IN THE SPOTLIGHT

Lineage-Specific Biomarkers Predict Response to FGFR Inhibition

David C. Loch and Pamela M. Pollock

Summary: In this issue of Cancer Discovery, Guagnano and colleagues use a large and diverse annotated collection of cancer cell lines, the Cancer Cell Line Encyclopedia, to correlate whole-genome expression and genomic alteration datasets with cell line sensitivity data to the novel pan-fibroblast growth factor receptor (FGFR) inhibitor NVP-BGJ398. Their findings underscore not only the preclinical use of such cell line panels in identifying predictive biomarkers, but also the emergence of the FGFRs as valid therapeutic targets, across an increasingly broad range of malignancies. Cancer Discov; 2(12); 1081–3. ©2012 AACR.

Commentary on Guagnano et al., p. 1118 (5).

Despite the established limitations of monolayer cultures as models for anticancer drug response, large-scale cancer cell line collections, well annotated at the DNA, RNA, and chromosome levels, represent powerful resources in identifying biomarkers of drug sensitivity. Building upon the success of the original NCI60 cancer cell line panel, several groups have established larger and more diverse cancer cell line libraries to better capture the lineage and genomic diversity of cancer. These efforts largely reflect the well-accepted idea that by and large, cancer is a disease of genetics—as well as the clinically unmet need to identify reliable predictive biomarkers for patient stratification. To underscore their use in this endeavor, large well-annotated cell line platforms have already revealed both known and novel predictors of preclinical efficacy to various cancer therapeutics (2–4). An apt illustration of such a cell line platform is the study by Garnett and colleagues (3), whose analyses of 639 human tumor cell lines across 130 drugs not only confirmed several expected genomic correlates of drug response, such as lapatinib sensitivity in cell lines with ERBB2 amplification, but also uncovered novel associations, including PARP inhibitor sensitivity in Ewing sarcoma cells with an EWS-FLI1 gene translocation.

Given the high cost of oncology drug development, there exists a financial, as well as ethical, imperative to use our understanding of cancer biology to incorporate predictive biomarkers into patient stratification at the earliest stages of clinical drug development. Using such a strategy in phase I or II clinical trials is expected to alleviate to some extent the high cost and prolonged time associated with oncology drug approval, and ultimately lead to the coapproval of accompanying diagnostic tests for patient preselection. Examples of drugs that have successfully used predictive biomarker assays within the original phase I trial, as well as subsequent phase II or III trials, include the anaplastic lymphoma kinase (ALK) inhibitor crizotinib in patients with EML4-ALK fusions and the BRAF inhibitor vemurafenib in BRAF-mutant melanoma patients (1).

Given the high cost of oncology drug development, there exists a financial, as well as ethical, imperative to use our understanding of cancer biology to incorporate predictive biomarkers into patient stratification at the earliest stages of clinical drug development. Using such a strategy in phase I or II clinical trials is expected to alleviate to some extent the high cost and prolonged time associated with oncology drug approval, and ultimately lead to the coapproval of accompanying diagnostic tests for patient preselection. Examples of drugs that have successfully used predictive biomarker assays within the original phase I trial, as well as subsequent phase II or III trials, include the anaplastic lymphoma kinase (ALK) inhibitor crizotinib in patients with EML4-ALK fusions and the BRAF inhibitor vemurafenib in BRAF-mutant melanoma patients (1).

Despite the established limitations of monolayer cultures as models for anticancer drug response, large-scale cancer cell line collections, well annotated at the DNA, RNA, and chromosome levels, represent powerful resources in identifying biomarkers of drug sensitivity. Building upon the success of the original NCI60 cancer cell line panel, several groups have established larger and more diverse cancer cell line libraries to better capture the lineage and genomic diversity of cancer. These efforts largely reflect the well-accepted idea that by and large, cancer is a disease of genetics—as well as the clinically unmet need to identify reliable predictive biomarkers for patient stratification. To underscore their use in this endeavor, large well-annotated cell line platforms have already revealed both known and novel predictors of preclinical efficacy to various cancer therapeutics (2–4). An apt illustration of such a cell line platform is the study by Garnett and colleagues (3), whose analyses of 639 human tumor cell lines across 130 drugs not only confirmed several expected genomic correlates of drug response, such as lapatinib sensitivity in cell lines with ERBB2 amplification, but also uncovered novel associations, including PARP inhibitor sensitivity in Ewing sarcoma cells with an EWS-FLI1 gene translocation.

In this issue of Cancer Discovery features a study by Guagnano and colleagues (5) at the Novartis Institutes for Biomedical Research that aimed to identify predictive biomarkers for the novel and selective FGFR inhibitor NVP-BGJ398, using the Cancer Cell Line Encyclopedia (CCLE), which they developed in conjunction with the Broad Institute. To this end, they conducted 2 independent high-throughput cell viability screens, using a panel of 541 cell lines from the CCLE, followed by confirmatory manual cell proliferation assays in a subset of lines. In this manner, they identified a subgroup (n = 32) of cancer lines sensitive to NVP-BGJ398. Of note, these 32 cell lines constituted approximately 6% of all cell lines tested and 13 different cancer types, including the novel finding of NVP-BGJ398 sensitivity in single cell lines derived from osteosarcoma and colon cancer. By using gene expression and genomic alteration datasets from the 541 cell lines, the authors then sought to identify predictive biomarkers of drug sensitivity to NVP-BGJ398, using a predictive categorial model approach with more than 50,000 input features. These features included individual genomic characteristics; expression signatures; and a composite “FGFR genetic alteration” feature that combined 8 distinct types of FGFR genetic alterations, including copy number gains, activating mutations, and chromosomal translocations. From the more than 500 cell lines studied, approximately 7% (37 of 541) harbored genetic aberrations in 1 of the 4 FGFRs, consistent with the reported frequency of FGFR activation in cell lines derived from various tumor types (6, 7).

Perhaps not surprisingly, integrative analysis revealed the composite “FGFR genetic alteration” as the most robust predictive input of NVP-BGJ398 sensitivity, confirming the expected relationship between NVP-BGJ398 and previously characterized models of FGFR dependence (7, 8). Data with other clinically relevant FGFR dependents have been reported previously for most of the FGFR inhibitor–sensitive cell lines with FGFR genetic alterations (8–11). However, the...
current study adds to the field by showing that very few cell lines without a known dependency on an FGFR, or a gene expression signature characteristic of FGF signaling, do indeed show sensitivity to NVP-BGJ398. The authors also show that the presence of an FGFR alteration by no means guarantees drug sensitivity, as only half of all FGFR-altered lines (17 of 37; 46%) were found to be sensitive. With respect to new indications, this study was also the first to report an FGFR1 amplification in 1 of 7 osteosarcoma cell lines and functional validation of FGFR1 dependence, using shRNA knockdown of FGFR1. Screening of primary tumors showed FGFR1 amplification in 1 of 17 primary osteosarcoma samples. Although no functional data are presented, the authors expand on the known importance of FGFR2 amplification in gastric cancer by reporting amplification of FGFR2 in 1 of 22 esophageal tumors, suggesting yet another tumor type for which FGFR inhibition may have a therapeutic value.

Of clinical significance, approximately half of the cancer cell lines with FGFR genomic alterations did not respond to NVP-BGJ398. Perhaps surprisingly, the majority of nonresponsive cell lines carried FGFR1 amplifications and were derived from lineages previously shown to be dependent on FGFR1 (e.g., breast and lung cancer cell lines). Of the 13 cell lines identified with FGFR1 amplification, only 5 of 13 (38%) were found to be sensitive to NVP-BGJ398, in contrast with 3 of 3 cell lines with FGFR2 amplifications. The authors propose that additional genetic alterations may bypass FGFR dependency (e.g., one lung cancer cell line carrying an FGFR1 amplification also carried a KRAS mutation) or that other genes found in the genomic identification of significant targets in cancer (GISTIC) peak may be the bona fide “oncogenic driver.” Given the current existence of multiple trials of anti-FGFR agents in which FGFR1 amplification is one of several FGFR biomarkers being used to stratify patients upfront [e.g., NVP BGJ398 (NCT01004224)] or in phase IIb expansion cohorts [e.g., E3810 (NCT01283945) and AZD4547 (NCT00979134)], the field eagerly awaits mature patient response data from these early trials. It may be that the work presented in this article foretells the necessity for additional biomarkers of sensitivity or resistance for better stratification of patients with FGFR1-amplified tumors. The difficulty in distinguishing which gene in an amplicon is the biologic “driver” is especially true for FGFR19, located within the same amplicon as CCND1 and amplified in 49 of 541 cell lines. To circumvent this problem, the authors looked for lineage-specific coexpression of other genes and identified the combination of FGFR1 amplification and β-Klotho expression as a predictive biomarker of drug sensitivity in liver cancer cell lines. In contrast with the development of BRAF inhibitors, in which patient stratification and the development of a companion diagnostic were relatively straightforward, the success of FGFR inhibitors will likely involve the development of predictive biomarkers that are largely lineage specific. With distinct mechanisms of FGFR activation presenting in particular tumor types, it is quite possible that the various FGFR inhibitors developed by industry will have different companion diagnostics, depending on the major tumor type for which they were initially approved, such as FGFR1 amplification in breast cancer, FGFR2 amplification in gastric cancer, or FGFR3 mutation in bladder cancer.

To better refine biomarkers of sensitivity and resistance, one possible approach might characterize additional cell lines or patient-derived xenograft models carrying the range of aberrations in FGFR/FGFR family members associated with sensitivity to FGFR inhibition. However, in many cases, this is cost and time prohibitive. Alternatively, as evidenced from a large number of molecularly targeted agents, much can be learned from reverse translation studies, with clinical tumor material obtained from patients either before or after treatment, or both (1). To truly increase our knowledge in this area, benefit the most patients in the shortest time, and gain insights into both intrinsic and acquired mechanisms of resistance, all trials of anti-FGFR agents should focus on collecting tumor biopsy specimens, ideally before treatment, after treatment, and if or when resistance emerges.

As FGFR inhibitors gain increasing clinical recognition, one major question that remains unanswered is whether multitargeted inhibitors or specific FGFR inhibitors will provide the most durable clinical responses in those patients preselected with FGFR activation. How does the potential for more durable clinical responses, as a result of inhibiting alternate kinase pathways associated with intrinsic and acquired resistance, balance against the increased toxicity associated with a broader inhibition profile? The answer will, of course, require using appropriate pharmacodynamic markers (e.g., antibodies to detect pan-phospho FGFR1) to show that a multitargeted inhibitor can successfully hit the FGFR target or modulate the downstream pathway to the same extent. Finally, the evaluation of specific FGFR inhibitors with narrow toxicity profiles may prove especially important in those FGFR-dependent malignancies associated with other comorbidities, such as patients with endometrial cancer with obesity or type 2 diabetes or both.

This cancer cell line screen also draws attention to one of the known bottlenecks originally associated with large-scale sequencing studies and soon to be increasingly associated with clinical trial enrollment, that is, how to interpret novel missense mutations. Specifically, if we see a novel mutation in a “druggable” kinase, how do we know if it results in “oncogene dependence” and is therefore “actionable”? If it occurs in a disease setting characterized by FGFR mutations (e.g., bladder or endometrial cancer), we would certainly be more hopeful. Nonetheless, inclusion of these patients would likely add unnecessary complexity to interpreting patient response data (e.g., in the absence of a clinical response, did they have an irrelevant passenger mutation or intrinsic resistance to FGFR inhibition?)

Of the 4 NVP BGJ398-insensitive cell lines, 3 carried novel and uncharacterized mutations in either FGFR1 or FGFR2. Indeed, 2 of these cell lines were derived from tumor types associated with a high “passenger” mutation rate owing to environmental mutagen exposure (e.g., lung cancer and melanoma) and thus exemplifying the inherent difficulty in distinguishing passenger from oncogenic driver mutations. As such, until functional data on novel FGFR missense mutations are published, we would propose that early proof-of-principle trials of anti-FGFR agents enroll only those patients with known activating mutations.

In summary, Guagnano and colleagues (5) provide strong evidence that FGFR genetic alterations drive carcinogenesis in a broad range of malignancies and, in doing so, show not only the preclinical use of large, genetically annotated cell line panels to identify predictive biomarkers but also the specificity of NVP-BGJ398. Ongoing work to be conducted alongside the clinical development of anti-FGFR agents includes,
among other tasks, the following: further refining the level of gene amplification associated with a clinical response for each receptor; validation of either in situ hybridization or immunohistochemistry in formalin-fixed paraffin-embedded pathology specimens as a companion diagnostic for receptor amplification; and, finally, correlative studies between different mutations in different FGFRs and patient response.

Disclosure of Potential Conflicts of Interest

P.M. Pollock has filed a patent regarding the detection of FGFR2 mutations in endometrial cancer samples (20100111944, Method of Diagnosing, Classifying and Treating Endometrial Cancer and Precancer, filed 24 March 2008) that is relevant to this topic. P.M. Pollock is a consultant/advisory board member in Five Prime Therapeutics. No potential conflicts of interest were disclosed by the other author.

Grant Support

P.M. Pollock is supported by a National Health and Medical Research Council (NHMRC) CDF2 grant, and D.C. Loch is supported by NHMRC grant 1011301.

Published online December 10, 2012.

REFERENCES


Lineage-Specific Biomarkers Predict Response to FGFR Inhibition

David C. Loch and Pamela M. Pollock


Updated version
Access the most recent version of this article at:
http://cancerdiscovery.aacrjournals.org/content/2/12/1081

Cited articles
This article cites 11 articles, 7 of which you can access for free at:
http://cancerdiscovery.aacrjournals.org/content/2/12/1081.full.html#ref-list-1

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.