Intrahepatic Cholangiocarcinoma: State of the Art of FGFR Inhibitors

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Abstract

Objective: Intrahepatic cholangiocarcinoma (iCCA), the second most common type of primary liver tumor, has an increasing incidence in the past few decades. iCCA is highly malignant, with a 5-year survival rate of approximately 5-10%. Surgical resection is usually the prescribed treatment for patients with early stage iCCA; however, patients are usually in an advanced stage iCCA upon diagnosis. Currently, targeted therapy combined with chemotherapy and other comprehensive treatment measures have been mainly adopted as palliative treatment measures. As a common candidate of targeted therapy, FGFR inhibitors have demonstrated their unique advantages in clinical trials. At present, the prospect of FGFR targeted therapy is encouraging. The landscape of FGFR inhibitors in iCCA is needed to be showed urgently.

Methods: We searched relative reports of clinical trials on FGFR inhibitors in PubMed as well as Web of Science. We also concluded other available clinical trials of FGFR inhibitors (Data were collected from clinicaltrials.gov).

Results: Several relatively effective targeted drugs are being used in clinical trials. Some preliminary results indicate the outlook of targeted therapy such as BGJ398, TAS120, and HSP90 inhibitors.

Conclusions: In summary, FGFR targeted therapy has broad prospects for the treatment of iCCA.

Keywords
intrahepatic cholangiocarcinoma (iCCA), fibroblast growth factor receptor (FGFR), cholangiocarcinoma (CCA), BGJ398, pemigatinib

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Introduction

Primary liver tumors mainly consist of hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA).1 As the second most common primary liver tumor, iCCA can also be classified as a type of cholangiocarcinoma.2 Intrahepatic cholangiocarcinoma (iCCA) accounts for 5-30% of primary liver tumors, and its morbidity has been observed to have an upward trend in the past few decades.3,4 This may be due to the increasing clinical diagnosis of iCCA, which leads to the increasing incidence of iCCA.5,6 However, this explanation alone is insufficient as Klatskin tumor, which was previously...
classified as an intrahepatic tumor, is now classified as a type of extrahepatic cholangiocarcinoma.5

iCCA has a high degree of malignancy and a low 5-year survival rate of approximately 5-10%.8 Surgical resection is usually prescribed for patients with early stage iCCA, but only less than 30% of the patients achieve negative tumor margins.9

Moreover, most patients already have an advanced stage iCCA upon diagnosis.

For the treatment of iCCA at such stage, targeted therapy combined with chemotherapy and other treatment measures are mainly adopted.2,10 Systemic chemotherapy with gemcitabine and cisplatin is the standard of care for patients with advanced biliary cancer.10 Similar to hepatocellular carcinoma, iCCA has a metastatic predilection for the liver and, therefore, locoregional therapy may be a reasonable palliative approach such as radiofrequency ablation,11,12 transarterial chemoembolization (TACE),13 and selective intra-arterial radiotherapy with radioactive 90Y.14 However, all of these approaches have been found to have limitations in clinical studies. Radiofrequency ablation may provide poor local tumor control in patients with iCCA of large (> 5 cm) diameter.15 A retrospective study conducted by Brandi and colleagues analyzed 29 patients with unresectable iCCA.16 All these patients were treated with radiofrequency ablation. Interestingly, statistical analysis revealed that tumor size larger than 2 cm was significantly associated with poorer PFS (the median PFS in this study was 9.27 months). They suggested 2 cm to be a threshold value for radiofrequency ablation for the first time.16 Another retrospective study on 27 iCCA patients enrolled reported the similar result. The iCCA patients with single tumor(< 2 cm) could have an encouraging long-term survival after thermal ablation,17 though recurrence rate was 77.8% (21 patients). 10 patients with single tumor(< 2 cm) got the OS of 94.5 months, which was significantly different from patients with single tumor(>2 cm).17 Department of Exp TACE is a palliative and safe treatment option for patients with unresectable iCCA.18 Selective intra-arterial radiotherapy with radioactive 90Y is associated with a high rate of treatment-related complications, including acute radiation-induced liver dysfunction, biliary strictures, and gastrointestinal mucosa damage.19,20 According to published treatment outcomes, liver transplantation for cholangiocarcinoma has historically been contraindicated given its high recurrence rates.21 Patients with cholangiocarcinoma also present a diagnostic challenge that often leads to the detection of more aggressive lesions on explants after liver transplantation higher recurrence rates, and worse post-liver transplantation survival.22 Liver transplantation can be a curative option for selected patients with perihilar cholangiocarcinoma as reported in a multicenter study in USA.23 Another large multicenter study has also confirmed that patients who are diagnosed with very early iCCA at explant pathology after liver transplantation tend to have an acceptable 5-year survival and a low recurrence rate.24 However, all these studies apply to only a small proportion of patients with iCCA. Among all kinds of molecular therapeutic targets for iCCA, FGFR (fibroblast growth factor receptor) and IDH (isocitrate dehydrogenase) have attracted the attention of researchers. Approximately 10-28% of iCCA tumors are characterized by IDH genetic mutations; this is higher than the occurrence of iCCA tumors with FGFR mutations (7-14%).25 Promising trial reports regarding the use of FGFR inhibitors have supported the potential roles of FGFR inhibitors in the treatment of iCCA.25 As a known candidate for targeted therapy, FGFR has demonstrated its unique advantages in some clinical trials.26 This review focuses on the brief pathogenesis of iCCA, the molecular correlation between FGFR and iCCA, and the current position and progress of targeted therapy of FGFR to treat iCCA. In addition, we specially described the application of circulating tumor DNA (ctDNA) detection on resistance during FGFR inhibitors’ therapy.

Pathogenesis of iCCA

There are many risk factors for cholangiocarcinoma: cholestatic liver diseases such as primary sclerosing cholangitis,27 liver cirrhosis, biliary calculus diseases, and certain bacterial, viral, or parasitic infections, including hepatitis B and C, and liver fluke disease.28 Most of these risk factors can cause chronic inflammation, cholestasis, or both and lead to the activation of intracellular pathways that result in cell matrix changes, gene mutation, chromosome aberration, epigenetic and miRNA changes, and ultimately iCCA occurrence.29,30 Currently, some signal molecule mutations are often detected in iCCA: fibroblast growth factor receptor (FGFR) mutation, isocitrate dehydrogenase gene (IDH (IDH 1/2) mutation, and BAP1 (a gene involved in chromatin remodeling) mutation. Some clinical research on IDH and FGFR-related mutations have achieved encouraging results and entered clinical trial stages.31

Molecular Correlation Between FGFR and iCCA

Research Progress on FGFR:

FGFR belongs to the tyrosine kinase receptor family. This receptor consists of 5 members (FGFR1-5).32 Extracellular FGFR is ubiquitous in humans and participates in various cellular pathways such as organ formation during embryonic development glycolipid metabolism, angiogenesis and migration, tissue repair, and regeneration.31,33,34 The binding of ligands to the extracellular domain of FGFR induces the dimerization of FGFR. which in turn allosterically activates the receptor tyrosine kinase domain (TKD) and triggers a series of downstream signaling. In this biological process, heparin cofactor is considered a bridge that connects 2 FGFRs and plays a key role in the dimerization of FGFR and FGFR.3,35

Connection Between FGFR and iCCA

Understanding the key signaling pathways and genetic changes involved in iCCA is essential in identifying new drug targets.
The FGF-FGFR signaling pathway is closely linked to a series of biological activities such as cell proliferation, apoptosis, and migration; therefore, when the key points of this signaling pathway are abnormal, they may lead to the occurrence of tumors. FGFR signals can be abnormally activated in tumor cells in several ways: amplification, translocation, fusion, or mutation of the genes of FGFR family members. Moreover, the overexpression of FGFR is attributable to changes or aberrations in the non-coding regions of epigenetic and/or transcriptional regulatory factors, or to the upregulation of FGF ligands in the tumor microenvironment via tumor-matrix interaction. Studies have found that high-level focal amplification of FGF19 and FGFR2 gene fusion occurs in approximately 5% of liver cancers, preferentially in HCC and iCCA. Many studies have revealed that approximately 15% of iCCA patients harbor mutations of FGFR2, whereas mutations in FGFR1 and FGFR3 can also be detected in some iCCA patients. These findings open a new door to the development of targeted therapy. Many progressive fusion targets have been found between iCCA and FGFR: FGFR2-BICC1, FGFR2-AHCYL1, FGFR2-MGEA5, FGFR2-TACC3, FGFR2-KIAA1598, FGFR2-CREB5, FGFR2-KIAA1967, FGFR2-CCDC6, FGFR2-AFF3, FGFR2-CASP7, FGFR2-OFD1, SLC45A3-FGFR2.

**FGFR Targeted Therapy for iCCA**

**Current Situation of FGFR Targeted Therapy**

FGFR clinical progress and outcomes of targeted treatment of iCCA. Currently, the first-line treatment for iCCA is the use cisplatin and gemcitabine combined with other chemotherapies, whereas a second-line treatment standard remains unestablished. A clinical phase II study report (NCT00262769) revealed the advantages of cisplatin/gemcitabine combination therapy, leading to the application in clinical therapy. Notwithstanding, the median OS after this treatment remains to be less than a year, and the 5-year survival rate is approximately 5%. This may be attributed to the advanced stage of the tumor upon diagnosis or progression on gemcitabine-based chemotherapy in some patients; the efficacy of this classic treatment scheme in CCA is beyond doubt. In addition, some targeted inhibitors are still in clinical trials; these inhibitors include targeting on FGFR, IDH1/2, HER2, and EGFR. And the characteristics of the clinical effects of some of them have demonstrated. The inhibitors that have been reported to have therapeutic effects include BGJ398, pemigatinib, ARQ-087, heat shock protein-HSP90 inhibitor. We summarized these effective results of clinical trials of FGFR inhibitors in Table 1. We also provided other available clinical trials of FGFR Inhibitors in Table 2 (The data comes from ClinicalTrials.gov).

**BGJ398 (Infigratinib).** BGJ398 is a pan-FGFR tyrosine kinase inhibitor. Recently, a phase II study of BGJ398 (NCT02150967) conducted in the United States showed encouraging results. Sixty-one patients with advanced or metastatic cholangiocarcinoma containing FGFR2 fusion (n = 48), mutation (n = 8), or amplification (n = 3) after ineffective first-line treatment were selected. The number of patients with iCCA was not specified. The results showed that the overall response rate (ORR) was 14.8%, (disease control rate) DCR 75.4%, and the median PFS was estimated to be 5.8 months (95% CI: 4.04-9.2 months). Adverse events include hyperphosphatemia (72.1%), fatigue (36.1%), stomatitis (29.5%), and alopecia (26.2%). As FGFR pathway signals is essential in FG23-mediated phosphate homeostasis, hyperphosphatemia is considered to have targeted therapeutic effects. The toxicity of the drug was reported to be within a controllable range.

These favorable results from this study have led to the further investigation of BGJ398 as a Phase III multicenter, open label, randomized trial (NCT03773302). Recently, Japanese scholars reported a case of drug resistance to the FGFR targeting inhibitor BGJ398 in 3 patients. Through a series of cfDNA analyses, they found many repeated point mutations in the FGFR2 kinase region during progression. Each mutation leads to the augmentation of drug resistance. This discovery may provide insight regarding future strategy selection for FGFR target inhibitor therapy.

**Derazantinib (ARQ 087).** ARQ 087 is an oral bioavailable multi-kinase inhibitor with strong pan-FGFR activity; it exerts strong effects on FGFR2, FGFR1, and FGFR3 kinases. Mazzaferro et al. completed a multi-center, 1/2-phase, open-label study in which 29 iCCA patients harboring FGFR2 fusion in the United States and Italy were included. Among the patients, 27 patients progressed after at least 1 prior systemic therapy and were not tolerant or suitable for first-line chemotherapy, whereas only 2 patients were treatment-naive. Based on the conclusion of Phase I, a recommended phase II dose of 300 mg of ARQ 087 was reported. The ORR was 20.7%, and the DCR was 82.8%. The median PFS was estimated to be 5.7 months (95% CI: 4.04-9.2 months). The regression of tumors was observed in 19 patients. Treatment-related adverse events were observed in 27 patients (93.1%), and those included weakness/fatigue (69.0%), eye toxicity (41.4%), and hyperphosphatemia (75.9%). However, in the 27 patients, compared with their first-line chemotherapy, the duration of treatment with derazantinib was not significantly prolonged, and promising results were observed. Currently, a targeted phase II trial is underway (NCT03230318).

**JNJ-42756493 (Erdafitinib).** JNJ-42756493 is an oral tyrosine kinase inhibitor of the FGFR. To evaluate its safety, pharmakokinetics, and pharmacodynamics, a non-randomized, open-label, multi-center phase I clinical trial study (NCT01703481) was conducted by Tabernero et al. who mainly targeted patients with advanced malignant solid tumors such as lung cancer and breast cancer. The RP2D determined in the study was the administration of 10 mg for 7 days followed by another 7 days but without treatment; its therapeutic strategy attracted further research. They reported that 187 patients participated in the trial, including 11 patients with cholangiocarcinoma. Interestingly, cholangiocarcinoma and urothelial carcinoma were most responsive to erdafitinib, with an objective response...
rates (ORR) of 27.3% (3/11) and 46.2% (12/26), both of which were detected as FGFR mutations or fusions. For all patients, the most common treatment-related AEs were hyperphosphatemia (64%), dry mouth (42%), and asthenia (28%). In another report of a phase IIa study of erdafitinib in advanced CCA patients with FGFR alterations (NCT02699606), 34 patients with CCA (15.3% of 222) with FGFR alterations were detected, 14 among whom were treated with 8 mg once daily. 13/14 and 12/14 patients received prior platinum- or gemcitabine-based therapy, respectively. In 12 patients, 6 patients had partial response (PR), 4 had stable disease (SD), and 2 had progressive disease (PD). The median PFS was 5.59 months (95% CI: 1.87, 13.67). In 10 evaluable patients with FGFR2 fusions and mutations, ORR (CR+PR) was 6/12 (50.0%) as well as DCR 10/10 (100.0%). No adverse complications emerged in the report. Nonetheless, the curative effect erdafitinib on iCCA requires further research.

**Ponatinib.** As a pan-FGFR inhibitor, ponatinib has gained attention owing to its remarkable curative effect not only on breast cancer, lung cancer, and genital system tumors, but also on leukemia. In a patient with FGFR gene translocation harboring FGFR2- tacc3 fusion, it is the preliminary antitumor activity of pazopanib was identified. However, the patient experienced stable disease (SD) after treatment with ponatinib subsequent to the onset the diminishing effects of pazopanib. In another patient with FGFR2-MGEA5 fusion, gemcitabine and cisplatin were administered during the first 6 months; then, cisplatin was replaced with capecitabine. However, the effects of all these substances began diminishing after another 6 months. The presence of a fusion gene was identified by using gene analysis. After 6 weeks of rescue therapy with ponatinib monotherapy, the condition of the latter patient remained stable, and the sum of the maximum diameters of tumors decreased by 14% and the CA19-9 tumor markers by 89.8%. Based on these findings, many clinical trials on ponatinib have been started, and several studies on solid tumors including iCCA are currently underway (NCT02272998; NCT02265341). NCT02265341 has already been completed with 12 patients enrolled, who were diagnosed with advanced biliary cancer harboring FGFR2 fusions.

**TAS-120 (futibatinib).** TAS-120, an irreversible pan-FGFR inhibitor, has demonstrated promising therapeutic effects in patients with drug resistance to BGJ398 or Debio 1347. Recently, the Goyal doctors’ team reported the therapeutic effects of TAS-120 in 4 patients with positive iCCA harboring FGFR2 fusion. Examination of a series of biopsy sections, circulating tumor DNA (ctDNA), and iCCA cells from patients was performed, and the frequency of mutant alleles in several patients decreased after treatment with TAS-120, thereby indicating that TAS-120 exerts effects on these alleles. Recently, Bahleda et al. reported the latest results of a phase I study of futibatinib on advanced solid tumors. 71 patients (83% of 86) with tumors harboring FG/FGR aberrations were divided into 2 groups: one group received 8-200 mg futibatinib 3 times a week, and the other group 4-24 mg once daily. Across the cohorts, 5 patients experienced an overall response of confirmed PR, and 41 (21 with TIW and 20 with QD dosing) experienced stable disease. 18 patients (75% of 24) with CCA experienced PR or SD, which pronounced the particular anti-tumor activity of futibatinib in iCCA. The most common treatment-emergent AEs were hyperphosphatemia (59%), diarrhea (37%), and constipation (34%). Based on the recommended phase II dose of 20 mg, futibatinib is currently being investigated in ongoing phase 2/3 trials in patients with advanced cancers harboring FGFR aberrations. Another phase III trial of futibatinib (NCT04093362) for patients with iCCA is also ongoing.

**Debio 1347/CH5183284.** Debio 1347 is an oral and selective inhibitor of FGFR 1-3. It showed anti-tumor activity against cancer cells of a mouse containing FGFR aberrations in both in vivo and in vitro experiments. Recently, a phase I trial of Debio 1347 on advanced solid tumor patients with FGFR1-3 alterations was conducted by Voss et al. 71 patients were screened and 58 were enrolled in the trial, including 8 (14%) patients with CCA. Debio 1347 was administered at a dose of 10-150 mg/day, and 5 among them were diagnosed with breast cancer or biliary tract cancer and have 6 dose-limiting toxicities (dry mouth/eye, hyperamylasemia, hypercalcemia, hyperbilirubinemia, hyperphosphatemia and stomatitis) at 3 dose levels. 52% of the patients experienced AEs, mainly owing to dose dependence and asymptomatic hyperphosphatemia (22%), thereby requiring dose adjustment. Six patients, including 3 with FGFR fusions, experienced PR. Interestingly, a change in response from PR to CR was observed in only 1 patient with CCA. Tumor sizes in another 10 patients regressed by 30%, and the maximum tolerated dose was 80 mg/d. The toxicity of Debio 1347 remains under control and the curative effect is encouraging. Based on these achievements, the next phase of clinical research would continue.

**INCB054828 (pemigatinib).** The FDA approved INCB054828 as the first targeted therapy for second-line treatment in locally advanced and metastatic CCAs harboring FGFR2 fusions or rearrangements. INCB054828 is a selective FGFR1-3 inhibitor. Recently, Krook et al. found that iCCA developed in a patient after using the first-line chemotherapy scheme. After that, an FGFR2-CLIP1 fusion in the patient was found by using gene analysis; INCB054828 treatment was conducted by administering 13.5 mg INCB054828 per day for 14 days per 21 days. According to the Response Evaluation Criteria in Solid Tumors (RECIST) standard, the disease assessment after the 3rd and 6th cycles indicated strong partial responses. Two target lesions (posterior hepatic dome lesion and left hepatic lobe lesion) were tracked during the whole treatment process, which were 34.8% and 46.5% lower than baseline, respectively, after the 3rd and 6th cycles. However, after a total of 5 months (7 cycles) of treatment with INCB054828, CT scans indicated a 41.3% increase in the size of the 2 target lesions, thereby confirming the progression of the disease. The heterogeneity of the tumor and the further development of drug resistance by a secondary mutation in FGFR was determined.
through a final autopsy.\(^67\) Most recently, an inspiring clinical trial report of INCB054828 for CCA uncovered another window for iCCA treatment.\(^68\) This phase II trial focused on patients with advanced or metastatic cholangiocarcinoma and 146 patients, including 107 with FGFR2 fusions or rearrangements were enrolled. The median follow-up period was 17.8 months. The OR was 38 (35.5%), including 3 complete responses (CR). Moreover, hyperphosphatemia was the most common all-grade adverse event (observed in 88 [60\%] among 146 patients). Notably, no patients with other FGFR/FGFR alterations or no FGFR/FGFR alterations achieved a response, and overall survival and PFS remained poor in these cohorts. Based on the findings from this study, an international, phase III, randomized, active-controlled trial was opened and is currently recruiting patients to compare the treatment with INCB054828 against treatment with gemcitabine and cisplatin chemotherapy as first-line therapy for patients with unresectable or metastatic cholangiocarcinoma with FGFR2 rearrangements (NCT03656536).

**HSP90 inhibitor.** Having been proven a partner of the FGFR family, HSP90 helps in the folding and protein packaging of FGFR. Once it is inhibited, the production of downstream proteins would be reduced. Interestingly, HSP90 is upregulated in 44.6\% iCCA and 32.8\% extrahepatic CCA, especially in poorly differentiated iCCA, where it has been reported to have a high expression.\(^69\) Such a situation provides a possible theoretical basis for the targeted treatment of FGFR. Test data indicated that the combination of BGJ398 and ganetespib (an HSP90 inhibitor) as a treatment is superior monotherapies, either in vivo and in vitro.\(^70\) The analysis revealed that the addition of Akt inhibitor with BGJ398, the phosphorylation levels of Akt (T308 and S473) in 2 agent-resistant CCA cell lines to BGJ398, thereby indicating that the Akt pathway plays a role in mediating acquired resistance to FGFR inhibition.\(^71\) This finding also reveals a possible mechanism of drug resistance for FGFR inhibitors.

**Drug resistance mechanism of FGFR targeted therapy.** After treatment with FGFR inhibitors, a large number of secondary mutations in FGFR or stimulations of other signaling pathways have been identified in patients. These secondary mutations lead to drug resistance.

**Heterogeneity of iCCA may refer to the resistance of FGFR targeted therapy.** The heterogeneity of iCCA indicates drug resistance. Reasons related to heterogeneity include changes in genetic and epigenetic mechanisms and tumor microenvironment.\(^81\)\(^82\) Due to the presence of multiple subclones in tumors or clonal evolution during treatment, the presence of independent FGFR clones may lead to the failure of targeted FGFR therapy for patients with HCC or iCCA.\(^83\)

**Abnormal activation of other pathways result in the resistance against FGFR targeted therapy.** In an experiment of drug resistance of cell lines with FGFR fusion/amplification mutation to BGJ398, the phosphorylation levels of Akt (T308 and S473) and its downstream target GSK3 (S9 and S21) in 2 agent-resistant cell lines were reportedly increased with the use of reverse-phase protein array (RPPA) analysis. The results of RPPA analysis were further confirmed by western blotting.\(^84\) The analysis revealed that the addition of Akt inhibitor (GSK2141795) or siRNA can restore the sensitivity of cell lines to BGJ398, thereby indicating that the Akt pathway plays a role in mediating acquired resistance to FGFR inhibition.\(^85\)

**Secondary mutation in FGFR2 kinase domain results in acquired resistance.** Secondary mutations in FGFR are the most common causes of resistance against therapeutic agents.\(^50\) Acquired
| Agents          | NCT Number       | Function                  | Phase | Number | Outcomes n (%)       | Adverse Effects n (%)                                                                 | Other active Clinical Trials |
|-----------------|------------------|---------------------------|-------|--------|----------------------|---------------------------------------------------------------------------------------|-----------------------------|
| BGJ398 (infigratinib) | NCT02150967     | pan-FGFR inhibitor (1-3)  | II    | n = 61 | CR 0                 | hyperphosphatemia 44 (72.1), fatigue 22 (36.1), stomatitis 18 (29.5), alopecia 16 (26.2) | NCT03773302                 |
|                 |                  |                           |       |        | PR 9 (14.8)          |                                                                                        | NCT04233567                 |
|                 |                  |                           |       |        | SD 37 (60.7)         |                                                                                        | NCT04424966                 |
|                 |                  |                           |       |        | PD 11 (18.0)         |                                                                                        |                             |
|                 |                  |                           |       |        | ORR 9 (14.8)         |                                                                                        |                             |
|                 |                  |                           |       |        | DCR 46 (75.4)        |                                                                                        |                             |
|                 |                  |                           |       |        | PFS 5.8 months       |                                                                                        |                             |
| Derazantinib (ARQ 087) | NCT01752920     | pan-FGFR inhibitor       | III   | n = 29 | CR 0                 | weakness/fatigue 20 (69.0), eye toxicity 12 (41.4), hyperphosphatemia 22 (75.9)       | NCT03230318                 |
|                 |                  |                           |       |        | PR 6 (20.7)          |                                                                                        | NCT04087876                 |
|                 |                  |                           |       |        | SD 18 (62.1)         |                                                                                        |                             |
|                 |                  |                           |       |        | PD 5 (17.2)          |                                                                                        |                             |
|                 |                  |                           |       |        | ORR 6 (20.7)         |                                                                                        |                             |
|                 |                  |                           |       |        | DCR 24 (82.8)        |                                                                                        |                             |
|                 |                  |                           |       |        | PFS 5.7 months (95% CI: 4.0-9.2) |                                                                                        |                             |
| JNJ-42756493 (erdafitinib) | NCT02699606    | pan-FGFR inhibitor       | Ila   | n = 14 | CR 0                 | Hyperphosphatemia, dry mouth, stomatitis, dry skin (all > 30%)                         | NCT02699606                 |
|                 |                  |                           |       |        | PR 6 (50)            |                                                                                        | NCT03210714                 |
|                 |                  |                           |       |        | SD 4 (33.3)          |                                                                                        | NCT04083976                 |
|                 |                  |                           |       |        | PD 2 (16.7)          |                                                                                        | NCT02465060                 |
|                 |                  |                           |       |        | ORR 6 (50)           |                                                                                        |                             |
|                 |                  |                           |       |        | DCR 10 (83.3)        |                                                                                        |                             |
|                 |                  |                           |       |        | PFS 5.59 months (95% CI: 1.87, 13.67) |                                                                                        |                             |
| Ponatinib       | NCT02265341     | pan-FGFR inhibitor       | II    | n = 12 | –                   |                                                                                        | NCT02272998                 |
| TAS-120 (futibatinib) | NCT02052778     | pan-FGFR inhibitor       | I     | n = 86 | CR 0                 | hyperphosphatemia 51 (59), diarrhea 32 (37), constipation 29 (34)                    | NCT04093362                 |
|                 |                  |                           |       |        | PR 5 (5.8)           |                                                                                        | NCT04507503                 |
|                 |                  |                           |       |        | SD 41 (48)           |                                                                                        | NCT04189445                 |
|                 |                  |                           |       |        | PD 40 (46.5)         |                                                                                        |                             |
|                 |                  |                           |       |        | ORR 5 (5.8)          |                                                                                        |                             |
|                 |                  |                           |       |        | DCR 46 (53.5)        |                                                                                        |                             |
| Debio 1347 (CH5183284) | NCT01948297     | selective inhibitor of FGFR1-3 | I     | n = 58 | CR 1 (1.7)           | hyperphosphatemia, diarrhea, nausea, fatigue, constipation, decreased appetite, nail changes, dry mouth (all > 25%) | NCT03834220                 |
|                 |                  |                           |       |        | PR 5 (8.8)           |                                                                                        |                             |
|                 |                  |                           |       |        | SD 16 (28.1)         |                                                                                        |                             |
|                 |                  |                           |       |        | PD 35 (61.4)         |                                                                                        |                             |
|                 |                  |                           |       |        | ORR 6 (10.5)         |                                                                                        |                             |
|                 |                  |                           |       |        | DCR 22 (38.6)        |                                                                                        |                             |
| INCBO54828 (pemigatinib) | NCT02924376     | selective inhibitor of FGFR1-3 | II    | n = 146 | CR 3 (2.8)           | Hyperphosphatemia 81 (55), Alopecia 67 (46), Dysgeusia 55 (38), Diarhrea 49 (34)    | NCT03011372                 |
|                 |                  |                           |       |        | PR 35 (32.7)         |                                                                                        | NCT04003623                 |
|                 |                  |                           |       |        | SD 50 (46.7)         |                                                                                        | NCT03822117                 |
|                 |                  |                           |       |        | PD 16 (14.9)         |                                                                                        | NCT03656536                 |
|                 |                  |                           |       |        | ORR 38 (35.5)        |                                                                                        | NCT04096417                 |
|                 |                  |                           |       |        | DCR 88 (82.2)        |                                                                                        | NCT04258527                 |
|                 |                  |                           |       |        | PFS 6.9 months       | Dry mouth 42 (29%)                                                                     | NCT04088188                 |
| NCT Number | Agent | Title | Tumor | Phase or Study Type | Status |
|------------|-------|-------|-------|---------------------|--------|
| NCT03773302 | BGJ398 Gencitabine Cisplatin | Phase 3 Study of BGJ398 (Oral infgratinib) in first fine cholangiocarcinoma with FGFR2 gene fusions/translocations | Advanced Cholangiocarcinoma | III | Recruiting |
| NCT04233567 | BGJ398 | Infigratinib for the treatment of advanced or metastatic solid tumors in patients with FGFR gene mutations | Advanced, Metastatic, or Refractory Malignant Solid Neoplasm Cholangiocarcinoma | II | Recruiting |
| NCT03230318 | Derazantinib | Derazantinib in subjects with FGFR2 gene fusion-, mutation- or amplification-positive inoperable or advanced intrahepatic cholangiocarcinoma | Intrahepatic Cholangiocarcinoma Combined Hepatocellular and Cholangiocarcinoma | II | Recruiting |
| NCT02699606 | JNJ-42756493 | A study to evaluate the clinical efficacy of JNJ-42756493 (Erdafitinib), A pan-fibroblast growth factor receptor (FGFR) tyrosine kinase inhibitor, in Asian participants with advanced non-small-cell lung cancer, urothelial cancer, esophageal cancer or cholangiocarcinoma | Neoplasm | IIa | Active, not recruiting |
| NCT03210714 | JNJ-42756493 | Erdafitinib in treating patients with relapsed or refractory advanced solid tumors, non-Hodgkin lymphoma, or histiocytic disorders with FGFR mutations (a pediatric MATCH treatment trial) | Advanced Malignant Solid Neoplasm | II | Recruiting |
| NCT04083976 | JNJ-42756493 | A study of erdafitinib in participants with advanced solid tumors and fibroblast growth factor receptor (FGFR) gene alterations | Advanced Solid Tumor | II | Recruiting |
| NCT02465060 | JNJ-42756493 | Targeted therapy directed by genetic testing in treating patients with advanced refractory solid tumors, lymphomas, or multiple myeloma (the MATCH screening trial) | Advanced Malignant Solid Neoplasm | II | Recruiting |
| NCT02272998 | Ponatinib | Ponatinib for patients whose advanced solid tumour cancer has activating mutations involving the following genes: FGFR1, FGFR2, FGFR3, FGFR4, RET, KIT. | Malignant Neoplasm | II | Recruiting |
| NCT02265341 | Ponatinib | Ponatinib hydrochloride in treating patients with advanced biliary cancer with FGFR2 fusions | Malignant Hepatobiliary Neoplasm | II | Completed |
| NCT04093362 | TAS-120 Gencitabine Cisplatin | Futibatinib vs gencitabine-cisplatin chemotherapy as 1st-line treatment of patients with advanced cholangiocarcinoma (CCA) harboring FGFR2 gene rearrangements | Advanced Cholangiocarcinoma FGFR2 Gene Rearrangements | III | Not yet recruiting |
| NCT04507503 | TAS-120 | Expanded access study of tas-120 in patients with advanced cholangiocarcinoma harboring FGFR2 gene rearrangements | Advanced Cholangiocarcinoma | Expanded Access | Available |
| NCT04189445 | TAS-120 | Futibatinib in patients with specific FGFR aberrations | Advanced or Metastatic Solid Tumor | II | Not yet recruiting |
| NCT03834220 | Debio 1347 | Basket trial in solid tumors harboring a fusion of FGFR1, FGFR2 or FGFR3 (FUZE clinical trial) | Solid Tumor | II | Active, not recruiting |
| NCT04003623 | Pemigatinib | Efficacy and safety of pemigatinib in participants with solid tumors with FGFR mutations or translocations (FIGHT-208) | Advanced or Metastatic Solid Tumors | II | Recruiting |

(continued)
| NCT Number  | Agent       | Title                                                                 | Tumor                                      | Phase or Study Type | Status       |
|-------------|-------------|-----------------------------------------------------------------------|--------------------------------------------|---------------------|--------------|
| NCT03822117 | Pemigatinib | Efficacy and safety of pemigatinib in previously treated locally advanced/metastatic or surgically unresectable solid tumor malignancies harboring activating FGFR mutations or translocations (FIGHT-207) | Solid Tumor Malignancy                     | II                  | Recruiting   |
| NCT03656536 | Pemigatinib | A study to evaluate the efficacy and safety of pemigatinib versus chemotherapy in unresectable or metastatic cholangiocarcinoma - (FIGHT-302) | Unresectable Cholangiocarcinoma Metastatic Cholangiocarcinoma FGFR Gene Changes | III                 | Recruiting   |
| NCT04096417 | Pemigatinib | Pemigatinib for the treatment of metastatic or unresectable colorectal cancer harboring FGFR Alterations | Metastatic Colorectal Carcinoma            | II                  | Recruiting   |
| NCT04256980 | Pemigatinib | Pemigatinib in treating patients with advanced/metastatic or surgically unresectable cholangiocarcinoma including FGFR2 rearrangement | Cholangiocarcinoma                         | II                  | Recruiting   |
| NCT04258527 | Pemigatinib | Phase I study of pemigatinib in patients with advanced malignancies with FGFR/FGFR alterations | Solid Tumor                               | I                   | Not yet recruiting |
| NCT04088188 | Cisplatin   | Gemcitabine and cisplatin with ivosidenib or pemigatinib for the treatment of unresectable or metastatic cholangiocarcinoma | Cholangiocarcinoma                         | I                   | Not yet recruiting |
resistance is often linked to tumor heterogeneity and the occurrence of secondary mutations in the FGFR2 kinase domain. This phenomenon can be regarded as the stress response of cancer cells to therapeutic drugs. Recently, Krook et al. reported a patient with metastatic cholangiocarcinoma and altered FGFR2 who was enrolled in a phase II clinical trial of the FGFR inhibitor BGJ398. The treatment was effective in the initial 8 months but ended with regrowth and disease progression. Targeted sequencing of tumor DNA revealed that the FGFR2 kinase domain p.E565A and p.L617M single-nucleotide variants (SNV) contributed to drug resistance. The expression of these FGFR2 SNVs was also detected after the application of other clinically relevant FGFR inhibitors, including AZD4547, erdafitinib (INJ-42756493), dovitinib, ponatinib, and TAS120. Furthermore, they proved that combination therapy strategies with FGFR and mTOR inhibitors might be used to overcome resistance to FGFR inhibition. Interestingly, analysis of the post-progression in ctDNA samples revealed that both p.E565A and p.L617M mutation while only p.E565A mutation was detected in the tumor biopsy and comparing with pre-(BGJ398) treatment. Non-p.L617M mutation detected in the post-progression tumor biopsy samples suggested the limitations of tumor biopsies in capturing tumor heterogeneity. As small biopsy cannot represent the whole tumor, not to mention some multiple metastatic tumors. What's more, liquid biopsy accounts for its advantage, especially when serial assessment of patients is needed and/or an invasive tumor biopsy is not practicable.

ctDNA, a critical component detected in peripheral blood of cancer patients, is also a hot topic to discuss, in liquid biopsy. Normally, similar components relative to primary tumors including circulating tumor cells (CTCs), circulating free DNA (cfDNA) and exosomes. The majority of cfDNA is usually derived from normal healthy leukocytes and stromal cells and ctDNA represents the part of cfDNA which derives from primary tumors and the metastatic sites, carrying tumor-specific genetic or epigenetic alterations. With convenient access of liquid biopsy, ctDNA can be used for tracking therapy resistance and analyzing resistance mechanisms, according to the mutations and copy number alterations detection.

Recently, a study aiming to characterize the ctDNA genomic alteration landscape in patients with biliary tract cancers was conducted by Mody and colleagues. A total of 138 samples from 124 patients (including 85 iCCA patients) were enrolled in the study. Therapeutically relevant alterations were detected in 76 patients (55%), demonstrating the feasibility of ctDNA testing in iCCA. Goyal and his colleagues analyzed ctDNA, primary tumors and metastases of 3 iCCA patients who participated in the phase II trial of BGJ398 (NCT02150967) before involved and after disease progression. All 3 cases demonstrated new point mutations in FGFR2 gene that conferred resistance to BGJ398 at the time of testing upon experiencing disease progression. Interestingly, the p.V564P point mutation was identified in all 3 cases. Molecular modeling and in vitro studies indicated that each mutation lead to BGJ398 resistance and was surmountable by structurally distinct FGFR inhibitors. This study glimpsed the significance of ctDNA analysis to monitor treatment responses so as to regulate the therapy scheme. In another similar study, Goyal and colleagues reported the efficacy of TAS-120 in 4 iCCA patients with FGFR2 fusion who developed resistance to BGJ398 or Debio1347. Of the 4 cases, some gene mutations were detected after progression with the application of BGJ398 or Debio1347, reflected by ctDNA. The expressions of these mutations decreased with the intervention of TAS-120. Unfortunately, subsequent ctDNA analysis indicated the reoccurrence of original mutations and the attendance of new mutations after TAS-120 lost control. With the help of serial biopsies and ctDNA detection, the strategic sequencing of FGFR inhibitors may prolong the duration benefits from FGFR inhibition in iCCA patients with FGFR2 fusion. The 2 studies above revealed the correlations between genotypes and drug sensitivities with the help of liquid biopsy. However, there were certain limitations of ctDNA, such as wide variations of the preanalytical variables, assay characteristics (PCR-based versus next-generation sequencing-based techniques), bioinformatic analysis methods. There is still a long way to go for the establishment of ctDNA-based biopsy standard. And abundant prospective clinical trials data are needed to evaluate the clinical utility of ctDNA in the management of iCCA. Establishing a deeper understanding of the specific molecular mechanisms is essential to continuously develop targeted drugs capable of overcoming multiple secondary drug resistance and completing comprehensive treatment.

**Prospect of FGFR Targeted Therapy for iCCA**

Altogether, the prospect of FGFR targeted therapy is encouraging. Many target agents are currently undergoing phase I or phase II clinical trials, and the clinical efficacy of BGJ398 (which is already in phase 3 clinical trial), pemigatinib and ARQ 087 have already been demonstrated. Clinical trials have proven the increasingly prominent role of FGFR inhibitors with many targeted therapeutic drugs. The frequency of FGFR mutations or fusions is higher in iCCA than in other solid tumors such as perihilar or extraphepatic CCA. However, the number of patients with iCCA enrolled in clinical trials is usually low, owing to its low incidence and high malignance, compared with other tumors such as hepatocellular carcinoma. Therefore, the early screening of iCCA and more detailed criteria for clinical trials may be the new focus of attention. For instance, specific agents being the only cure for aberrations in FGFR in iCCA is the sole criterion of “Targeted Therapy,” similar to the therapeutic effects of imatinib effect on chronic myeloid leukemia. Although some researchers have reported longer overall survival in patients with tumors and FGFR alterations than in those without FGFR alterations, especially for iCCA compared with other biliary tract cancers, the findings accomplished with the use of FGFR inhibitors must not be ignored. The gap between the selection of first-line and second-line treatments does exist; however, the efforts related to FGFR inhibitors are narrowing now the gap, as an accelerated
approval of the FDA in April 2020 considering pemigatinib as a second-line treatment for advanced cholangiocarcinoma was revealed. Nonetheless, based on various drug resistance mechanisms, some agents still fail potential therapies. In view of this situation, research on relevant molecular mechanisms should be continuously improved in order to identify new molecular drugs. For the downstream signaling pathways of FGFR, a therapeutic agent combined with other therapeutic strategies or agents for inhibiting multiple pathways can be an effective treatment. Otherwise, with rapid evolvement of liquid biopsy, ctDNA can be applied to monitor tumor responses to treatment and regulates the scheme of targeted therapy, as a unique kind of biomarkers. Understanding the spectrum of activity of nowadays FGFR inhibitors against commonly observed secondary mutations may lead to strategies to overcome the resistance.61 What’s more, future development of FGFR inhibitors should focus on agents that is capable of secondary resistance mutations reflected by liquid biopsy.50 The development of biologic agents for treatment while developing inhibitory agents with accurate pharmacological targets are also new approaches. As a representative of this aspect, promising data obtained from the investigations of the effects of FGFR inhibitors may support the postulation that patients with FGFR2 gene fusion may benefit from targeted therapy of FGFR.

Both tables are listed in another document.

Abbreviations

| Abbreviation | Definition |
|--------------|------------|
| AE           | adverse events |
| CR           | complete response |
| ctDNA        | circulating tumor DNA |
| DCR          | disease control rate |
| OS           | overall survival |
| ORR          | overall response rate |
| PD           | progressive disease |
| PFS          | progression-free survival |
| PR           | partial response |
| RP2D         | recommended phase II dose |
| SD           | stable disease |

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