Gatekeeper mutations and intra-tumoural heterogeneity in *FGFR2*-translocated cholangiocarcinoma.

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Competing interests:

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 intra-tumoral heterogeneity drive resistance in patients with *FGFR2*-translocated intrahepatic cholangiocarcinoma, which will have important implications for management of the disease in clinic.

Editorial

Oncogenic chromosomal rearrangements in the fibroblast growth factor receptors (FGFRs) present a potential therapeutic target, with translocations in *FGFR2* found most frequently in cholangiocarcinoma (1,2) and translocations in *FGFR3* prevalent in bladder 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 only been investigated preclinically. Goyal *et al*(6) demonstrate *FGFR2* translocations in intrahepatic cholangiocarcinoma (ICC) 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 dichotomised by anatomical location into intra- and extra-hepatic subtypes. Recent developments in genomic and transcriptomic technologies have revealed fundamental molecular differences between these anatomic sub-sites, reflecting distinct aetiologies (7). Intrahepatic cholangiocarcinoma, a disease with growing incidence for yet unknown reasons, harbours *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 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 NVP-BGJ398(4). In previously treated *FGFR2-*translocated cholangiocarcinoma patients, the selective pan-FGFR inhibitor NVP-BGJ398 revealed an objective response rate of 22% and median duration of treatment of approximately six months (4). As progression-free survival in most second line cholangiocarcinoma studies is in the region of three months, these results are promising.

Despite these encouraging findings, *FGFR2*-translocated cholangiocarcinoma tumours appear to relatively swiftly develop acquired resistance to FGFR inhibitors. In this issue of Cancer Discovery, Goyal and colleagues report on a proposed mechanism of resistance to FGFR inhibition in three patients treated with NVP-BGJ398. Although the number of patients studied is small, these patients revealed

consistent acquisition of gatekeeper mutations in FGFR2 and polyclonal resistance reflective of diverse intra-tumoural genetic heterogeneity in cholangiocarcinoma.

Using molecular barcoded sequencing on progression plasma DNA and tumour, they describe the acquisition of polyclonal *FGFR2* mutations, with a gatekeeper p.V564F mutation found in all three patients. Mutations at this residue had been identified as potential gatekeeper mutation in prior preclinical studies (8,9), and this study confirms the importance of p.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 modelling and functional *in vitro* studies, the authors show that the mutations identified have the potential to hinder binding of NVP-BJG398 either through stabilizing the kinase in an active or inactive conformation, producing an unfavourable binding conformation, or direct steric hindrance such as that created by the commonly present p.V564F gatekeeper mutation.

Goyal *et al*. identified two other major factors contributing to resistance. Intra-tumoral genetic heterogeneity contributed substantially to acquired resistance in at least two of the three patients, although *FGFR2* p.V564F was the only selected mutation detected in plasma, in the patient with the longest duration of response. Overt geographical intra-tumoural heterogeneity was further demonstrated from multi-region sequencing of a rapid autopsy, with lesion-specific acquisition of distinct resistance mutations. The study suggests that ICC has substantial intra-tumoral

genetic heterogeneity, which presents a major challenge to targeted therapy for these cancers. Amongst 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 tumours or cut-off one of the mechanisms to acquired resistance. Goyal *et al.* 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 clear weakness in the manuscript. Nevertheless, the results are interesting. Polyclonal gatekeeper mutations develop in acquired resistant *TEL-FGFR3* at low doses of NVP-BGJ398, but at higher doses only V555M mutation develops (equivalent to V564 in FGFR2). The authors proceeded to evaluate the differential sensitivity of BaF3 cells expressing FGFR3 resistance mutation to several other FGFR inhibitors, of which pan-FGFR inhibitor LY2874455 displayed the most activity against cells harbouring TEL-FGFR3_V555M mutant, and also in FGFR2_V564F. This finding may have direct clinical relevance in terms of treatment sequencing for cholangiocarcinoma patients in future.

The contribution of Goyal and colleagues to the field of *FGFR2*-translocated cholangiocarcinoma is important. Although the manuscript 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 development of superior second and third generation EGFR and ALK tyrosine kinase inhibitors and improved survival for non-small cell lung cancer patients. 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*, emphasising 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 tumour heterogeneity. As the sensitivity of circulating DNA sequencing increases, this tool may become the primary method through which the genomic evolution of tumours is evaluated, as the limitations imposed by the spatial boundaries of biopsies may needlessly limit our capacity to fully comprehend the complexity of heterogeneous tumours.

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