFR alterations in an anchorage-independent growth assay. All FGFR-activating translocations tested conferred the ability to promote soft-agar growth compared with wild-type FGFRs (Fig. 3A). The transformation activity of these translocations was associated with constitutive activation of FGFR and downstream signaling. The effects of three pan-FGFR inhibitors, erdafitinib, AZD4547, and NVP-BGJ398, on the MAPK signaling pathway in RK3E cells expressing stable FGFR2 and FGFR3 fusion constructs and empty vector was assessed by immunoblotting. All FGFR inhibitors tested reduced the levels of MAPK and S6 phosphorylation induced by the transfection of RK3E cells with FGFR3 and FGFR2 fusion proteins (Fig. 3B). In contrast, the effect of AZD4547 and NVP-BGJ398 on proliferation of these FGFR fusion gene-expressing cells was weaker than the growth inhibition by erdafitinib (Supplementary Table S2). The detection of total FGFR2 by the FGFR2 antibody was not successful in any of the FGFR2 fusion harboring RK3E cells, but we could detect the HA tag expression in each of the FGFR2-overexpressing cell lines, thus suggesting that the FGFR2 protein was expressed (Supplementary Fig. S2). The failure to detect FGFR2 by FGFR2 antibody could

potentially be due to the masking of the specific epitope the FGFR2 antibody targets caused by the tertiary conformation of the fusion protein. Overall, these results demonstrate that the FGFR2 and FGFR3 translocations tested are oncogenic and confer sensitivity to FGFR inhibitors.

Assessment of response markers in patients treated with erdafitinib

To confirm the utility of pharmacodynamic markers identified in this study, immunohistochemical analysis for phospho-Erk (pErk), a downstream mediator of the FGFR pathway, was carried out on pretreatment and posttreatment biopsies from patients treated with erdafitinib (30). Samples were obtained from two patients enrolled in the phase I clinical trial of erdafitinib. One breast cancer patient with archival tumor harboring an FGFR1 amplification was treated with erdafitinib at 12 mg daily for 2 weeks and biopsied after 7 days of drug interruption (Fig. 4A). An approximate 40% reduction in pErk posttreatment was observed. In another patient with pleural mesothelioma of unknown FGFR status (Fig. 4B), a 45% reduction in pErk was observed after 3 weeks of treatment with erdafitinib at 6 mg daily. Other pharmacodynamic markers such as calcium, phosphate, soluble FGF23, soluble VEGFR, soluble FGFR2, 3 and 4, and vitamin D, were also measured from sera of patients undergoing the trial. Of those, only phosphate levels showed significant dose proportional changes upon treatment (30). The increase of serum phosphate concentrations in response to erdafitinib, due to renal tubular FGFR inhibition (31), extends similar findings on serum phosphate levels observed for other FGFR inhibitors (32).

In the phase I study (30), a 52-year-old bladder cancer patient, enrolled in the dose escalation cohort, with metastases to the lung who had failed three prior therapies was treated with erdafitinib. This patient had tumor shrinkage of 38% (Fig. 5) with a confirmed partial response by RECIST criteria (33) and stayed on treatment (at 9 mg daily) for about 10 months. The tumor shrinkage was calculated based on target lesion measurements at baseline and posttreatment efficacy assessment time point. The FGFR3–TACC3 translocation was detected in this patient’s tumor sample, extending the observation that these alterations are a predictor of sensitivity to erdafitinib clinically.

Discussion

A detailed analysis of sensitive and resistant cell lines identified FGFR gene amplification and translocations as potential markers of response to FGFR inhibitors. FGFR2 and FGFR3 translocations were shown to be potent oncogenes and resulted in constitutive FGFR phosphorylation and downstream signaling. Transformed cells expressing these FGFR alterations were sensitive to FGFR small molecule inhibition with several inhibitors in clinical development, but erdafitinib showed the highest potency in a comparative analysis. Interestingly, a patient whose tumor harbored an FGFR translocation, FGFR3–TACC3, showed a durable partial response to erdafitinib by RECIST criteria, demonstrating that the effects observed in cell lines were also applicable to cancer patients in the clinic.

FGFR amplifications were shown to be frequent genetic events in a variety of malignancies. Preclinically, sensitivity was also seen with FGFR amplifications in tumor cell lines and xenograft models, where a decrease in signaling downstream of FGFR was demonstrated upon administration of FGFR inhibitors. A recent report of positive clinical activity of the FGFR inhibitor AZD4547.
in gastric and breast cancer patients with high-level amplification of FGFR2 is very encouraging (34). Interestingly, in the current study the high-level amplification of FGFR2 was observed in breast, NSCLC, and ovarian cancers at a frequency of 6.6%, 5.6%, and 6.0%, respectively. But, in a recent report (35) using next-generation sequencing method, researchers reported the amplification of FGFR2 in breast, NSCLC, and ovarian cancers at a frequency of 0.8%, 1.7%, and 0.3%, respectively. Similarly, a high-level amplification of FGFR1 was reported in breast, ovarian, and NSCLC at a frequency of 14%, 5%, and 9% (NSCLC squamous) or 4% (NSCLC adenocarcinoma; ref. 35), respectively, whereas our data using the FISH method showed a frequency of 11.7%, 2.9%, and 21.6%, respectively. The discrepancy of the findings may be due to the different methodologies used in the assessment of the FGFR high-level amplifications. The SNU-16 gastric cell line with high-level of FGFR2 amplification has been shown to be sensitive to erdafitinib, which is in concordance with the data reported for AZD4547 (34). However, overexpression of FGFRs in transformation assays is not sufficient to confer a potent oncogenic effect without ligand expression (27), suggesting that FGFR gene amplifications may be less dominant driver pathway events compared with translocations.

A key aspect of drug development is generating clinical data to support what dose levels are taken forward into late clinical development. To this end, we examined FGFR-mediated signal transduction and functional endpoints to determine maximal target engagement as a component of dose selection. In preclinical models, downregulation of the pharmacodynamic biomarkers (pErk and pS6) was demonstrated in the SNU-16 model after treatment with an FGFR inhibitor. These endpoints were subsequently shown to be modulated in two patients receiving erdafitinib, confirming the activity of the inhibitor on suppressing FGFR-mediated signal transduction (36). Serum phosphate homeostasis has been reported to be controlled by FGFR activity in the renal tubules through the FGF23–klotho signaling axis and that genetic deletion of FGF23 results in upregulation of serum phosphate (32). We showed that erdafitinib regulating serum phosphate levels in a dose dependent manner in patients was managed clinically through chelation therapy and was not associated with significant adverse events (30), an observation consistent with this being an on-mechanism class effect (26). Interestingly, FGFR-mediated phosphate increases provide an interesting on-mechanism endpoint for monitoring clinical target engagement to support subsequent dose selection.

In conclusion, the data from this study credential FGFR amplifications and C-terminal translocations as dominant oncogenic events that confer sensitivity to small molecule FGFR inhibitors. These FGFR activating alterations resulted in constitutive pathway activation that was observed preclinically and clinically. On-target FGFR-mediated changes in phosphate regulation were observed clinically and a patient expressing a FGFR3–TACC3 translocation received clinical benefit from treatment with erdafitinib. Collectively, these observations highlight erdafitinib as a selective

Figure 5.
CT scan images of a patient with FGFR3–TACC3-translocated bladder cancer with a confirmed partial response in lung metastases (marked with white arrows on baseline scans) responding to erdafitinib. A and C are baseline lung images, and B and D are posttreatment lung images at week 6 for the same patient.
FGFR family inhibitor and support its ongoing clinical development in patients with activating FGFR genetic alterations.

Disclosure of Potential Conflicts of Interest
F.R. Luo is an employee of Janssen and has an ownership interest in the company stock. J.D. Alvarez is a senior scientific director at Janssen Research and Development and has an ownership interest (including patents) in the same. J.D. Karkera and A. Santiago-Walker have an ownership interest in the company stock (including patents). No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions
Conception and design: J.D. Karkera, G.M. Cardona, K. Bell, D. Gaffney, P. King, R. Bahleda, M.V. Lorenzi, S.J. Platero
Acquisition of data (provided animals, acquired and managed patients, facilities, etc.): J.D. Karkera, G.M. Cardona, K. Bell, D. Gaffney, J.C. Portale, P. King, R. Bahleda, F.R. Luo, J.D. Alvarez, M.V. Lorenzi, S.J. Platero

References

Writing, review, and/or revision of the manuscript: J.D. Karkera, G.M. Cardona, K. Bell, D. Gaffney, J.C. Portale, A. Santiago-Walker, P. King, M. Sharp, R. Bahleda, F.R. Luo, J.D. Alvarez, M.V. Lorenzi, S.J. Platero

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J.D. Karkera, G.M. Cardona, K. Bell, D. Gaffney, J.C. Portale, M. Sharp, J.D. Alvarez

Study supervision: J.D. Karkera, F.R. Luo, M.V. Lorenzi, S.J. Platero

Acknowledgments
This study was funded and supported by Janssen Research and Development, LLC. The authors would like to thank Harry Ma for writing and editorial assistance, and Clifford O. Motley, Dan Rhodes, Timothy Perera, Chris Takimoto, Vijay Peddaredigari, and Marcus Ote for their support and guidance.

Grant Support
This study was financially supported by Janssen Research and Development, LLC.

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 August 3, 2016, revised January 6, 2017; accepted April 5, 2017; published OnlineFirst April 17, 2017.
Karkera et al.

Molecular Cancer Therapeutics

Oncogenic Characterization and Pharmacologic Sensitivity of Activating Fibroblast Growth Factor Receptor (FGFR) Genetic Alterations to the Selective FGFR Inhibitor Erdafitinib

Jayaprakash D. Karkera, Gabriela Martinez Cardona, Katherine Bell, et al.


Updated version
Access the most recent version of this article at: doi:10.1158/1535-7163.MCT-16-0518

Supplementary Material
Access the most recent supplemental material at:
http://mct.aacrjournals.org/content/suppl/2017/04/15/1535-7163.MCT-16-0518.DC1

Cited articles
This article cites 35 articles, 19 of which you can access for free at:
http://mct.aacrjournals.org/content/16/8/1717.full#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
http://mct.aacrjournals.org/content/16/8/1717.full#related-urls

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, use this link http://mct.aacrjournals.org/content/16/8/1717. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.