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EVOLVING THERAPIES (RM BUKOWSKI, SECTION EDITOR)

FGFR Inhibitors: Clinical Activity and Development in the Treatment of Cholangiocarcinoma

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Abstract

Purpose of Review Cholangiocarcinoma is an aggressive cancer with a poor prognosis and limited treatment. Gene sequencing studies have identified genetic alterations in fibroblast growth factor receptor (*FGFR*) in a significant proportion of cholangiocarcinoma (CCA) patients. This review will discuss the *FGFR* signaling pathway's role in CCA and highlight the development of therapeutic strategies targeting this pathway.

Recent Findings The development of highly potent and selective FGFR inhibitors has led to the approval of pemigatinib for FGFR2 fusion or rearranged CCA. Other selective FGFR inhibitors are currently under clinical investigation and show promising activity. Despite encouraging results, the emergence of resistance is inevitable. Studies using circulating tumor DNA and on-treatment tissue biopsies have elucidated underlying mechanisms of intrinsic and acquired resistance. There is a critical need to not only develop more effective compounds, but also innovative sequencing strategies and combinations to overcome resistance to selective FGFR inhibition. Therapeutic development of precision medicine for *FGFR*-altered CCA is a dynamic process of involving a comprehensive understanding of tumor biology, rational clinical trial design, and therapeutic optimization.

Summary Alterations in FGFR represent a valid therapeutic target in CCA and selective FGFR inhibitors are treatment options for this patient population.

Keywords Cholangiocarcinoma · Fibroblast growth factor receptor · Targeted therapy · Tyrosine kinase inhibitors · Biliary tract cancer · Genetic rearrangement

Introduction: The Global Burden of Cholangiocarcinoma and Unmet Needs

Cholangiocarcinomas (CCA) comprise a heterogeneous group of biologically distinct cancers arising from the biliary tract. They are anatomically subdivided into intrahepatic cholangiocarcinoma (iCCA), perihilar cholangiocarcinoma (pCCA), and distal cholangiocarcinoma (dCCA). Specifically, iCCAs arise above the second-order bile ducts, while dCCA arise from below the cystic duct insertion and pCCAs arise from the space in between [1]. After hepatocellular carcinoma (HCC), CCA represents the second most common primary malignancy arising from the liver, with an incidence of 1.26 per 100,000 person-years in the United States (US) [2]. The incidence of iCCA is rising worldwide but the reason for this is not clearly understood [3]. Although considered rare in the US, cholangiocarcinoma is more common in Asian countries such as China, Korea, and Thailand where the incidence in some regions is as high as > 6 per 100,000 inhabitants [4].

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Complete surgical resection and liver transplantation are the only curative treatments and are reserved for fit patients with localized disease. The BILCAP trial demonstrated improvement in survival with adjuvant capecitabine chemotherapy vs. observation alone [5]. Adjuvant chemoradiation is often considered for resected patients with margin-positive and/or node-positive disease, although a definitive benefit is unclear [6, 7]. Despite multi-modal therapy, recurrence rates are high and prognosis is poor with median overall survival (mOS) of 28 months and 5-year survival of 10–30% [8–10]. Unresectable and metastatic disease is treated palliatively with chemotherapy. The ABC-02 trial established gemcitabine and cisplatin as first-line therapy over gemcitabine monotherapy, demonstrating improved mOS of 11.7 months vs. 8.1 months on gemcitabine alone (HR 0.64, $p < 0.001$) [11]. Subsequently, the ABC-06 trial demonstrated a modest 5% objective response rate (ORR) and survival advantage of FOLFOX vs. best supportive care (6.2 vs. 5.3 months, adjusted HR 0.69, $p = 0.03$) when used in the second-line setting [12].

Although options beyond the second-line setting are limited, next-generation sequencing studies (NGS) have identified several potentially targetable molecular alterations that are rapidly changing the treatment landscape of CCA. Actionable genetic alterations are identifiable in over 50% of iCCA cases, including *IDH1/2*, *FGFR*, *BRAF*, *BRCA1/2*, *HER-2*, *ALK*, *RET*, and *NTRK* [13, 14]. Recently, the US Food and Drug Administration (FDA) approved pemigatinib for CCA with FGFR2 fusions or rearrangements, making it the first targeted therapy for these aggressive cancers [15]. As such, molecular profiling with NGS is rapidly being incorporated in the management of these cancers.

This review will discuss the FGFR signaling pathway's role in CCA and highlight the development of therapeutic strategies to target this oncogenic pathway. We will also highlight the biologic nuances of this pathway and challenges in the management of *FGFR*-altered CCA.

FGFR Alterations in Cholangiocarcinoma

The FGFRs form a family of four highly conserved transmembrane receptor tyrosine kinases (FGFR 1–4) [16]. Signaling through the FGFR pathway has an important role in mediating several physiological processes involved in metabolism, tissue homeostasis, endocrine function, and wound repair [17]. Dysregulated FGFR activity can lead to malignant transformation and oncogenesis in a variety of different cancer types. Oncogenic signaling through FGFR is typically mediated through fibroblast growth factor receptor substrate 2 (FRS2), mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase 1/2, (ERK1/2), phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) signaling pathways, Janus kinase–signal transducer and activator of transcription (JAK–STAT), phospholipase Cγ (PLCγ), and ribosomal protein S6 kinase 2 (RSK2) 1,2 [18, 19].

Studies through NGS have identified a spectrum of *FGFR* alterations in CCA that occur through amplification, activating mutations, or fusions through gene rearrangements [20]. Overall, gene fusions are the most frequently encountered (3.5%), followed by amplification (2.6%) and activating mutation (0.9%) [21]. The majority of fusions involve genes encoding FGFR2 and occur almost exclusively in iCCA, accounting for about 10–16% of this subtype [22–24].

FGFR2 Fusions in Cholangiocarcinoma

The *FGFR2* gene is located on chromosome 10 and about half of FGFR fusions evolve from intrachromosomal events. These typically result in an in-frame fusion between the 5′ end of the *FGFR2* gene fusing with another partner gene [25]. The FGFR2 moiety of the fusion product retains the extracellular and kinase domains while the fusion partner imparts the dimerization signal promoting a constitutive ligand-independent activation. There are more than 150 known *FGFR2* fusion partners reported in the literature with the FGFR2-BICC1 fusion being the first and most frequently encountered [26, 27].

Testing for *FGFR2* fusions in CCA can be performed by a variety of methods, each with their own merits and limitations. Sequencing DNA to detect the FGFR2 fusion transcript via NGS is commonly used in clinic. This approach uses a hybrid capture-based methodology to generate target-enriched DNA libraries from fresh frozen paraffin-embedded (FFPE) tumor tissue. This is applicable to *FGFR2* fusions as translocations resulting in *FGFR2* fusions almost always occur in intron 17 or exon 18, which allows the design of specific capture probes close to the fusion breakpoints [27].

Fluorescence in situ hybridization (FISH) is another commonly used method. This method employs fluorescently labeled DNA probes that bind to specific complementary target sequences which can be detected using fluorescence microscopy. The break-apart FISH assay can identify gene translocations using probes specific for loci of interest. The wild-type signal pattern shows two pairs of closely approximated or fused signals, whereas the two colors split apart in the presence of rearrangements. This can be easily performed on limited FFPE samples within a short time frame and is relatively inexpensive. Disadvantages include false negative rates with complex and intrachromosomal rearrangements.

A limitation of DNA NGS is the potential to miss fusion events that occur at the RNA splicing level. RNA sequencing (RNA-Seq) only sequences a small portion of the genome that is transcribed and spliced into mature mRNA. In addition to the traditional gene fusions, RNA-Seq can detect spliced fusions that only occur at RNA level and can detect multiple

alternative splice variants resulting from fusions. It is relatively low-cost and expeditious, and is therefore becoming the test of choice for the detection of fusions. Other methodologies like immunohistochemistry for FGFR expression or RT-PCR for individual fusions are not validated/useful in clinical practice.

Clinical Phenotype of *FGFR*-Altered Cholangiocarcinoma

Cholangiocarcinoma harboring *FGFR* alterations has several features worth highlighting. Retrospective studies showed *FGFR*-altered CCA patients are more likely to be female, Caucasian, and younger at diagnosis. These patients also had a high rate of normal CA 19-9 levels (42.6%), bone metastases (30.6%), and a short median time on first-line chemotherapy with gemcitabine and cisplatin (6.2 months) [28•]. With regard to survival, a sequencing study across major centers reported that in comparison to wild-type *FGFR* CCA, patients with *FGFR* alterations presented with early-stage disease (38.5% vs. 22%) and had a longer OS in this early disease setting (37 vs. 20 months). This survival advantage over wild-type FGFR was also seen in advanced and metastatic disease (24 vs. 17 months), suggesting that *FGFR* alterations confer a more indolent clinical course. The OS did not significantly differ between those with *FGFR* fusions vs. other *FGFR* alterations [29]. Moreover, FGFR inhibitors appear to have better anti-tumor efficacy in patients with *FGFR2* fusion in comparison to other *FGFR* alterations. This observation has encouraged the development of more potent FGFR inhibitors targeting this specific subgroup of patients.

Targeting *FGFR* Alterations in Cholangiocarcinoma

A variety of therapeutic strategies exist for targeting dysregulated FGFR signaling in cancer, including small-molecule tyrosine kinase inhibitors (TKIs), monoclonal antibodies, and FGFR ligand traps [30]. Of these agents, TKIs have demonstrated the most promising clinical activity. These can be classified into type I or type II TKIs based on their binding behaviors to the FGFR kinase domain. Type I inhibitors bind FGFRs in the Asp-Phe-Gly (DFG) in-state active enzymatic conformation in an ATP-competitive manner, while type II inhibitors bind to the flipped DFG out-of-state motif [31]. Majority of the FGFR inhibitors belong to the type I category of small-molecule TKIs (Table 1). FGFR inhibitors can be further classified into non-selective or selective FGFR inhibitors, the latter is further classified depending on the FGFR subtype inhibited, either selectively inhibiting all FGFR subtypes (pan-FGFR 1–4 inhibitors) or those that are selective for FGFR2 only. Lastly, FGFR inhibitors may be either reversible ATP-competitive inhibitors or irreversible covalently bound inhibitors.

Non-selective FGFR inhibitors

The kinase domains of the FGFR, vascular endothelial growth factor receptor (VEGFR), and platelet-derived growth factor receptor (PDGFR) families are phylogenetically related and share a relatively conserved ATP-binding domain. The first generation of small-molecule TKIs lacked kinase selectivity for FGFR and blocked the activity of other kinases including VEGFR and PDGFR. Notably, some of these TKIs were initially developed for targeting other kinases and were later found to have potent activity against FGFR [31]. For example, the type II small-molecule TKI ponatinib was initially developed to overcome the BCR-ABL T315I gatekeeper mutation in chronic myelogenous leukemia and was later shown to have nanomolar binding potency against FGFR 1–4 [37]. Other notable non-selective FGFR inhibitors are nintedanib, dovitinib, brivanib, TSU-68, masitinib, lenvatinib, and regorafenib [38–43]. Although blockade of multiple pathways may be desirable to maximize anti-tumor coverage, clinical activity and development are limited by off-target toxicity at therapeutic levels necessary for FGFR-driven cancers [43–45].

Selective FGFR inhibitors

The next generation of FGFR TKIs was developed to exhibit a more selective and more potent inhibition of FGFR. Many of these are reversible ATP-competitive type I pan-FGFR TKIs. As mentioned, CCA is of particular interest in developing these agents due to the presence of FGFR2 fusions which have shown higher rates of response compared to other FGFR alterations. Table 1 lists a selection of relevant compounds that have been or are currently being studied in CCA.

A number of these selective FGFR inhibitors were studied in multi-cohort phase I/II trials or basket studies that included FGFR alterations. The selective FGFR 1–3 inhibitor AZD4547 was evaluated in a tumor agnostic strategy in subprotocol W (FGFR alterations) of the NCI-MATCH trial (EAY131). The study failed to meet its objective response rate (ORR) endpoint of 16% across tumor types (ORR was 8%), but described responses to be limited to FGFR point mutations and fusions. Notably, one of the four patients with a confirmed partial response (PR) was a patient with iCCA and an FGFR fusion. In addition, the 6-month progression-free survival (PFS) rate was highest among patients with FGFR fusions (56%) as compared to patients with single-nucleotide variants (SNVs) or amplifications (0%) [46]. A similar trend was seen in the first-in-human studies of another selective FGFR inhibitor, infigratinib (BGJ398), which produced a tumor shrinkage in all of the *FGFR2* fusion patients [47]. These results underscore the biological variation of *FGFR* alterations and the importance of patient selection in trials developing these agents.

Table 1 Selective FGFR inhibitors in clinical development for cholangiocarcinoma

Compound, references	Inhibitor class, potency vs. FGFR isotypes	FGFR population in phase 1/2	ORR, DCR	mPFS in months (95% CI months) or mDOR, mDOT	mOS in months (95% CI)	Current phase of drug development
Pemigatinib (INC054828) Abou-Alfa [32]	Type I TKI, ATP-competitive Selective FGFR 1–3 FGFR1 IC50 0.4 nM FGFR2 IC50 0.5 nM FGFR3 IC50 1 nM	Previously treated CCA with *FGFR* alterations FGFR2 fusions n = 107, other FGFR alteration n = 20, no FGFR alteration n = 18	*FGFR2* fusions: ORR 35.5% Other *FGFR* alteration: ORR 0% No *FGFR* alteration: ORR0%	*FGFR2* fusions mPFS 6.9 (6.2–9.6) Other *FGFR* alteration mPFS 2.1 (1.2–4.9) No *FGFR* alteration mPFS 1.7 (1.3–1.8)	*FGFR2* fusions mOS 21.2 (14.8–NE) Other *FGFR* alteration mOS 6.7 (2.1–16.6) No *FGFR* alteration mOS 14 (2.3–6.5)	FDA-approved for previously treated CCA with FGFR2 fusions/rearrangements Ongoing first-line phase III pemigatinib vs. gemcitabine and cisplatin *FGFR2*-rearranged CCA (FIGHT-302, NCT036565360)
Infigratinib (BGJ398) Javle [33•]	Type I TKI, ATP-competitive, Selective FGFR 1–3, FGFR1 IC50 0.9 nM FGFR2 IC50 1.4 nM FGFR3 IC50 1 nM	Cohort 1: Prev treated CCA with *FGFR2* fusions and rearrangements n = 108 (fusions n = 83, other rearrangements n = 20)	Overall population: ORR 23.1%, 1 CR and 24 PRs Prespecified subgroup analysis: ORR 34% (17/50) in 2nd line, ORR13.8% (8/58) in 3rd line (3–8 prior treatments)	mPFS 7.3 (5.6–7.6) mDOR 5.0 (0.9–19.1)	n/a	Ongoing first-line phase III infigratinib vs. gemcitabine cisplatin *FGFR2*-rearranged CCA (PROOF, NCT03773302)
Futibatinib (TAS-120) Goyal [34••]	Type I TKI Covalent, irreversible Selective FGFR 1–4 FGFR1 IC50 3.9 nM FGFR2 IC50 1.43 nM FGFR3 IC50 1.6 nM FGFR4 IC50 8.3 nM	Previously treated CCA with FGFR alterations (N = 103). 80 (78%) with *FGFR2* fusion, 23 (22%) rearrangement	Overall population: ORR: 41.7% DCR: 82.5%	mPFS 9 (6.9–13.1) mDOR 9.7 (7.6–17.0)	21.7mo (14.5-NR)	First-line phase III futibatinib vs. gemcitabine cisplatin *FGFR2*-rearranged CCA (FOENIX-CCA3, NCT04093362), not yet recruiting
Erdafitinib (JN-J-42756493) Park [35•]	Type I TKI ATP-competitive Selective FGFR4 FGFR1 IC50: 1.2 nM FGFR2 IC50: 2.5 nM FGFR3 IC50: 3.0 nM FGFR4 IC50: 5.7 nM	Previously treated CCA, Asian patients *FGFR2* fusions n = 8 *FGFR2* mutations n = 3 *FGFR3* fusions: n = 1 *FGFR3* mutations n = 2	3 PR: 50% (6/12) *FGFR2* alterations: 10 evaluable ORR 60% (6/10)	mPFS 2.35 (3.15–19.38)	n/a	
Derazantinib (ARQ 087) Mazzaferro [36•]	Type 1 TKI ATP-competitive Non-selective FGFR 1–3, RET, PDGFR, KIT, Src FGFR1 IC50: 4.5 nM FGFR2 IC50: 1.8 nM FGFR3 IC50: 4.5 nM	Previously treated CCA with *FGFR2* fusions n = 27 Treatment naïve n = 2	*FGFR2* fusions (entire study population) ORR 20.7%	mPFS 5.7 (4.04–9.2)	n/a	Ongoing phase II *FGFR2* fusions, mutations, and amplifications, pretreated CCA (FIDES-01, NCT03230318)

Subsequently, updated results of the proof-of-concept phase II study of infigratinib in 122 CCA patients with *FGFR2* fusions (n = 108) or other alterations (n = 14) demonstrated an ORR of 23%, a disease control rate (DCR) of 84%, and estimated median progression-free survival (mPFS) of 7.3 months in patients with *FGFR2* fusions. Grade 3 or 4 treatment-related adverse events (TRAEs) were observed in 25 (41%) patients. ORRs in the second and third/later line (3–8 prior treatments) settings were 34% (17/50) and 13.8% (8/58), respectively, suggesting that infigratinib was more effective in an earlier line of therapy setting. The most common treatment-emergent adverse events (any grade) were hyperphosphatemia (76.9%), eye disorders (67.6%, excluding central serous retinopathy/retinal pigment epithelium detachment [CSR/RPED]), and stomatitis (54.6%) [33•]. Similar results were observed with derazantinib (ARQ-087), which, although described as a multi-kinase inhibitor, has potent FGFR 1–3 inhibitory activity. A phase I/II open-label study evaluated derazantinib in *FGFR2* fusion CCA (n = 29) and demonstrated an ORR of 20.7%, DCR of 82.8%, and an estimated mPFS of 5.7 months (95% CI: 4.04–9.2 months) [36•]. Of note, TRAEs were observed in 93.1% of patients (all grades), including ocular toxicity in 41.4%. Grade $\geq$ 3 adverse events (AEs) occurred in 8 patients (27.6%). An expansion cohort for 300-mg dose of derazantinib is currently ongoing (NCT03230318).

At present, only two selective FGFR inhibitors are FDA-approved for the treatment of cancer: erdafitinib and pemigatinib. Erdafitinib was approved for patients with locally advanced or metastatic urothelial carcinoma with susceptible *FGFR3* or *FGFR2* alterations, following progression on platinum-containing chemotherapy [48]. Activity in *FGFR2*-altered CCA has been described but not yet established. Preliminary results from phase II study in Asian patients with FGFR alterations (n = 12) showed promising activity with a 50% ORR. Activity was more pronounced in *FGFR2* fusion CCA with ORR of 60%, DCR of 100%, and mPFS of 12.35 months (NCT02699606) [35•].

Pemigatinib was FDA-approved specifically for CCA based on FIGHT-202 which was an open-label single-arm phase II study that evaluated pemigatinib in patients with *FGFR* alterations, including fusions, mutations, and amplifications. In a cohort of 107 CCA patients with FGFR2 fusions or other rearrangements, pemigatinib demonstrated an ORR of 36%, including 3 complete responses. All of the observed responses were limited to *FGFR2* fusion-positive CCA and no confirmed responses were seen in other *FGFR* alterations. The median duration of response (mDOR) was 9.1 months, with 24/38 responding patients having a mDOR of 9.1 months. Only patients with *FGFR2* fusions derived survival benefit with pemigatinib. This *FGFR2* fusion-selected population compared favorably with outcomes obtained with second-line FOLFOX chemotherapy, with the caveat that OS data is immature for pemigatinib [12]. Based on these results, pemigatinib received accelerated FDA approval as a treatment option for patients with previously treated CCA harboring *FGFR2* fusions or rearrangements [15].

Futibatinib (TAS-120) is an irreversible, highly selective FGFR inhibitor that inhibits all four FGFR subtypes at nearly equal sub-nanomolar concentrations [49]. Unlike other reversible ATP-competitive FGFR inhibitors, futibatinib forms a covalent adduct with the cysteine in the highly conserved P-loop of the kinase domain (C492 in the *FGFR2*-IIIb) [50]. In vitro cell line studies demonstrated potent inhibition of wild-type *FGFR* and some *FGFR* mutants resistant to ATP-competitive inhibitors at nearly the same potency, including the *FGFR* V565L gatekeeper mutation. Moreover, fewer resistant clones emerged with more prolonged FGFR inhibition with futibatinib [51]. Results from the first-in-human phase 1 basket study in refractory solid tumors showed a manageable safety profile and preliminary responses. Among 28 patients with CCA and *FGFR* fusions, 20 (71%) experienced tumor shrinkage, 7 (25%) experienced a confirmed partial response (PR), and 15 (54%) had stable disease as their best response producing an ORR of 25% and DCR of 79% [52]. Of note, 13 patients had prior exposure to a reversible FGFR inhibitor and 4 of these patients achieved a confirmed PR to futibatinib, suggesting that covalent FGFR inhibitors can overcome prior resistance to ATP-competitive inhibitors. A subsequent phase II registrational trial (FOENIX-101, FOENIX-CCA2, NCT02052778) evaluated futibatinib 20 mg daily in the second-line treatment of iCCA harboring *FGFR2* fusions and other genetic aberrations. Recently presented results for the 103 enrolled patients show robust activity, demonstrating an ORR of 41.7% and DCR of 82.5%. The median time to response was 2.5 months and the mDOR was 9.7 months. The mPFS was 9 months and the mOS at the time of presentation was 21.7 months, although OS data is immature and further follow-up is ongoing [34••]. Toxicity profile was similar to the other FGFR inhibitors. Based on these results, the FDA has granted futibatinib a breakthrough designation for the treatment of advanced cholangiocarcinoma [53].

Randomized phase III trials are currently ongoing to evaluate the efficacy of selective FGFR inhibitor monotherapy in the first-line setting vs. gemcitabine and cisplatin. The PROOF trial (NCT03773302) is evaluating infigratinib, the FIGHT-302 trial (NCT03656536) is evaluating pemigatinib, and the FOENIX-CCA3 will be investigating futibatinib (NCT04093362). These trials are selecting CCA patients with *FGFR2* fusions or rearrangements (excluding other alterations) and have PFS as their primary outcome measure. The integral biomarker selection of *FGFR2* fusions in these trials seek to achieve a mPFS that outperforms the 8-month mPFS achieved with first-line gemcitabine and cisplatin in an unselected biliary tract cancer population [11].

Targeting Other FGFR Alterations: Activating Mutations and Amplifications

In contrast to the more frequently encountered *FGFR* fusions, mutations and amplifications are less frequent and show a lower likelihood of achieving an objective response from FGFR inhibitors. Data from the phase II study of infigratinib in CCA patients with *FGFR* alterations showed mutations to be present in about 13% (8/61 patients) and amplifications in 5% (3/61 patients), consistent with other reported series of *FGFR* alterations in CCA. Unfortunately, no objective responses were seen in CCA patients with a mutation or amplification in *FGFR*, even in the presence of a co-occurring *FGFR2* fusion. Furthermore, all four patients with an *FGFR3* amplification showed tumor growth compared to baseline. It is worth noting that although objective responses were not achieved, tumor shrinkage compared to baseline was observed in patients with *FGFR2* amplification (3/3) and *FGFR2*-activating mutations (6/8) [54]. Primary resistance through alternative signaling pathways may be contributing to the less robust tumor response seen in this population.

On-Target Dose-Limiting Toxicities

Most of the toxicities observed in FGFR inhibitor trials are related to on-target toxicities caused by disruption of physiologic FGFR signaling. The most prominent and frequent is the development of hyperphosphatemia in three quarters of patients. Under physiologic conditions, fibroblast growth factor-23 (FGF23) released from bone interacts with FGFRs in the kidney (mainly FGFR1) to inhibit reabsorption in the proximal tubules [55]. Blockade of renal FGF23/FGFR signaling by FGFR inhibitors leads to increased reabsorption of phosphate and subsequent hyperphosphatemia. In fact, the development of hyperphosphatemia can be seen as a pharmacodynamic biomarker for FGFR inhibition specifically reflecting the inhibition of the FGFR axis vs. other growth-stimulating pathways [56]. Most of the hyperphosphatemia observed in FGFR inhibitor trials are typically of low severity (grade 1–2) and uncommonly result in treatment interruption or dose modifications, although earlier studies with infigratinib reported more frequent dose adjustments/interruptions (42.6% of study patients) [32•, 36•, 54]. On rare occasions, chronic hyperphosphatemia can result in ectopic calcinosis in skin, soft tissue, and internal organs (Fig. 1) [57]. Ironically, hypophosphatemia has been observed and, in some studies, represents the most common grade 3–4 AE. This was hypothesized to be a consequence of either treating hyperphosphatemia with low phosphate diets and phosphate binders or from negative feedback effects on phosphate homeostasis [32•].

Ocular toxicity is another on-target and dose-limiting toxicity of FGFR inhibition occurring in approximately 20–40% of patients in FGFR inhibitor trials in CCA. In general, ocular toxicities can be categorized into whether these are related to CSR. The accumulation of subretinal fluid in CSR, which can eventually lead to RPED, is similar to that observed in patients treated agents intervening in the MAPK pathway [58]. Most patients who develop CSR/RPED on FGFR inhibitors are often asymptomatic, but more severe cases present with acute central vision loss or decrease and metamorphopsia [32•, 59, 60]. In FGFR trials that included baseline and serial ophthalmologic examination, CSR/RPED was found to occur in 21% of patients, majority were low grade and reversible with drug interruption or discontinuation. Non-CSR-related ocular toxicities are more common, accounting for more than half the cases of ocular toxicity including dry eyes, increased lacrimation, and conjunctivitis [60]. Keratopathy is proposed to be related to dysregulation of FGFR2 signaling in the cornea [36•].

Cutaneous toxicities related to FGFR inhibitors have also been described, most prominently involving appendages such as nails and hair. Nail changes, including onychomadesis, onycholysis, and onychoclasis (Fig. 2), occur in approximately 15–20% of patients, occasionally requiring dose modifications and delays [32•, 36•, 52, 54]. Severe straightening of scalp hair and trichomegaly with FGFR inhibitors have also been described [61, 62].

Resistance: Primary and Secondary

More potent and selective inhibition with TKIs has increased the therapeutic window and clinical efficacy of FGFR inhibitors as compared to non-selective inhibitors. It is apparent, however, that only a subset of patients will respond to selective FGFR inhibition, suggesting the presence of primary resistance mechanisms that render these TKIs ineffective at the onset of treatment. Alternatively, in patients who do achieve a response to selective FGFR inhibition, the duration of response is typically only 7–9 months, suggesting the development of secondary resistance. This is a situation where patients respond at the beginning of treatment and later fail to maintain this response in the midst of consistent drug exposure.

Preclinical cell line studies have elucidated potential mechanisms of FGFR TKI primary resistance. However, many of these studies have been conducted in lung, breast, and urothelial cell lines and may not be specifically applicable to CCA. As such, assessment of resistance mechanisms in clinical trials and individual patients can be a useful tool in elucidating patterns of resistance. Studies using circulating tumor DNA (ctDNA) and tissue biopsies on progression have demonstrated more specific and thorough evaluation of secondary resistance mechanisms in CCA patients on FGFR inhibitors.

Existing co-mutations have also been implicated to confer primary resistance to FGFR inhibitors in CCA. In a

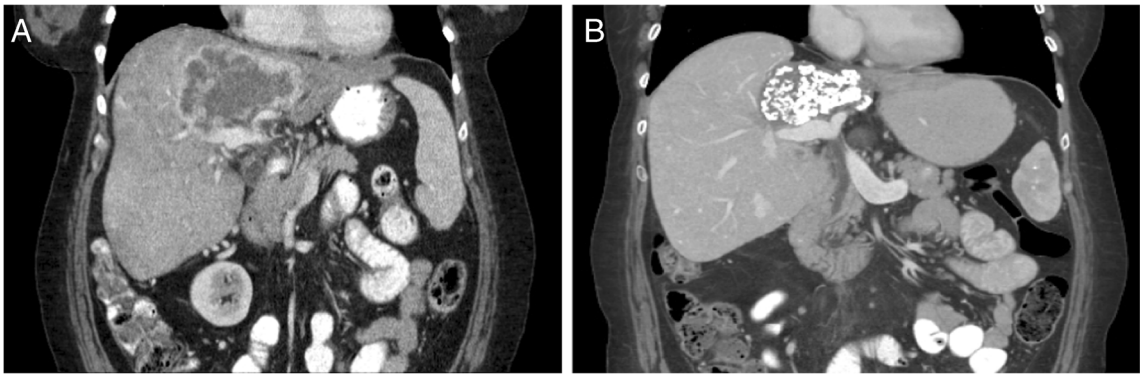

Fig. 1 Ectopic tumor-related hepatic calcification in a patient with FGFR2-BICC1 fusion on infigratinib. **A** Cholangiocarcinoma prior to treatment. **B** Ectopic calcification after treatment and response to infigratinib

comprehensive genomic profiling study of *FGFR2*-rearranged CCA in the FIGHT-202 trial, mutations in *BAP1* were the most frequently encountered co-mutation and was associated with a somewhat shorter mPFS (6.9 months vs. 9.1 months, p = 0.06). Patients with *CDKN2A/B* or *PBRM1* mutations had a significantly shorter mPFS (*CDKN2A/B*, 6.4 months vs. 9.0 months, p = 0.03; *PBRM1*, 4.7 months vs. 7.0 months, p = 0.05) [25]. None of the patients with a coexisting mutation in *TP53* had a response to pemigatinib. Moreover, patients with *TP53* mutations also had a significantly shorter mPFS as compared to those without, a trend also seen in *EGFR* mutation-driven lung cancers treated with anti-EGFR TKIs [63, 64]. It remains to be elucidated as to how these signaling networks lead to primary resistance.

Secondary Gatekeeper Mutations

One of the major mechanisms of resistance to FGFR inhibitors is the emergence of gatekeeper mutations. Similar to other TKIs, selective FGFR inhibitors have exploited the conserved residue within the ATP-binding site of TKI for binding specificity [65]. This residue controls access of inhibitors to a hydrophobic pocket in the active in-state confirmation that is not contacted by ATP, hence the "gatekeeper" function of this residue [66]. In FGFRs, the gatekeeper residue is a valine residue and substitutional mutations in this residue can result in the formation of bulky side chains that prevent inhibitor access into the binding pocket (steric hindrance) and contributes to resistance to ATP-competitive inhibitors [31]. Preclinical studies have identified gatekeeper mutations in *FGFR3* V555M and comparable residues in *FGFR1* V561M and *FGFR2* V564 induce resistance to multiple FGFR inhibitors in vitro [67, 68]. Many of these studies, however, have been conducted in non-CCA cell lines. Contrastingly, much of the insightful data on gatekeeper mutations in CCA have emerged from patient-specific in vivo studies involving serial tissue biopsies and ctDNA.

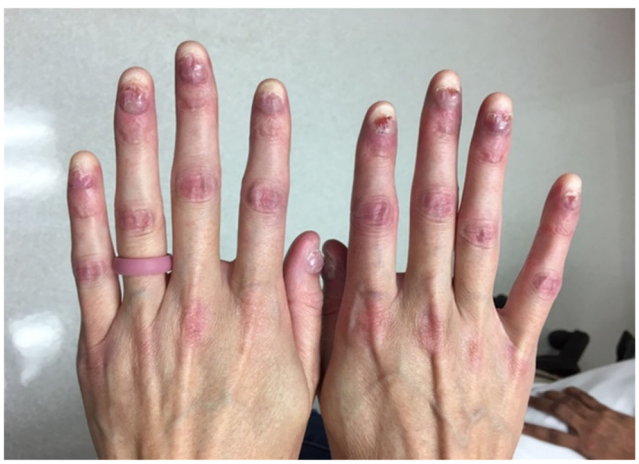

Fig. 2 Onycholysis and onychoclasis of the fingernails on a selective FGFR inhibitor

A serial ctDNA study of 8 CCA patients with *FGFR* alterations (7 fusions, 1 amplification) receiving competitive FGFR inhibitors (Debio1347 and infigratinib) detected a diverse spectrum of *FGFR* mutations emergent on clinical progression. A total of 19 acquired mutations were detected in 5/8 patients (1–9 mutations in each patient), all of which involved the kinase domain of FGFR [69]. Similarly, an insightful study involving 3 patients receiving infigratinib as part of a clinical study showed that serial ctDNA detected the emergence of multiple recurrent point mutations of the FGFR2 kinase domain at progression. The presence of the V564F gatekeeper mutation was common to all of the patients and conferred resistance to infigratinib via steric hindrance in the binding pocket, as predicted by structural modeling. The development of multiple gatekeeper resistance mutations was recapitulated in mutagenesis screens using BaF3 cell lines engineered to express a TEL-FGFR2 fusion protein. The emergence of a V555M mutation, exclusively at higher doses of infigratinib, conferred the highest degree of resistance and was also detected in all three patients by ctDNA [70]. The investigators also performed post-progression biopsies and rapid autopsy which confirmed the presence of marked inter- and intralesional heterogeneity, with various *FGFR2* mutations in individual resistant clones. Together, these

findings suggest significant tumor heterogeneity and evolutionary convergence of resistance mechanisms in CCAs treated with FGFR inhibitors.

Different selective FGFR inhibitors appear to have variable binding to their kinase targets and some have been shown to have the ability to overcome *FGFR* gatekeeper mutations. The selective pan-FGFR inhibitor LY2874455 has demonstrated an almost equal binding affinity to wild-type *FGFR* and a variety of *FGFR* gatekeeper mutations including *FGFR1* V561M, *FGFR2* V564F, *FGFR3* V555M, and *FGFR4* V550M, V550L [71]. Translational studies in patients who received futibatinib after prior competitive FGFR inhibitors showed that futibatinib retained activity against several of the acquired mutations by altering conformational dynamics of FGFR2 rather than directly interacting with the mutant residues. The exception is the V565F gatekeeper mutation which conferred resistance futibatinib, even in increasing concentrations. In silico structural modeling indicated that the dimethoxy phenyl group of futibatinib is in close contact with the V565F gatekeeper residue and that the V565F mutation confers resistance due to steric clash preventing access of futibatinib (and other inhibitors) into the ATP-binding pocket [72••].

Activation of Alternate Intracellular Signaling Pathways

Off-target resistance via activation of alternative intracellular signaling pathways that bypass oncogenic FGFR addiction is another mechanism described in cancers developing resistance to anti-FGFR therapy. Activation in the AKT, MAPK, STAT3, and phosphatase and tensin homolog (PTEN) pathways have been implicated to mediate resistance to FGFR inhibition in preclinical studies across various cancer types [70, 73, 74]. Specifically for *FGFR2*-rearranged CCA, in vitro proteomic studies of tissue samples obtained at progression have identified upregulation of the PI3K/AKT/mechanistic target of rapamycin (mTOR) pathway as a resistance mechanism to FGFR inhibitor therapy. Moreover, the degree of PI3K/AKT/mTOR appears to vary with the underlying kinase domain mutation that developed during progression with the FGFR E565A mutation producing the most pronounced activation in PI3K/AKT/mTOR [75]. Post-progression ctDNA sequencing studies have also identified silencing or loss of *PTEN* as another mechanism where dysregulation in alternate signaling pathways convey resistance to FGFR inhibitors [70, 76].

Combination Strategies with FGFR inhibitors

Despite the encouraging response and disease control seen with selective FGFR inhibitors in clinical trials, the emergence of acquired resistance is inevitable. There is thus a critical need to develop innovative combination therapies to overcome resistance.

One strategy is to address resistance mechanisms that either arise or are concomitantly active in parallel with FGFR signaling. As previously mentioned, the PI3K/AKT/mTOR pathway has been implicated as both a primary and secondary resistance mechanism in FGFR-altered CCA, making combination PI3K and FGFR inhibition a rational strategy. In vitro studies of tissue biopsies obtained on progression in an FGFR2 fusion CCA patient treated with infigratinib showed that the development of an E565A resistance mutation significantly upregulated activity in the PI3K/AKT/mTOR signaling pathway. Subsequent treatment with the potent mTOR inhibitor sapanisertib (INK128, TAK-228, MLN0128) resensitized these cells to FGFR inhibition [75]. However, clinical translation into a feasible combination strategy has been challenging as evidenced by a phase 1b study of alpelisib and infigratinib in patients with *PIK3CA* mutated solid tumors, with or without concurrent FGFR alterations. Results showed sporadic responses and a challenging safety profile necessitating treatment interruption or dose reduction in 71% of patients. In addition, the responses observed were seen in tumor types and genotypes previously demonstrated to be sensitive to either agent alone. Clinical studies in patients with specifically defined CCA genotypes have yet to be conducted.

Targeting the immune tumor microenvironment (TME) with immune checkpoint inhibitors in addition to inhibition of FGFR signaling is another promising combination for FGFR-altered CCA. Preclinical mouse models of *FGFR2* and *TP53* mutated lung cancer treated with the combination of erdafitinib and anti-PD-1 showed significant tumor regression and increase in survival that was not observed with either agent alone. An increase in T-cell infiltration, decrease in regulatory T-cells, and downregulation of PD-L1 expression on tumor cells were observed with combination treatment in the FGFR mutant model. These changes in the TME were not observed in an FGFR-insensitive *KRAS* G12C mutant mouse model, indicating that the immune changes mediated by erdafitinib may have been initiated as a consequence of tumor cell death induced by erdafitinib treatment. A phase I/II trial of lucitanib plus nivolumab in multiple tumor types (NCT04042116) and a phase II pemigatinib plus pembrolizumab in urothelial carcinoma is currently ongoing (FIGHT-205, NCT04003610). However, there are no ongoing trials for CCA.

It is also worth noting that some non-selective FGFR inhibitors have immunomodulating properties that make them particularly attractive to consider for combination therapy. For instance, derazantinib inhibits colony-stimulating factor-1 receptor (CSF1R) at similar concentrations required for in vitro inhibition of FGFR. One of the dominant immune cells in the CCA TME is the alternatively activated tumor-associated macrophage (M2-TAM) [27]. These immunosuppressive

macrophages signal through the CSF1/CSF1R pathway and inhibition has led to enhanced T-cell infiltration, function, and anti-tumor response in preclinical models [77]. Combining derazantinib with immune checkpoint inhibitors has the potential to render CCAs more responsive to immune checkpoint blockade.

Tumor-associated angiogenesis has a significant role in promoting cancer progression and decreasing survival in CCA. A retrospective study in 114 CCA patients showed that 5-year survival rate was significantly longer in patients with low microvessel density (42.1%) as opposed to those with high microvessel density (2.2%) [78]. In addition, dysfunctional vasculature and increased VEGF levels have been associated with poor T-cell infiltration and increased M2-TAM. Therapeutic targeting of VEGF in CCA is thus an attractive strategy, but has unfortunately demonstrated inconsistent and modest clinical activity, even in combination with other agents [79, 80]. CCA patients with FGFR alterations may be a population worth exploring with an anti-FGFR and anti-VEGF combination as many studies have implicated significant cross talk between these pathways [81]. In particular, FGF2 has been shown to be twice as potent as VEGF in inducing angiogenesis in preclinical models [82]. Clinical studies specifically exploring FGFR and anti-VEGF combinations in FGFR-altered CCA are lacking.

Last but not least, combining specific FGFR inhibitors with standard chemotherapeutic agents used to treat cholangiocarcinoma has sound rationale for a synergistic strategy. Cisplatin has been shown to increase sensitivity to anti-FGFR inhibition in patient-derived xenograft models of squamous cell lung cancer. Other preclinical studies have implicated the FGF/FGFR pathway as a cisplatin resistance mechanism, specifically in patients with tumor overexpressing the anti-apoptotic gene *API5* [83]. Whether these explain the relatively short duration of response of FGFR-altered CCA to first-line gemcitabine and cisplatin is unclear and needs to be explored further.

Future Directions and Challenges

The FDA approval of pemigatinib for FGFR2-rearranged CCA is the first targeted therapy to be approved for the treatment of CCA. This approval heralds the advent of precision medicine in CCA and has borne out of the collaboration of basic science, industry, physicians, and most importantly the patients who participated in clinical trials. The significance of this milestone is further emphasized when considering the limited patient population with FGFR-altered CCA patients and the degree of coordination involved in running multicenter trials.

Other promising FGFR inhibitors in trials are likely to follow pemigatinib approval and the decision tree regarding the choice of an appropriate agent in the first and sequential lines of therapy will require clarity. Primary or innate resistance limits the efficacy of FGFR inhibitors and the development of secondary resistance limits the durability of response. On- and off-target toxicity, though manageable, makes combination with other agents like chemotherapy and other TKIs challenging in the clinic. The incorporation of FGFR inhibitors in the multidisciplinary setting, such as with surgery, radiation, or other liver-directed therapy, remains undefined at this time. The unique clinical phenotype of these patients also needs to

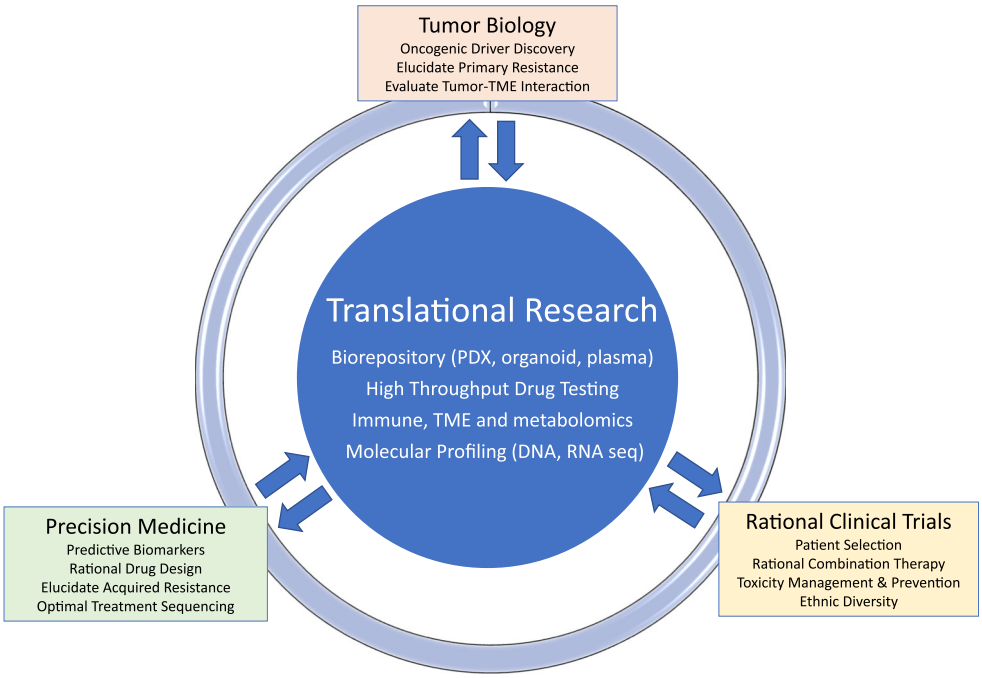

Fig. 3 Hub and spoke model of research priorities for the FGFR pathway in cholangiocarcinoma

be accounted for better in treatment planning, beyond the usage of targeted therapeutics.

Despite these shortcomings, the development of FGFR inhibitors represents an important advancement in the management of CCA. Therapeutic development, with the goal of delivering precision medicine to CCA patients, is a dynamic process of learning and refinement involving comprehensive understanding of tumor biology, rational clinical trial design, and therapeutic optimization to deliver precision medicine. Translational research that brings discoveries from the bench to bedside and vice versa is the hub that links the spokes of these research priorities (Fig. 3).

Conclusion

FGFR is a valid molecular target in the treatment of CCA. Several potent and selective FGFR inhibitors have demonstrated significant activity and clinical benefit for patients with CCA, specifically in patients with FGFR2 fusions or rearrangements. The eventual development of resistance to these small-molecule TKIs limits the potential for more durable responses. Elucidating and overcoming mechanisms of resistance to FGFR inhibitors is an active field of research. Circulating tumor DNA is an emerging tool to interrogate evolving mutations and mechanisms of resistance in FGFR inhibitor therapy of CCA. The rapid development of targeted FGFR therapy and serial interrogation through sequential treatments make precision oncology a valid strategy in the treatment of CCA.

Declarations

Conflict of Interest Gentry King declares that he has no conflict of interest. Milind Javle has received research funding from QED Therapeutics, Taiho Pharmceutical Group, Basilea Pharmaceutical AG, EMD Serono, Meclun, AstraZeneca, and Merck; and has received compensation for service as a consultant from QED Therapeutics, Taiho, EMD Serono, AstraZeneca, and Merck.

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- • Of importance
- •• Of major importance

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