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REVIEW ARTICLE

Mechanisms of resistance to FGFR inhibitors in the treatment of advanced cholangiocarcinoma with FGFR mutations: A literature review

Marcelo P. Sunagua Aruquipa*

Department of Gastrointestinal Oncology, Oncoclinicas, São Paulo, Brazil

Abstract

Advanced cholangiocarcinoma harbors various genetic alterations, one of the most common being fibroblast growth factor receptor (FGFR) fusions. Although FGFR inhibitors have a good response rate, their median progression-free survival is about 6 – 9 months in most trials. The present manuscript is a non-systematic review that explores the mechanisms of resistance and the possible strategies to overcome this rapid resistance. From the findings, resistance to FGFR inhibition can be classified into either primary or secondary resistance. Primary resistance is less common, explained mostly by the presence of co-mutations. In contrast, secondary resistance mechanisms are similar to other tyrosine kinase inhibitors: On-target mutations alter the binding site of the FGFR protein (gatekeeper resistance); off-target mechanisms are multifactorial and involve the activation of related intracellular pathways and the loss of differentiation, leading to a mesenchymal phenotype. Various strategies are in development in order to maintain the efficacy of targeted therapy for patients by overcoming FGFR inhibition resistance, including the coinhibition of the related pathway, the use of pan-FGFR inhibitors, and the development of covalent or dual FGFR inhibitors.

Keywords: Cholangiocarcinoma; FGFR inhibitor; Resistance; Mechanism

***Corresponding author:**
Marcelo P. Sunagua Aruquipa (marcelo.aruquipa@medicos.oncoclinicas.com)

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1. Introduction

Cholangiocarcinoma (CCA) is defined as a tumor that originates from epithelial cells in the bile ducts and is typically characterized by a poor prognosis, with an estimated 5-year overall survival (OS) of about 5% in the advanced stage.[1] CCA treatment has advanced from previously limited systemic treatment options (surgery and traditional chemotherapy with limited efficacy) to personalized medicine. Especially in the case of intrahepatic CCA, there is a higher chance (up to 40%) of detecting actionable somatic alterations using wide genomic analysis techniques.[2,3] The most commonly reported gene alterations are *IDH1*, *ARID1A*, *BAP1*, *TP53*, and *FGFR*.[4]

The causes of CCA remain unclear and multifactorial; there is no unique oncogene driver mutation, and data for hereditary predisposition are not conclusive.[5] However, the known risk factors for CCA include conditions with chronic inflammation of bile ducts generating bile stasis: primary sclerosing cholangitis, liver cirrhosis, intra or extrahepatic biliary stones, and bile duct anatomic abnormalities (Caroli's disease).[6] Some infectious

conditions related to CCA are hepatitis B and C virus and liver fluke parasites (*Clonorchis sinensis*, *Opisthorchis felineus*, and *Opisthorchis viverrine*), which are endemic in some Asian regions.[7,8]

The only curative-intent treatment for localized CCA is surgery. According to the pathological stage, capecitabine can be used for adjuvant treatment, as reported in the phase III trial BILCAP for the occidental population.[9] In the Asian population, the chemotherapy S-1 is used as an adjuvant treatment, as investigated in the phase III ASCOT trial.[10] Unfortunately, some patients do not go to surgery either due to poor clinical conditions or technically unresectable tumors.[11] Even patients who undergo surgery with adjuvant treatment have a high recurrence rate of about 50%, as reported in most trials and databases.[12]

Before immunotherapy, the primary treatment method for advanced CCA was a doublet of platinum-based chemotherapy, with a median OS (mOS) of 11.7 months, as reported in the ABC-02 trial in 2010.[13] Currently, the standard of care in the first-line treatment of advanced CCA is the combination of chemotherapy (platinum and gemcitabine) and durvalumab, as reported in the TOPAZ-1 trial, with a median progression-free survival (mPFS) of 7.2 months and an mOS of 12.8 months.[14] Hence, while immunotherapy offers a statistical benefit, the proportion of long-term responders remains low in most clinical trials, and the majority of patients eventually experience disease progression.

Currently, there are no phase III studies comparing chemotherapy regimens for second-line treatment in patients with negative or unknown actionable mutations. Lamarca *et al.*[15] compared FOLFOX to the best supportive care and demonstrated slightly better results, with an mOS of 6.2 months. Recently, the phase IIB study NIFTY compared the combination of 5-fluorouracil/leucovorin (5FU/LV) and liposomal irinotecan to 5FU/LV alone; the study reported an mOS of 8.6 versus 5.3 months, respectively.[16] Indeed, intrahepatic CCA is a tumor type in which next-generation sequencing plays a crucial role in treatment decision-making, as approximately 30% of cases harbor genetic alterations with approved targeted therapies or are currently being evaluated in clinical trials.[17,18]

2. Methods

For this literature review about the mechanisms of resistance to fibroblast growing factor receptor (FGFR) inhibitors in advanced CCA, an electronic search was performed to retrieve studies using the keywords: "cholangiocarcinoma," "biliary tract cancer," "advanced," "metastatic," "FGFR inhibitor," and "mechanisms of resistance." The Boolean operators "OR" and "AND" were utilized in various combinations with these keywords to refine the search. The electronic databases utilized for this review included PubMed, ScienceDirect, and Medline. Additionally, meeting abstracts from the European Society for Medical Oncology and the American Society of Clinical Oncology annual congresses were reviewed. Zotero 6.0 software was used to manage and exclude repeated references.

The inclusion criteria encompassed prospective studies with patients having metastatic or locally advanced CCA published in peer-reviewed journals between 2017 and 2024, written in English, and providing essential information, such as overall response rate (ORR) according to RECIST 1.1 criteria and mPFS. Phase I and II studies, as well as original research papers on preclinical and translational studies focusing on resistance mechanisms to FGFR inhibitors in CCA and other gastrointestinal malignancies, were included. The exclusion criteria were papers not written in English, without complete information on ORR and mPFS, and retrospective studies with FGFR inhibitors. The initial screening process involved the evaluation of articles based on their title and abstract, applying the inclusion criteria, and removing duplicate sources. Selected papers then underwent a full-text review. Ethical approval was not required for the present manuscript, as it is a review of data from previously published papers.

3. FGFR inhibitors in second-line treatment

The *FGFR2* gene mutation is one of the possible actionable genetic alterations present in about 20 – 30% of patients, more prevalent in intrahepatic CCA. In addition, it is also possible to find *FGFR1* and *FGFR3* mutations unless they are less common.[19] Targeted therapy has demonstrated longer mPFS and mOS in other solid malignancies compared to chemotherapy. However, these results are from previous randomized phase II and basket trials (including advanced CCA), as it is challenging to recruit patients with advanced CCA and driver mutations for phase III trials. A phase II single-arm trial using infigratinib (BGJ398), a pan-FGFR inhibitor, reported an mPFS of 7.3 months.[20]

The confirmatory phase III trial (PROOF 301) compared infigratinib to standard chemotherapy (cisplatin + gemcitabine) was closed prematurely due to difficulties in recruiting patients; the preliminary results with only 48 participants displayed no difference in mPFS (7.4 months for infigratinib and 8 months for chemotherapy), but there was a significant improvement in ORR for infigratinib (37.9%) versus chemotherapy (15.8%).[21] A similar problem was experienced by the phase III trial FOENIX-CCA3, which compared futinatinib to chemotherapy as first-line treatment; the trial was terminated by the sponsor due to poor recruitment.[22]

Currently, the FIGHT-302 trial is the only phase III trial that has completed recruitment, comparing FGFR inhibitor pemigatinib to chemotherapy as a first-line treatment, the results of which are eagerly expected.[23]

The performance of various FGFR inhibitors as second-line treatment has been evaluated in phase II trials (Table 1). Research has demonstrated that the cross-talk in various signaling pathways, both within and beyond the targeted receptor, contributes to the development of cancer resistance mechanisms.[24] In fact, targeted therapy has a rapid effect on symptom relief and tumor control; however, the duration of response is often short-lived, as resistance mechanisms develop quickly.[25] As observed, the mPFS of various drugs is between 6 and 9 months (Table 1).

4. Primary resistance

Most of the first generation of FGFR inhibitors had limited clinical activity, with an mPFS of 4 – 7 months; these drugs were non-selective FGFR inhibitors. In contrast, second-generation FGFR inhibitors with more *FGFR*-selective inhibition have demonstrated better clinical activity. However, there are other types of alterations in the *FGFR* gene beyond fusion: Aberration and amplification, which are being studied as the next step to understanding the resistance mechanisms and achieving better responses.[34,35]

Alternatively, primary resistance to FGFR inhibitors can be attributed to the presence of co-mutations, as observed in urothelial and CCA cancer models, where *FGFR2* and *KRAS* co-mutations lead to a more aggressive and undifferentiated phenotype that is resistant to FGFR inhibitors *in vitro*.[36] In the FIGHT-202 trial with pemigatinib, the post-hoc analysis revealed that CCA patients with a co-mutation in *BAP-1*, *CDKN2A/B*, *TP53*, and *PBRM1* had a shorter PFS and response rate.[37,38]

Preclinical evidence also suggests the role of the *EGFR* pathway in the resistance to FGFR inhibitors. A translational study with gastric cancer cell lines exposed to *FGFR3* inhibition with AZD4547 expressed an upregulation of the *EGFR* pathway, which subsequently became the predominant signaling pathway. This is not a direct interaction; rather, it appears that downregulation of FGFR signaling facilitates the phosphorylation and consequent activation of the EGFR pathway.[39]

5. Secondary or acquired resistance

5.1. On-target resistance

The activity of FGFR inhibitors depends on the binding to the ATP-binding pocket region on the tyrosine kinase domain; this region is called the "gatekeeper," as any structural change can reduce the specificity and activity of the FGFR inhibitors. In the case of the FGFR protein, the main gatekeeper is a valine residue, which is prone to secondary mutations after being exposed to continuous FGFR inhibition.[40,41] Initial studies in CCA performed biopsies at the time during FGFR inhibitor treatment. The principal mutations detected – either by liquid biopsy or tissue analysis—were: V561M for *FGFR1*, V564F/V564M for *FGFR2*, V555M for *FGFR3*, and V560L for *FGFR4*. All of these mutations affect the gatekeeper domain of the FGFR protein and confer resistance to first-generation tyrosine kinase inhibitors (TKIs).[42,43]

An infigratinib phase II trial with CCA patients expressing the *FGFR2* mutation revealed that the main resistance mutation found in the gatekeeper domain at the time of progression was V546F. There were other *FGFR* mutations outside of the gatekeeper domain, but their significance remains unknown, suggesting the heterogeneity of the resistance mechanisms.[44]

Another study reported that pemigatinib could overcome gatekeeper resistance only in *FGFR1–3*-mutated cell lines that develop the V564I mutation, but it was not effective in cases with *FGFR4* mutations or other

Table 1. Phase II trials with FGFR inhibitors in the second-line treatment of advanced CCA

Author, year	Drug	ORR (%)	mPFS (months)	Phase 3 trial	References
Abou-Alfa, 2020	Pemigatinib	37	7	FIGHT-302 (ongoing)	26
Javle, 2021	Derazatinib	21	7.3	No	27
Javle, 2021	Infigratinib	23	6.7	PROOF-301 (closed due to poor recruitment)	28
Xu, 2023	HMPL-453*	50	5.7	No	29
Guo, 2023	Gunagritinib*	52.9	6.9	No	30
Goyal, 2023	Futibatinib	42	9.7	FOENIX-CCA3 (closed due to poor recruitment)	31
Pant, 2023	Erdafetinib	60	8.4	No	32
Furuse, 2024	Tasurgatinib*	30	5.4	No	33

Note: *Only tested in an Asian population.
Abbreviations: CCA: Cholangiocarcinoma; ORR: Objective response rate; mPFS: Median progression-free survival; FGFR: Fibroblast growth factor receptor.

gatekeeper mutations.[45] Erdafetinib and infigratinib have similar molecular structures in their binding site compared to pemigatinib, and preclinical models using cell lines from various solid tumors, including CCA, have demonstrated that both agents can overcome gatekeeper resistance only in cases of *FGFR1* with V561I mutation.[46]

5.2. Off-target resistance

5.2.1. Activation of alternate receptors and intracellular pathways

Continuous FGFR inhibition can lead to the upregulation of alternative receptors in tumor cells, resulting in reduced dependence on the FGFR signaling pathway. This upregulation has been observed in preclinical models of CCA, lung, and gastric cancer cell lines; some examples of alternate pathways include *MET, Eph3B, ERBB2/3,* and *EGFR*.[47] The activation of these pathways depends on the type of FGFR alteration. For instance, the upregulation of *ERBB2/3* and *EGFR* is predominantly associated with *FGFR3* alterations, as observed in a preclinical study with urothelial carcinoma cell lines.[48] Preclinical models in solid tumors, including CCA, using RNA interference screens suggest that the *EGFR* pathway gains function after continuous *FGFR* inhibition, leading to an increase in the activity of *RAS/MEK* intracellular signaling. Interestingly, the EGFR gain-of-function is due to gene expression modulation rather than gene amplification.[49,50]

The intracellular PI3K/AKT/mTOR signaling pathway can also be upregulated after *FGFR* inhibition, as it can also be activated by other ligands, like the hepatocyte growth factor, which binds to the c-MET receptor in the tumor cells, resulting in CCA cell proliferation.[51] This was also observed in lung and urothelial cancer cell lines with *FGFR1* amplification and *FGFR3* fusions exposed to infigratinib.[52]

A similar mechanism was reported in preclinical studies with gastric cancer cell lines, where the constitutive

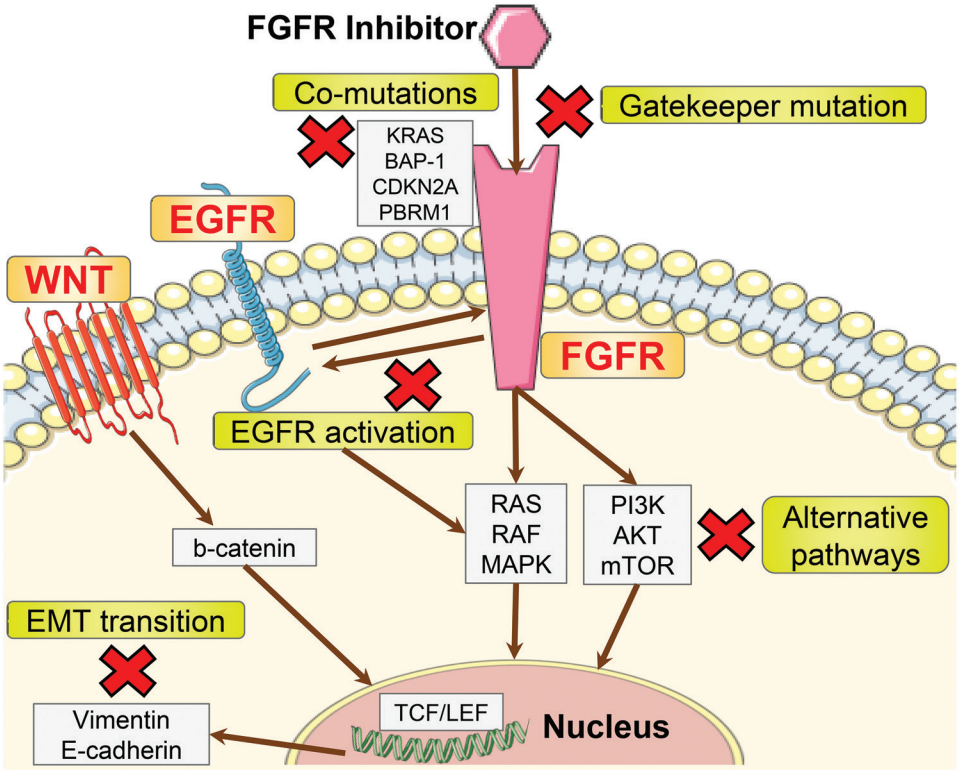

Figure 1. Resistance mechanisms to FGFR inhibitors. Primary resistance is mediated by co-mutations of BAP-1, CDKN2, and PBRM1. Secondary resistance is divided into: (i) On-target mechanism/gatekeeper mutations in the ATP-binding pocket and (ii) off-target mechanisms, including the activation of the PI3K/AKT/mTOR, EGFR/RAS, and MAPK pathways. EMT is mediated by the WNT/b-catenin pathway that interacts with the transcriptional factor TCF/LEF, leading to an increase in vimentin and a decrease in E-cadherin. This Figure was created using Power Point 2021.
Abbreviations: FGFR: Fibroblast growth factor receptor; BAP-1: BRCA-associated protein 1; CDKN2: Cyclin-dependent kinase inhibitor 2; PBRM1: Protein polybromo 1; EGFR: Epidermal growth factor receptor; RAS: Rat-sarcoma; RAF: Rapidly accelerated fibrosarcoma; MAPK: Mitogen-activated protein kinase; PI3K: Phosphoinositide 3 kinase; AKT: Serine/threonine kinase; mTOR: Mammalian target of rapamycin; WNT: Wingless-related integration site; TCF/LEF: T-cell factor/lymphoid enhancer factor; EMT: Epithelial-mesenchymal transition.

activation of the MAPK pathway in *FGFR1*- and *FGFR2*-amplified tumors could be mediated by a secondary mutation in *NRAS* or *BRAF* genes.[53,54] A retrospective study analyzing plasma and tissue samples from participants from the phase I trial of futinatinib in CCA (including mostly *FGFR2* fusions) demonstrated that in almost half of the patients, diverse point mutations in *BRAF*, *NRAS*, and *KRAS* genes were responsible for resistance to the FGFR inhibitor.[55]

In other gastrointestinal malignancies, such as gastric cancer, inactivation of the GSK3B pathway in *FGFR2*-amplified cell lines has been reported to contribute to resistance to the selective FGFR1–3 inhibitor AZD4547.[56]

5.2.2. Epithelial-mesenchymal transition

The chronic inhibition of an oncogenic driver, in this case *FGFR*, can lead to morphological changes in the epithelial cancer cells, transforming them into a mesenchymal phenotype.[57] This phenomenon is reported in various cancer types and is related to the activation of the Wnt/beta-catenin pathway that causes epigenetic changes in the tumoral cell DNA, leading to the activation of transcription factors TCF/LEF, which upregulate vimentin production and downregulate E-cadherin. In various gastrointestinal malignancies, these changes in the microfilament distribution cause the transition to a mesenchymal phenotype. Additionally, this transition appears to be more related to the inhibition of *FGFR1* and *FGFR4* and is less commonly associated with *FGFR2* alterations.[58,59] A summary of resistance mechanisms is described in Figure 1.

6. Strategies to overcome resistance

Most strategies to overcome resistance remain in the preclinical or *in vitro* stage. Currently, the most clinically relevant approach is the use of multi- or pan-FGFR inhibitors (Table 2), as the most common mechanisms of resistance are gatekeeper mutations. This approach is currently being evaluated in ongoing clinical trials using TKIs in advanced solid tumors with actionable mutations, including CCA.[79]

Table 2. Proposed methods to overcome resistance to FGFR inhibitors

Method	Description	References
Combination with AKT inhibitors	Inhibition of the AKT pathway with small interfering RNA (siRNA) on pan-AKT inhibitors (e.g., GSK2141795); restored sensitivity to infigratinib in cell lines of various solid tumors harboring FGFR amplification and fusions	52
Combination with mTOR inhibitors	In cell lines from patients with progression to FGFR inhibitors, the use of the potent mTOR inhibitor sapanisertib could resensitize the tumor	60
Combination with immune checkpoint inhibitors	Results from an FGFR-2-driven mouse model suggest that TKI drugs reduce the regulatory T-cell density and increase the cytotoxic T-cell infiltration, enhancing the immune responsiveness of the tumor; this synergism seems to be dependent on the previous sensitivity to the anti-FGFR drug, as it was not seen in the case of primary resistance	61
	A Chinese study reported high levels of PDL1 expression in FGFR2-rearranged CCA, but this data can be biased by the high prevalence of viral hepatitis in Asian regions.	62
	An American retrospective study reported low levels of PDL1 expression in patients with FGFR2-rearranged CCA	63
Dual warhead covalent inhibition	Increasing the specificity of the drug with a double binding site on the FGFR structure.	64
	The CXF-009 molecule binds two serine residues on FGFR4 (Cys477 present in all FGFR subtypes in the ATP-binding pocket) and Cys522 (present in FGFR4); this technique makes it possible to have a highly specific active treatment in FGFR-related tumors	65
Dual inhibition of FGFR and VEGF	Preclinical studies in FGFR-driven solid tumors have demonstrated the intercorrelation of the FGFR and the VEGF pathways FGFR activation is a mechanism of resistance of antiangiogenics drugs; dual simultaneous inhibition could lead to more durable and synergic responses	66
Dual inhibition of FGFR and EGFR	EGFR inhibitor Erlotinib, either alone or in combination with chemotherapy and antiangiogenics, had negative results in unselected advanced CCA patients from previous trials	67-69
	Preclinical models in CCA cell lines with FGFR2 fusions demonstrated that the inhibition of EGFR signaling could potentiate the efficacy of FGFR inhibitors and reduce adaptive resistance	70
	A preclinical study demonstrated *in vitro* anti-tumor activity with a new pan-FGFR inhibitor (PD173074) in CCA cell lines with FGFR alterations; a synergistic effect was observed with the addition of Erlotinib (first-generation EGFR inhibitor)	71

(Cont'd...)

Table 2. ***(Continued)***

Method	Description	References
Dual inhibition of FGFR and RAS/BRAF/MEK	A preclinical study involving CCA cell lines with FGFR2 fusions was exposed to the FGFR inhibitor futibatinib and transduced with KRAS G12D and BRAF V600E gene mutations, followed by MEK inhibition; the *in vitro* results were positive, but the in vivo xenograft models did not achieve tumor regression	55
	A preclinical study using CCA cell lines with FGFR2 fusions displayed positive *in vitro* results with the combination of infigratinib with an MEK inhibitor	72
	A phase 1 trial using the combination of futibatinib and binimetinib in advanced solid tumors was terminated by the decision of the sponsor	73
Multi-FGFR inhibition	Inhibition of multiple FGFRs did not induce conformational change in the ATP-binding pocket, keeping the drug far from the gatekeeper resistance mutation region and the substrate-binding pocket	40
	In the Asian population, the FGFR1–3 inhibitor tasugratinib (E7090) displayed promising antitumor activity in solid tumors, including CCA, in a phase 1 trial	74
	A phase 2 trial in an Asian population, only including CCA with FGFR2 fusions, also demonstrated the antitumor activity of tasugratinib with an ORR of 30%, with a manageable safety profile	33
	In an occidental population, a preclinical study demonstrated the efficacy of the FGFR1–3 inhibitor KIN-3248 in overcoming the main gatekeeper mutations in CCA cell lines	75
Pan-FGFR inhibition	The first *in vitro* evidence of inhibition of all FGFRs to overcome gatekeeper resistance was the drug LY2874455, which exhibited antitumor activity in advanced solid tumors containing FGFR alterations; however, the drug was discontinued	76,77
	The pan-FGFR inhibitor futibatinib demonstrated clinical efficacy in patients previously treated with first-generation FGFR inhibitors in CCA cell lines	78
	A phase 2 trial with futibatinib reported a greater inhibition compared to first-generation FGFR inhibitors	31

Abbreviation: FGFR: Fibroblast growth factor receptor.

7. Conclusion

As a second-line treatment option, FGFR inhibition has become an established target therapy for advanced FGFR-positive CCA. FGFR inhibitors have a good response rate; however, resistance typically develops after approximately 6 months of treatment in most prospective trials.

Primary resistance is less common and is related to co-mutations in other receptors, such as the EGFR pathway. The mechanisms of secondary resistance are multifactorial; the most common are secondary gatekeeper mutations. Other mechanisms include the activation of parallel intracellular signaling pathways and epithelial-mesenchymal transition.

Various strategies to overcome this resistance are in development in order to preserve the efficacy of targeted therapies for advanced CCA patients. These include combinations with other antineoplastic drugs that have mechanisms of action different from FGFR inhibition, as well as the development of more specific and potent drugs targeting the FGFR pathway.

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