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FGFR2 Inhibition in Cholangiocarcinoma

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Abstract

Biliary tract cancer (BTC) is the second most common primary liver cancer after hepatocellular carcinoma and accounts for 2% of cancer-related deaths. BTCs are classified according to their anatomical origin into intrahepatic (iCCA), perihilar, or distal cholangiocarcinoma, as well as gall bladder carcinoma. While the mutational profiles in these anatomical BTC subtypes overlap to a large extent, iCCA is notable for the high frequency of IDH1/2 mutations (10–22%) and the nearly exclusive occurrence of FGFR2 fusions in 10–15% of patients. In recent years, FGFR2 fusions have become one of the most promising targets for precision oncology targeting BTC, with FGFR inhibitors already approved in Europe and the United States for patients with advanced, pretreated iCCA. While the therapeutic potential of nonfusion alterations is still under debate, it is expected that the field of FGFR2-directed therapies will be subject to rapid further evolution and optimization. The scope of this review is to provide an overview of oncogenic FGFR signaling in iCCA cells and highlight the pathophysiology, diagnostic testing strategies, and therapeutic promises and challenges associated with FGFR2-altered iCCA.

INTRODUCTION

FF: FGFR2 fusion

iCCA: intrahepatic cholangiocarcinoma

FGFR2: fibroblast growth factor receptor 2

RTK: receptor tyrosine kinase

The past few years have witnessed a rapid advancement of genomics-informed therapies in biliary tract cancer (1). A particularly exciting case is represented by pharmacological targeting of oncogenic FGFR2 fusions (FFs) in intrahepatic cholangiocarcinoma (iCCA) (1). FFs are generated by chromosomal rearrangements that fuse the C-terminal end of FGFR2 to heterologous sequences encoded by >100 fusion genes (2, 3). Herein, we review our current understanding of the genetics, biology, and pharmacological actionability of oncogenic FGFR2 alterations in iCCA.

CANONICAL ACTIVATION OF FGFR SIGNALING

The FGFR family includes four receptor tyrosine kinases (RTKs), namely FGFR1 through FGFR4. FGFR1–4 share a similar structural organization (**Figure 1**). A fifth member, FGFR5 (also known as FGFRL1), retains ligand binding capacity but lacks an intracellular catalytic domain (4).

The activity of FGFRs is regulated by the 18-member family of secreted fibroblast growth factors (FGFs). Most secreted FGFs interact with heparan sulfate proteoglycans present at the cell surface and in the extracellular matrix and therefore act locally as autocrine/paracrine growth factors. Endocrine FGFs (namely FGF15/19, FGF21, and FGF23) do not bind to heparan sulfate proteoglycans and therefore can diffuse freely, acting at a distance from their site of synthesis.

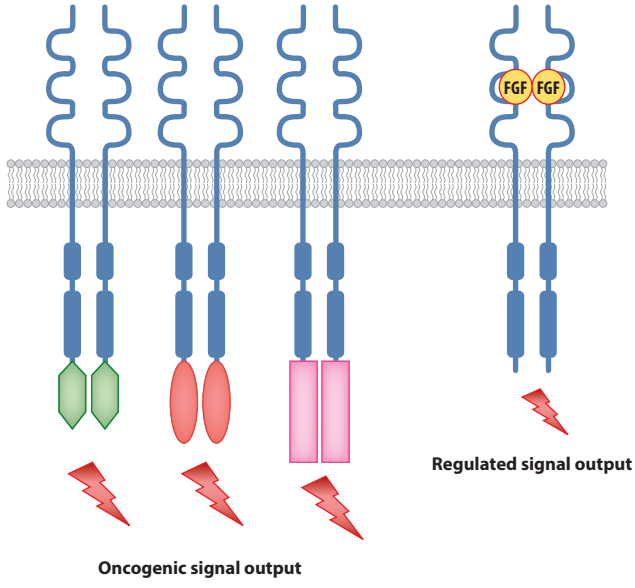

Figure 1

Subversion of ligand-regulated FGFR2 activation results in unabated downstream signaling and oncogenic transformation. *Right:* Outline of key structural features of FGFR2, including the three extracellular Ig-like domains, the intracellular juxtamembrane domain, the split tyrosine kinase domain, and the short regulatory C-tail. Binding of FGF (*yellow circles*) to FGFR2, at a 2:2 stoichiometry, induces receptor dimerization, catalytic activation, and downstream regulated signaling (*small red flash*). *Left:* In iCCA FGFR2 fusions, C-terminal sequences—which in the wild-type receptor exert negative control over kinase activity—are replaced by heterologous sequences contributed by a large number of genes (*green*, *red*, and *pink shapes*), which contribute protein–protein interaction surfaces capable of driving constitutive receptor dimerization and attendant oncogenic signaling (*large red flashes*). Figure adapted from images created with BioRender.com.

Restricted availability coupled to differences in receptor binding affinity/specificity among different FGFs allow for fine regulation of the FGF signal input (4).

In their unliganded inactive state, FGFRs are present at the cell surface as monomers or short-lived dimers, in which the kinase domain is held in a default autoinhibited state by a set of intramolecular interactions (5). Ligand engagement leads to the dimerization of FGFR extracellular domain, a structural transition that is translated intracellularly into the asymmetric dimerization of the kinase domains of adjacent FGFR protomers (6). This process overcomes the energetic barrier imposed by inhibitory intramolecular interactions within the kinase domain, ultimately allowing for FGFR catalytic activation and downstream signal transduction (6).

ABERRANT FGFR2 KINASE ACTIVATION IN CANCER

In cancer, the overarching regulatory principles outlined above can be disrupted by FGFR2 structural alterations that (*a*) increase ligand affinity and therefore overcome restricted FGF availability, (*b*) cause ligand-independent FGFR2 dimerization, and (*c*) disrupt the autoinhibited configuration of the FGFR kinase domain (7).

Ligand-independent dimerization is the mechanism responsible for constitutive catalytic activation of FFs. Here, protein–protein interaction surfaces contributed by C-terminal fusion sequences drive a dimerization process (2) that is thought to bring the tyrosine kinase domains of adjacent protomers in close proximity, so as to mimic the geometry and conformation of kinase dimers induced by ligand-regulated dimerization of the FGFR2 extracellular domains (6) (**Figure 1**). Such mimicry may not be perfect and in fact comes at a cost, because FFs, unlike wild-type (wt) FGFR2, require assistance by the HSP90-centered chaperone machinery for proper folding (8). It must be noted that, despite the molecular heterogeneity inherent to the multitude of FF partners detected in iCCA, the dimerization process described above appears to be a remarkably robust mechanism of oncogenic FGFR2 activation (**Figure 1**). One reason might be that residues 769–822, which are encoded by exon 18 and missing in the most prevalent configuration of iCCA FFs (9), are a critical locus of negative regulation of the FGFR2 kinase (**Figure 1**). Thus, Ser 780 is involved in ERK1/2-dependent negative feedback regulation of FGFR2 kinase activity (10), while residues 808–822 limit kinase activation of unliganded FGFR2 via a mechanism entailing recruitment of GRB2 via SH3-dependent molecular interactions (11, 12). Harmonious with these findings, *FGFR2* amplification in gastric cancer is associated with expression of an alternatively spliced oncogenic FGFR2 isoform that lacks exon 18–encoded C-terminal sequences (7). In summary, loss of a negative regulatory sequence located in the FGFR2 C-tail is envisioned to act in concert with receptor dimerization mediated by heterologous sequences in order to produce potent and unabated catalytic activation of FFs.

Could FF partner sequences provide more than a mere dimerization surface? Recent work has highlighted the role of biomolecular condensates as subcellular structures where RTK fusion proteins must be concentrated to transmit downstream oncogenic signals via RAS-ERK (13). Key to the generation of these biomolecular condensates is phase separation driven by the formation of large molecular complexes nucleated by multivalent protein–protein interaction surfaces (14) located in sequences fused to oncogenic RTKs (13). While this seminal work focused on cytoplasmic ALK and RET fusions, it is worth noting that wt FGFR2 was shown to signal at plasma membrane puncta, the formation of which was initiated through liquid–liquid phase separation by FGFR2 itself upon its catalytic activation (15). By analogy, FFs might nucleate the formation of biomolecular condensates at the plasma membrane and exploit them as privileged sites of oncogenic signaling in iCCA cells. Considering the essential role played by RAS-ERK signaling downstream of FFs (see below), it is particularly attractive that signaling granules generated

by EML4-ALK and CCDC6-RET fusions were found to be enriched in effectors of the RAS-ERK pathway, while being depleted of negative pathway regulators (13), a condition permissive for unrestrained oncogenic signaling.

IED: in-frame deletion in the extracellular domain

TKI: tyrosine kinase inhibitor

The notion that aberrant signaling by FGFR2 plays a role in iCCA pathogenesis has been reinforced by the recent identification in approximately 3% of iCCA patients of *FGFR2* insertions/deletions that generate in-frame deletions in the extracellular domain (IEDs) of FGFR2 (9). Most of these IEDs were predicted to impact ligand recognition and/or receptor dimerization (9, 16), resulting in FGFR2 oncogenic activation causally linked to iCCA pathogenesis. In contrast to IEDs, sporadic *FGFR2* point mutations have not been associated with meaningful clinical responses to FGFR inhibitors in iCCA, with the possible exception of the W290C and C282R substitutions (9, 17, 18). Perhaps most *FGFR2* point mutations detected in iCCA do not generate levels of FGFR2 activity sufficient to drive iCCA pathogenesis and establish oncogenic dependence. Whether these mutations may still be causative of iCCA by acting in obligate concert with other still unrecognized alterations remains an interesting possibility for future investigations.

ONCOGENIC SIGNALING BY FGFR2 IN INTRAHEPATIC CHOLANGIOCARCINOMA: OPPORTUNITIES FOR PHARMACOLOGICAL TARGETING

RAS-ERK, PI-3K-AKT, PLC-γ, and STAT are major signaling pathways that play necessary roles downstream of FGFR2 in different tissue contexts (19). Despite this complexity, the available evidence points to RAS-ERK being both necessary and sufficient for the oncogenic activity of FFs in iCCA cells (20, 21). This characterization fits with *KRAS* and *BRAF* being known mutationally activated oncogenic drivers in iCCA.

As in the case of wt FGFR2, FFs phosphorylate the membrane-located FRS2 scaffold in order to couple to RAS (9, 21) (**Figure 2**). Tyrosine phosphorylated FRS2, in turn, acts as a platform for recruiting SHP2 and GRB2. SHP2 itself becomes tyrosine phosphorylated, which enables further recruitment of GRB2 to the plasma membrane via SH2-pTyr interaction (**Figure 2**). Once relocated to FRS2, GRB2 enables GTP loading onto RAS proteins via the mSOS guanyl exchange factor, recruited by GRB2 itself through its SH3 domains. In turn, GTP-bound RAS triggers activation of the RAF-MEK1/2-ERK1/2 kinase cascade (**Figure 2**). This model was validated in FF-driven iCCA cells by showing that pharmacological blockade of FFs resulted in loss of Tyr-phosphorylated FRS2 and SHP2, along with loss of pMEK1/2 and pERK (21). Likewise, pharmacological inhibition of either SHP2 or MEK1/2 abrogated pERK detection in FF-expressing iCCA cells (21).

RAS-ERK signaling was found to be necessary for survival and growth of FF-driven iCCA cells, as indicated by comparable extent of growth inhibition obtained upon pharmacological targeting of FFs, Shp2, or Mek1/2 in iCCA murine cellular models (21). Sufficiency of RAS-ERK in sustaining the FF-driven oncogenic phenotype was demonstrated by the ability of mutationally activated KRAS to rescue iCCA cells from FF inhibition (20). Consistent with these results, BGJ398 and trametinib interacted synergistically in cultured FF-driven iCCA cells (20, 21). When compared to single agents, this combination provided more durable control of tumor growth in murine models of FF-driven iCCA (20, 21). Whether RAS-ERK signaling plays a necessary/sufficient role downstream of FGFR2 IED in iCCA cells is unclear. However, *BRAF* and *KRAS* mutations were hypothesized to drive secondary resistance to treatment with FGFR TKIs in a patient with FGFR2 IED+ iCCA (9), suggesting a key role for RAS-ERK output downstream of FGFR2 IED in iCCA cells.

While RAF and MEK1/2 kinases act nonpromiscuously within the RAS-ERK module, ERK kinases can phosphorylate a myriad of substrates in both cytosol and nucleus, ultimately

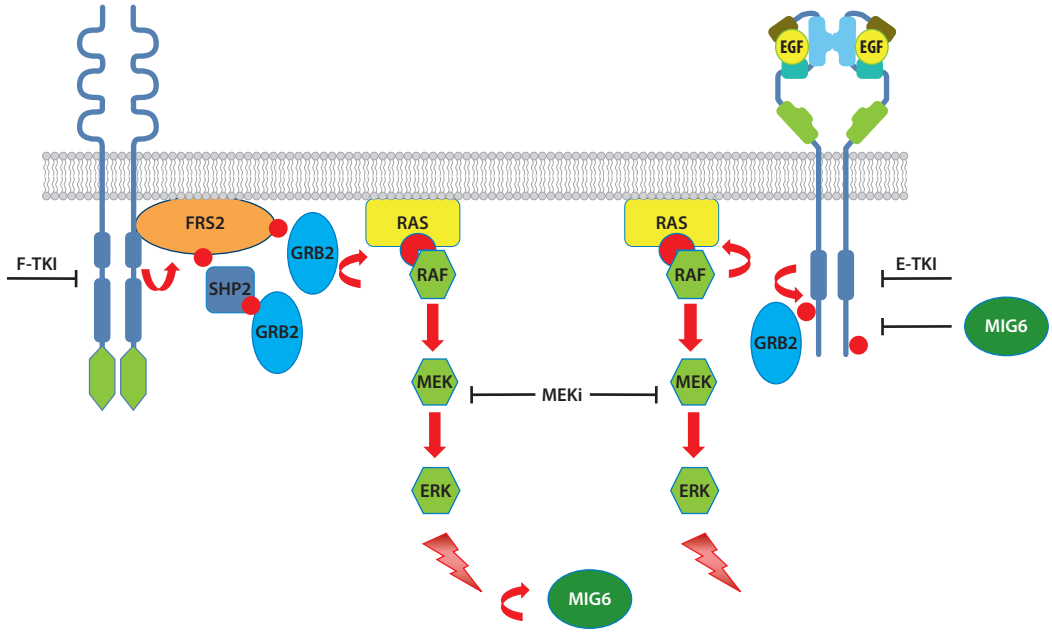

Figure 2

Oncogenic activity of FGFR2 fusions in iCCA is reliant on RAS-ERK activity. *Left:* FGFR2 fusions phosphorylate FRS2 on tyrosine residues (*red circles*), which recruit GRB2 and SHP2 to the plasma membrane, ultimately enabling RAS GTP loading and downstream activation of the RAF-MEK-ERK cascade. ERK signaling induces expression of MIG6, which is capable of inhibiting EGFR. FGFR-specific TKIs inhibit RAS-ERK output, which can relieve EGFR from MIG6 inhibition and enable parallel RAS-ERK reactivation by EGFR despite FF blockade. Combination therapies providing dual suppression of FGFR2 fusions and either EGFR or MEK1/2 aim at providing robust and durable suppression of RAS-ERK output in FF-driven iCCA cells. Figure adapted from images created with BioRender.com. Abbreviations: EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular regulated kinase; F-TKI, FGFR-specific tyrosine kinase inhibitor; FF, FGFR2 fusion; FGFR2, fibroblast growth factor receptor 2; GRB2, growth factor receptor bound 2; GTP, guanosine triphosphate; iCCA, intracellular cholangiocarcinoma; MEK, mitogen-activated protein kinase kinase; MEKi, MEK inhibitor; MIG6, mitogen-induced gene 6; RAF, rat accelerated fibrosarcoma; RAS, rat sarcoma viral oncogene homologue; SHP2, SH2 domain containing protein tyrosine phosphatase 2; TKI, tyrosine kinase inhibitor.

regulating both transcription-dependent and -independent key tumor cell programs. How signal propagation downstream of ERKs implements fundamental oncogenic traits of FF-driven iCCA cells is currently unclear. Keeping an eye on pharmacological targeting of the FF-RAS-ERK axis in iCCA cells, we highlight two key aspects of the above-discussed model. First, the RAS-ERK module can be activated by upstream positive regulators, which creates the opportunity for parallel pathway reactivation of RAS-ERK in iCCA cells subjected to FF pharmacological blockade (**Figure 2**). Second, as already shown in other cell types, RAS-ERK activation in iCCA cells brings about the activation of a suite of negative feedback regulators—including MIG6 and proteins of the DUSP and SPROUTY families (22–24)—that act at different steps in the pathway to limit ERK output. This is relevant because, by relieving the RAS-ERK module from transcriptionally dependent feedback inhibition, FF blockade could set the stage for pathway reactivation (24) (**Figure 2**). Exploiting the above models, Wu et al. (25) showed that downregulation of the ERBB feedback inhibitor MIG6 (23) upon FF blockade resulted in an increase of EGFR activity and downstream RAS-ERK output, which prevented iCCA cell death upon FF blockade (**Figure 2**). Consequently, therapeutic responses to FGFR inhibitors in mouse models of iCCA could be dramatically improved by coadministration of an EGFR TKI (25).

Notably, dependence on RAS-ERK signal output is maintained also in iCCA cells carrying resistance mutations in the kinase domain of FFs (21) (see next section). Thus, the findings discussed above collectively support a model wherein combined FF and MEK1/2 inhibition is predicted to delay or prevent the emergence of resistant iCCA cells, evolving through different mechanisms that converge on preservation of RAS-ERK signaling (26).

MODELS OF FGFR2 FUSION–DRIVEN TUMOR DEVELOPMENT IN MICE

Murine cholangiocarcinoma (CCA) models can be classified into five main categories according to technical approach: (*a*) chemically induced models; (*b*) transgenic animals in which cancer-driving alleles are inserted in the germline or delivered into adult liver cells through electroporation, tail vein injection, or intrabiliary injection; (*c*) orthotopic or subcutaneous transplantation of genetically manipulated liver organoids, whether of human or mouse origin; (*d*) transplantation of cultured iCCA cells, which can be traditional 2D cell lines or tumor organoid cultures propagated in 3D; (*e*) transplantation of tumor fragments obtained from surgically resected human CCA samples (patient-derived xenografts). Flanking the clinical discussion about the cell of origin of iCCA, lineage tracing has revealed that some murine models give rise to CCAs from biliary markers expressing cells, while in other models, hepatocytic cells give rise to iCCA through transdifferentiation into the biliary lineage.

Early studies using nonbiliary cells demonstrated that FFs are capable of transforming NIH3T3 mouse fibroblasts, resulting in tumor development upon transplantation into immunocompromised mice (3). Recently, a parallel approach supported the oncogenic potential of one of the few IED *FGFR2* alleles described thus far (9). In 2016, the first patient-derived CCA xenograft bearing an FGFR2-CCDC6 fusion was described (27). Two murine models published in 2021 confirmed the ability of FFs to drive cholangiocarcinogenesis, using two independent approaches (20, 21).

Kendre et al. (20) employed the liver electroporation technique (28), in which plasmid DNA is injected directly into the liver, and a short electric pulse is applied at the injection site to achieve in vivo transfection. Sleeping-beauty transposase-mediated stable genomic integration facilitated continuous expression of FFs, while transient expression of a single guide RNA targeting Trp53 from a CRISPR/Cas9-encoding plasmid resulted in efficient disruption of the tumor suppressor gene (20). Considering that only plasmid DNA is required, the model is flexible because it allows for rapid a la carte genetic modeling in the desired immunocompetent recipient strain background. In the presence of an endogenous *KrasG12D* mutation, which by itself was not sufficient for tumor development in the p53-deficient context, six different fusions were tested, yielding a similar median overall survival of approximately 3 months. In the absence of mutant Kras, latency was prolonged, and penetrance was incomplete. While hepatocytes are considered the bona fide target cells in liver electroporation (28), a necroptosis-dominant microenvironment due to electroporation was suggested to instruct tumor development along the cholangiocellular rather than hepatocellular lineage (29). This observation is pathophysiologically interesting, considering that an inflammatory environment is a common denominator of most established risk factors for CCA (29).

In a separate approach, Cristinziano et al. (21) chose a liver organoid system to generate FF-driven murine tumors (30). Four different fusions were retrovirally introduced into Trp53$^{-/-}$ liver organoids derived from C57/Bl6 mice and injected orthotopically and/or subcutaneously into NOD/Scid mice, resulting in tumor development with full penetrance in two of the FFs under investigation. While the organoid-based model demands slightly more effort to change the genetic setup or adapt the model to different strain backgrounds, it offers the opportunity to enrich for alterations during in vitro culture that are not necessarily positively selected during in vivo

tumorigenesis. Liver organoids are regarded as adult progenitor-type cells that display a biliary marker profile. Thus, the biliary lineage also appears to be prone to malignant transformation driven by FFs. Of note, the observation that FFs are almost exclusively, but with a relatively high frequency, detected in iCCA (and not in other hepatobiliary malignancies), in conjunction with the spatial cellular heterogeneity of the biliary system (31), might point toward a cell of origin that is unique within the hepatobiliary niche. However, its localization remains to be determined.

MOLECULAR TESTING

FFs are described as driver events in 10–15% of iCCAs, and more than 140 fusion partners are currently known. The breakpoints in *FGFR2* are mainly found in intron 17 but were also described for exons 17 and 18 and the 3′ untranslated region. Significant frequencies of *FGFR2* amplifications have been reported for gastric cancer (approximately 5%) and breast cancer (approximately 1–4%), and *FGFR2* amplifications have been identified in other cancer types as well (32–34). Mutations, which are located in the ligand-binding and transmembrane domains as well as in the kinase domains, have been primarily demonstrated in endometrial carcinomas (10%), non–small cell lung cancer (4%), and gastric cancer (4%) (35, 36) but are also subject to clinical investigation in patients with biliary cancers.

When choosing the appropriate detection method for FF, the preanalytical part is the first important aspect to consider. An abundance of tumor cells and high tumor DNA/RNA quantity and quality are essential to avoid false-negative results. Given the diverse landscape of FFs, a fusion partner–agnostic next-generation sequencing (NGS) approach (e.g., single primer extension or hybrid capture) at the RNA level is the method of choice. In addition to identifying the fusion partners, these approaches provide further relevant information such as the exons involved and whether the reading frame is conserved (in-frame fusion). The synoptic view of these data facilitates predictions about the function and functionality of the gene fusion. In addition, detection at the RNA level provides evidence of transcription of the fusion gene.

The DNA translocation analysis can be carried out as an alternative or in addition if the hotspot translocation areas are sufficiently covered by primers/probes. If one sample is not sufficient for NGS-based analysis, FGFR2 break-apart FISH (fluorescence in situ hybridization) should be used for analysis, as this analysis requires fewer cells for reliable detection of FFs. Ideally, a combinatorial approach interrogating both RNA and DNA is used in a diagnostic setting. Two FISH approaches, break-apart probes and the fusion-specific dual-fusion probes, may be used. Both have in common that no statement can be made about the functionality and transcription of the fused gene product. While the first approach (break-apart probes) is largely fusion partner–agnostic, the second is specific and restricted to exactly one partner gene. The latter is not suitable for the detection of FFs due to the large number of different fusion partners. The break-apart approach relies on the strategy that after a chromosomal rearrangement, the two probes are far enough apart to ensure the visibility of the two different color signals. Therefore, genomic rearrangements where the two probes are not sufficiently separated, as is the case with genes that are in close proximity to each other, can lead to false-negative results in FISH analyses.

Besides FFs, there is accumulating evidence that in-frame deletions in exons 3–8 encoding the extracellular domain of FGFR2 lead to oncogenic activation of the gene. Targeted NGS panels employed for DNA sequencing should ideally cover the full exonic region of *FGFR2* and specifically exons 3–8. For the detection of high-level amplifications, both ampliseq and hybrid capture approaches may be used; full coverage of *FGFR2* exons and adjacent genetic regions is preferred. For the detection of low- to mid-level amplifications, a hybrid capture approach is favorable. Mutations may also be identified by both approaches, but coverage of the appropriate genomic regions (i.e., primarily exons 5–9) has to be considered. In summary, the methods mentioned above each

have different advantages and limitations that must be considered when interpreting molecular data and diagnostic reports.

TARGETING FGFR2 FUSIONS IN INTRAHEPATIC CHOLANGIOCARCINOMA

Consistent with the nomination of FFs as iCCA drivers, discussed above, several phase II studies in FF+ iCCA patients have shown consistent efficacy for the pan-FGFR inhibitors pemigatinib, infigratinib, debio 1347, derazantinib, and futibatinib (37–40) (**Table 1**). In contrast to the ATP-competitive inhibitors, futibatinib covalently binds to the highly conserved P-loop cysteine residue in the ATP pocket of FGFR (C492 in the FGFR2-IIIb isoform). Although the phase II studies were conducted in pretreated patients, response rates between 21% and 42% could be achieved with a disease control rate above 80%. The median progression-free and overall survival in the studies ranged from 7 to 9 and 12 to 22 months, respectively, but due to their single-arm design and the unknown prognostic impact of FFs in iCCA, survival data should be interpreted with caution. FGFR inhibitor–associated toxicity profiles are comparable between the compounds and appear to be overall manageable: The most common adverse event reported across all trials was hyperphosphatemia due to the physiological involvement of the FGF23/FGFR signaling axis in phosphate homeostasis (**Table 1**). Further frequent adverse events included fatigue, alopecia, gastrointestinal toxicity, nail toxicities, stomatitis, and ophthalmological toxicities. The phase II studies led to approval of pemigatinib by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA), followed by an FDA approval for infigratinib, for the treatment of chemorefractory FF+ iCCA patients. In addition, breakthrough designation was granted to futibatinib, as the first irreversible FGFR inhibitor. Based on the high efficacy of the drugs in the chemorefractory setting, three phase III trials have been initiated to compare pemigatinib (FIGHT-302, NCT03656536) (41), infigratinib (PROOF, NCT03773302) (42), and futibatinib (FOENIX-CCA3, NCT04093362) (43) to gemcitabine and cisplatin ± durvalumab as the current standard of care as first-line monotherapy in FF+ iCCA patients.

In addition to FFs, oncogenic FGFR mutations, amplifications, and in-frame deletions/IEDs are observed in multiple solid tumors with the majority being gene amplifications, followed by mutations (9, 35). Common oncogenic point mutations in *FGFR2* include S252W, P253R, Y375C, C382R, N549K/H, and K659E. In the FIGHT-202 trial, no deep responses were seen in iCCA patients with *FGFR2* mutations treated with pemigatinib (38). Similarly, the overall response rate in the interim analysis of the phase II study FIDES-01 cohort 2 was low (8.7%) in nonfusion, FGFR2-altered patients under therapy with derazantinib. Nevertheless, the study drug, a multikinase inhibitor with potent pan-FGFR activity, achieved a notable disease control rate of 73.9% with a median progression-free survival of 7.3 months in this patient subgroup, and the final results of this study are awaited (44). The experience with FGFR inhibitors in patients with IEDs is currently limited to three cases, but the observation that all patients achieved long-lasting partial response appears promising (9). Potent and highly selective oral FGFR2 inhibitors such as RLY-4008, with an 80- to >5,000-fold selectivity for FGFR2 relative to FGFR1, FGFR3, and FGFR4 (45), are now entering the clinical stage, and there is hope that they will not only expand the repertoire of already available FGFR2-targeted agents but also provide an alternative, especially in patients with nonfusion alterations and secondary resistance.

PRIMARY RESISTANCE TO FGFR KINASE INHIBITORS IN INTRAHEPATIC CHOLANGIOCARCINOMA

Despite the overall encouraging results of the phase II trials, critical evaluation of the clinical data also highlights that not all patients respond deeply or durably to the targeted therapies. A

Table 1 Overview of clinical trials with FGFR inhibitors in FF-CCA

Drug (and trial if named)	MOA	IC50 (nM)	Phase	*n*	ORR%	DCR%	mPFS months (95% CI)	mOS months (95% CI)	Adverse events
Debio 1347 (FUZE)	Reversible FGFR1–3 inhibitor	FGFR1: 9.3 FGFR2: 7.6 FGFR3: 22 FGFR4: 290	I	4	25	N/A	N/A	N/A	Fatigue (50.0%), hyperphosphatemia (44.4%), anemia (38.9%)
Erdafitinib	Reversible FGFR1–4 inhibitor	FGFR1: 1.2 FGFR2: 2.5 FGFR3: 3.0 FGFR4: 5.7	IIa	11	54	81	12.3 (3.2–19.4)	N/A	Low-grade hyperphosphatemia (64%), stomatitis (37%), dry mouth (42%), skin changes, most commonly dry skin (29%)
Infigratinib	Reversible FGFR1–3 inhibitor	FGFR1: 0.9 FGFR2: 1.4 FGFR3: 1.0 FGFR4: 60.0	II	108	23.1	84.3	7.3 (5.6–7.6)	12.2 (10.7–14.9)	Hyperphosphatemia (76.9%), eye disorders (67.6%), stomatitis (54.6%), fatigue (39.8%), CSR/RPED (16.7%)
Derazantinib (FIDES-01, cohort 1)[a]	Reversible multikinase inhibitor	FGFR1: 4.5 FGFR2: 1.8 FGFR3: 4.5 FGFR4: 34	II	103	21.0	76	8.0 (5.5–8.3)	15.9 (12.5–22.6)	Hyperphosphatemia (73%), dry mouth and nausea (44.8%), fatigue (34.5%), dysgeusia, vomiting (31.0%), dry eye (17.2%), conjunctivitis (13.8%), blurred vision (10.3%)
Derazantinib (FIDES-01, cohort 2)	Reversible multikinase inhibitor	FGFR1: 4.5 FGFR2: 1.8 FGFR3: 4.5 FGFR4: 34	II	28	8.7	74	7.3 (3.5–16.7)	N/A	Hyperphosphatemia (75.9%), fatigue (34%), nausea (30%), dry eye (30%), dry mouth (27%), diarrhea (19%), ALT increase (24%)
Pemigatinib (FIGHT-202)	Reversible FGFR1–3 inhibitor	FGFR1: 0.4 FGFR2: 0.5 FGFR3: 1.0 FGFR4: 30	II	108	37.0	82.4	7.0 (6.1–10.5)	17.5 (14.4–22.9)	Hyperphosphatemia (60.3%), alopecia (49.3%), diarrhea (47.2%), fatigue (41.8%), dysgeusia (39.7%)
Futibatinib (FOENIX-CCA2)	Irreversible FGFR1–4 inhibitor	FGFR1: 3.9 FGFR2: 1.3 FGFR3: 1.6 FGFR4: 8.3	II	103	41.7	82.5	9.0 (6.9–13.1)	21.7 (14.5–NR)	Hyperphosphatemia (78%), increased ALT (29%), dry skin (29%), diarrhea (27%), dry mouth (27%)

[a]Includes patients with FGFR fusions.

Abbreviations: ALT, alanin-aminotransferase; CI, confidence interval; CSR, central serous chorioretinopathy; DCR, disease control rate; FF-CCA, FGFR2 fusion–cholangiocarcinoma; IC, inhibitory concentration; MOA, mechanism of action; MOS, median overall survival; MPFS, median progression-free survival; N/A, not available; NR, not reached; ORR, overall response rate; RPED, retinal pigment epithelium detachment.

better understanding of primary and secondary resistance mechanisms will lay the groundwork to fully exploit the potential of FGFR2-directed therapies. Primary resistance is likely influenced by co-occurring alterations. At this point, associations between response and mutational spectrum remain speculative. Furthermore, interpretation of the data is highly dynamic because (*a*) prospective clinical data from genetically characterized patients are only now starting to emerge, (*b*) the overall number of patients with comparable comutational profiles remains small, and (*c*) we still lack an understanding of how specific genetic alterations might influence clinical outcome parameters. For instance, a retrospective analysis from the FIGHT-202 trial suggested that FF+ patients harboring *TP53* mutations might have a shorter survival and an inferior response to the inhibitor pemigatinib. Although the FOENIX-CCA2 trial similarly reported an inferior overall response rate in this genetic subgroup, a complete response documented under futibatinib treatment also indicated that at this point no conclusion should be drawn concerning the potential negative predictive value of *TP53* comutations in FF+ patients (46)—especially given that retrospective studies imply that p53 may serve as an independent negative prognostic marker of overall survival (47).

Results from ongoing randomized controlled first-line studies, paired with evidence from revisited historical cohorts on the prognostic influence of distinct genetic alterations, will help to identify patients who benefit from targeting FGFR2, or select those who require upfront cotreatment approaches (17, 47).

SECONDARY RESISTANCE TO FGFR KINASE INHIBITORS IN INTRAHEPATIC CHOLANGIOCARCINOMA

Under the selective pressure of pan-FGFR inhibitors, secondary resistance inevitably evolves, reflected by a duration of response of 9.1 and 9.7 months in the FIGHT-202 (pemigatinib) and the FOENIX-CCA2 (futibatinib) trials, respectively. Acquired resistance can result from on- or off-target resistance, and resistance profiles of different FGFR inhibitors are likely influenced by their distinct binding properties. On-target resistance results from alterations that affect the activity of the drug at its target, and most patients who were responsive to FGFR inhibitors will eventually develop such mutations under treatment. Although data on secondary on-target resistance are still limited, recurrent acquired alterations in the FGFR2 kinase have been reported. Chief among the mutational hotspots is the V564 gatekeeper residue, which, if mutated, can prevent FGFR2 inhibitors from accessing the ATP binding pocket, thereby rendering the drugs nonfunctional. Other alterations stabilize or promote the active conformation of the kinase, counteracting the autoinhibitory molecular brake (N549X, E565X, and K641X). Preclinical studies indicate that RLY-4008 and, to a lesser degree, TAS-120 and debio 1347 retain potency against at least a subset of resistance mutations. In FF+ and F-IED+ patients with secondary on-target resistance under FGFR-targeted treatment, clinical proof-of-concept evidence supports the sequential use of FGFR inhibitors (48, 49), but the optimal choice of sequence is complicated by (*a*) an incomplete understanding of mutational fingerprints characteristic of the different inhibitors and their respective clinical activity against secondary mutations and (*b*) the complex coevolution of multiple modes of secondary resistance mutations and mechanisms. Resistance appears to be polyclonal in most cases, with multiple resistance mutations detected in blood and tumor samples from patients progressing on pan-FGFR inhibitors (50). Polyclonal resistance was impressively demonstrated by a rapid autopsy study that documented a profound inter- and intralesional heterogeneity, highlighting the limited diagnostic power of individual biopsies and the need for tumor site–agnostic liquid biopsies to monitor clonal dynamics in order to guide sequential treatments (50, 51).

In addition, the dynamic landscape of secondary resistance mechanisms is further diversified by the evolution of off-target resistance that bypasses dependency on FGFR2 signaling through the activation of alternative oncogenic pathways. Off-target resistance might also be propelled by nongenetic mechanisms, the identification and targeting of which would pose a substantial challenge in the clinic.

Off-target resistance—as well as primary resistance—will likely become the domain of combination therapies. It remains to be determined whether highly specific FGFR2 inhibitors with activity against a broad spectrum of mutations are more prone to off-target resistance and whether off-target resistance develops at the same pace as on-target resistance. If so, sequential therapy—starting with a first-generation FGFR inhibitor—may be a more effective strategy than an immediate start with a second-generation inhibitor.

COMBINATION THERAPIES IN FGFR2 FUSION–POSITIVE PATIENTS

Following the completion of the currently recruiting trials that address the role of FGFR-targeted monotherapy, the field will likely move toward combination approaches. As discussed above, combinations of F-TKI + MEKi and F-TKI + EGFRi are strongly supported by preclinical data mature enough for being tested in clinical trials (20, 21, 25, 26).

Building on promising data from other solid malignancies that support the combination of targeted therapy/TKIs and immune oncology, another concept to be explored might be the combination of immune oncology and FGFR inhibition. Along this line, it is encouraging that initial data in murine model systems imply that FGFR inhibition can alter the immune microenvironment of tumors and enhance the antitumor T cell responses (52–54). In addition, some FGFR inhibitory compounds also exhibit activity against other RTKs. For instance, derazantinib inhibits the colony stimulating factor 1 receptor (CSF1R) in vitro concentrations similar to those required for the inhibition of FGF receptors, and tumor macrophage modulation through CSF1R blockade may render tumors more responsive to T cell checkpoint inhibition. This concept is currently being evaluated in the ADVANCE study in biliary tract cancer (NCT05174650) and the FIDES-02 study in urothelial cancer (NCT04045613).

CONCLUSION AND FUTURE DIRECTIONS

The FGFR pathway has emerged as a promising target in precision oncology. While the current focus is on FF, the promise of FGFR mutations and of the recently discovered IEDs as targets for FGFR-directed therapies remains to be determined in larger cohorts. The strong oncogenic potential, especially of FFs, has been confirmed in preclinical model systems, and efficacy data unequivocally support the use of FGFR inhibitors in FF+ iCCA patients. Therefore, routine genetic testing that reliably identifies patients with FF alterations is fundamental to provide these iCCA patients with a viable and effective treatment option.

Remarkably, FGFR inhibitors were approved in FF+ biliary cancer not only by the FDA but also by the EMA based on evidence from single-arm phase II studies. Although it appears an obvious next step to evaluate FGFR inhibitors now in the first-line setting, it is debatable whether three ongoing parallel phase III trials testing FGFR inhibitors against the standard of care, with comparable phase II data, is the most reasonable approach to advance therapies for FF+ patients. Especially in small genetic subgroups of a rare cancer, patient-centered trial development appears mandatory to make timely and truly meaningful clinical progress. For FF+ iCCA, it is of utmost importance to develop interventional concepts that prospectively address codependencies in FF-driven iCCA, as well as secondary resistance mechanisms. The implementation of reasonable

sequential treatment strategies and rationale-based combinations offers the chance to realize and exploit the full potential of FGFR inhibitors.

The recent developments have positioned FGFR-directed therapies at the front line of precision oncology. They shine a spotlight on the clinical importance of molecular diagnostics and the promise of targeted approaches in a rare cancer entity.

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