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Lineage specific biomarkers predict response to FGFR inhibition

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Summary

In this issue of *Cancer Discovery*, Guagnano and colleagues utilise 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-FGFR inhibitor NVP-BGJ398. Their findings not only underscore the preclinical utility 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.

Given the high cost of oncology drug development, there exists a financial, as well as ethical, imperative to utilize 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/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 co-approval of accompanying diagnostic tests for patient preselection. Examples of drugs that have successfully utilized predictive biomarker assays within the original Phase I trial, as well as subsequent Phase II/III trials, include the 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 notion 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 utility in this endeavour, 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 this is the study by Garnett and colleagues, whose analyses of 639 human

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Disclosure of Potential Conflicts of Interest: PMP 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.

tumour 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's sarcoma cells with a *EWS-FLII* gene translocation (3).

This issue of Cancer Discovery features an study by Guagnano and colleagues at the Novartis Institutes for Biomedical Research that aimed to identify predictive biomarkers for the novel and selective FGFR inhibitor, NVP-BGJ398 utilizing the “Cancer Cell Line Encyclopedia” (CCLE), which they developed in conjunction with the Broad Institute (5). To this end, they performed two 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 comprised 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 utilizing 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 categorical model approach with more than 50,000 input features. These included individual genomic features, 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% (37/541) harboured genetic aberrations in one of the four FGFRs, consistent with the reported frequency of FGFR activation in cell lines derived from various tumour 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 inhibitors has 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 signalling, 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 (17/37- 46%) were found to be sensitive. With respect to new indications, this study was also the first to report an *FGFR1* amplification in 1/7 osteosarcoma cell lines and functional validation of FGFR1 dependence using shRNA knockdown of FGFR1. Screening of primary tumors showed *FGFR1* amplification in 1/17 primary osteosarcoma samples. Although no functional data is presented, the authors expand on the known importance of FGFR2 amplification in gastric cancer by reporting amplification of *FGFR2* in 1/22 oesophageal tumors, suggesting yet another tumour type where FGFR inhibition may have 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 non-responsive 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 5/13 (38%) were found to be sensitive to NVP-BGJ398 in contrast to 3/3 cell lines with *FGFR2* amplifications. The authors propose that additional genetic alterations may bypass FGFR dependency (e.g. one lung cancer cell lines with an *FGFR1* amplification also carried a *KRAS* mutation) or that other genes found in the gistic peak may be the bona fide “oncogenic driver”. Given there are currently multiple trials of anti-FGFR agents where *FGFR1* amplification is one of several FGFR biomarkers being used to stratify patients upfront e.g. NVP BGJ398 (NCT01004224) or in a Phase 1b 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 manuscript 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 biological “driver” is especially true for *FGF19*, located within the same amplicon as *CCND1* and amplified in 49/541 cell lines. To circumvent this problem, the authors looked for lineage specific coexpression of other genes and identified the combination of *FGF19* amplification and β -Klotho expression as predictive biomarkers of drug sensitivity in liver cancer cell lines. In contrast to the development of BRAF inhibitors where patient stratification and the development of a companion diagnostic was 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 approach could be to characterize additional cell lines or patient derived xenograft models carrying the range of aberrations in FGF/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 pre and/or post drug treatment (1). To truly increase our knowledge in this area, benefit the most patients in the shortest period of time, and gain insights into both intrinsic and acquired mechanisms of resistance, all trials of anti-FGFR agents should focus on collecting tumor biopsies, ideally before treatment, post treatment and if - or when - resistance emerges.

As FGFR inhibitors gain increasing clinical recognition, one major question that remains unanswered is whether multi-kinase 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. This will of course require employing appropriate pharmacodynamic markers (e.g. antibodies to detect pan-phospho FGFR) to show that a multi-kinase 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 endometrial cancer patients with obesity and/or type 2 diabetes.

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 i.e. 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 four NVP-BGJ398-insensitive cell lines, three carried novel and uncharacterized mutations in either *FGFR1* or *FGFR2*. Indeed, two 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 is published on novel FGFR missense mutations, we would propose that early proof-of-principle trials of anti-FGFR agents enroll only those patients with known activating mutations.

In summary, Gaugnano and colleagues provide strong evidence that FGFR genetic alterations drive carcinogenesis in a broad range of malignancies and in doing so demonstrate not only the preclinical utility of large genetically annotated cell line panels to identify predictive biomarkers but also the specificity of NVP-BGJ398. Ongoing work to be performed alongside the clinical development of anti-FGFR agents include among others: 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.

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