IN THE SPOTLIGHT

Gatekeeper Mutations and Intratumoral Heterogeneity in *FGFR2*-Translocated Cholangiocarcinoma

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Summary: *FGFR2* genetic translocations are frequent in cholangiocarcinoma, yet despite initial sensitivity to FGFR inhibitors in clinic, patients quickly become resistant to targeted therapies. The work published by Goyal and colleagues demonstrates that acquisition of gatekeeper mutations in *FGFR2* and intratumoral heterogeneity drive resistance in patients with *FGFR2*-translocated intrahepatic cholangiocarcinoma, which will have important implications for management of the disease in clinic. *Cancer Discov; 7(3); 248–9. ©2017 AACR.*

See related article by Goyal et al., p. 252 (6).

Oncogenic chromosomal rearrangements in the FGFRs present a potential therapeutic target, with translocations in *FGFR2* found most frequently in cholangiocarcinoma (1, 2) and translocations in *FGFR3* prevalent in bladder cancer and glioblastoma (2). Preclinical models of these fusion proteins have demonstrated sensitivity to FGFR inhibitors, and recent data from early-phase clinical trials substantiated these findings in the clinic (3–5). However, the extent of these translocations as true oncogenic drivers and the potential mechanisms of resistance to therapy have previously been investigated only preclinically. Goyal and colleagues (6) demonstrate that *FGFR2* translocations in intrahepatic cholangiocarcinoma are classic oncogene drivers, with response to FGFR inhibition in the clinic followed by development of common gatekeeper mutations in *FGFR2* resulting in clinical resistance.

Cholangiocarcinoma has historically been dichotomized by anatomic location into intrahepatic and extrahepatic subtypes. Recent developments in genomic and transcriptomic technologies have revealed fundamental molecular differences between these anatomic subsites, reflecting distinct etiologies (7). Intrahepatic cholangiocarcinoma, a disease with growing incidence for yet unknown reasons, harbors *FGFR2* translocations in up to 16% of cases (1, 2). Fusion proteins generated by genetic translocations generally fuse a variety of protein fragments to the cytoplasmic tail of FGFR2, thereby deleting the C-terminus. The fusion partners usually contain protein-binding domains, which most likely induce constitutive dimerization and ligand-independent activation of the FGFR kinase domain (2). Overexpression of FGFR fusion proteins results in increased sensitivity to FGFR inhibitors in *in vitro* and *in vivo* models (2). Furthermore, early-phase clinical trials have demonstrated clinical activity with a number of selective FGFR inhibitors, including JNJ-42756493 (3), AZD4547 (5), and BGJ398 (4). In patients with previously treated *FGFR2*-translocated cholangiocarcinoma, the selective pan-FGFR inhibitor BGJ398 revealed an objective response rate of 14% and median duration of treatment of approximately 6 months (4). As progression-free survival in most second-line cholangiocarcinoma studies is in the region of 3 months, these results are promising.

Despite these encouraging findings, *FGFR2*-translocated cholangiocarcinoma tumors appear to develop acquired resistance to FGFR inhibitors relatively swiftly. In this issue of *Cancer Discovery*, Goyal and colleagues report on a proposed mechanism of resistance to FGFR inhibition in 3 patients treated with BGJ398 (6). Although the number of patients studied is small, these patients revealed consistent acquisition of gatekeeper mutations in *FGFR2* and polyclonal resistance reflective of diverse intratumoral genetic heterogeneity in cholangiocarcinoma.

Using molecular barcoded sequencing on progression plasma DNA and tumors, they describe the acquisition of polyclonal *FGFR2* mutations, with a gatekeeper p.V564F mutation found in all 3 patients. Mutations at this residue had been identified as potential gatekeeper mutations in prior preclinical studies (8, 9), and this study confirms the importance of the V564 residue in mediating resistance to targeted therapy. In total, the study identified three *FGFR2* mutations that were common (p.N549H, p.V564F, and p.E565A, all in the kinase domain of the receptor) and further mutations unique to individual patients. Using computational modeling and functional *in vitro* studies, the authors show that the mutations identified have the potential to hinder binding of BJG398 through either stabilization of the kinase in an active or inactive conformation, production of an unfavorable binding conformation, or direct steric hindrance, such as that created by the commonly present p.V564F gatekeeper mutation.

Goyal and colleagues identified two other major factors contributing to resistance. Intratumoral genetic heterogeneity contributed substantially to acquired resistance in at least 2 of the 3 patients, although *FGFR2* p.V564F was the

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only selected mutation detected in plasma in the patient with the longest duration of response. Overt geographical intratumoral heterogeneity was further demonstrated from multiregion sequencing of a rapid autopsy, with lesion-specific acquisition of distinct resistance mutations. The study suggests that intrahepatic cholangiocarcinoma has substantial intratumoral genetic heterogeneity, which presents a major challenge to targeted therapy for these cancers. Among the lesion-specific mutations identified, acquired genetic loss of *PTEN* was selected in multiple lesions, confirming prior preclinical observation that silencing PTEN reduced sensitivity to FGFR inhibitors in *FGFR2*-amplified cancers (10).

The study provides a rationale for developing third-generation inhibitors that target FGFR2^{V564F} and other common mutations to treat resistant tumors or cut off one of the mechanisms to acquired resistance. Goyal and colleagues also profiled currently available first- and second-generation FGFR inhibitors to identify which may be more active against gatekeeper mutations. Here, the authors switched to using *TEL–FGFR3* fusions in BaF3 cell lines for inhibitor screening. Although the kinase domains of FGFR2 and FGFR3 are highly similar, the use of FGFR3 as a model is a weakness in this article. Nevertheless, the results are interesting. Polyclonal gatekeeper mutations develop in *TEL–FGFR3*-expressing cells with acquired resistance at low doses of BGJ398, but at higher doses, only the V555M mutation develops (equivalent to V564 in FGFR2). The authors proceeded to evaluate the differential sensitivity of BaF3 cells expressing an *FGFR3* resistance mutation to several other FGFR inhibitors, of which the pan-FGFR inhibitor LY2874455 displayed the most activity against cells harboring the TEL–FGFR3^{V555M} mutant, and also against FGFR2^{V564F}. This finding may have direct clinical relevance in terms of treatment sequencing for patients with cholangiocarcinoma in the future.

The contribution of Goyal and colleagues to the field of *FGFR2*-translocated cholangiocarcinoma is important. Although this article is limited by small sample size, shared gatekeeper mutations were characterized. Identification of similar gatekeeper mutations in *EGFR*- and *ALK*-positive lung cancer has led to the development of superior second- and third-generation EGFR and ALK tyrosine kinase inhibitors and improved survival for patients with non–small cell lung cancer. It will be important to extend these observations into more patients, and into other pan-FGFR tyrosine kinase inhibitors. Identification of gatekeeper mutations confirms beyond doubt that cholangiocarcinomas are addicted to translocated *FGFR2*, emphasizing the pressing clinical need to move inhibitors forward to pivotal studies. A more fundamental question that the current study raises is the utility of post-progression biopsies to evaluate mechanisms of resistance in the face of gross tumor heterogeneity. As the sensitivity of circulating DNA sequencing increases, this tool may become the primary method through which the genomic evolution of tumors is evaluated, as the limitations imposed by the spatial boundaries of biopsies may needlessly limit our capacity to fully comprehend the complexity of heterogeneous tumors.

Disclosure of Potential Conflicts of Interest

N. Turner reports receiving a commercial research grant from AstraZeneca and is a consultant/advisory board member for AstraZeneca, Lilly, and Novartis. E. Smyth has received honoraria for advisory board participation from Five Prime Therapeutics and for an advisory role from Bristol-Myers Squibb. No potential conflicts of interest were disclosed by the other author.

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