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## Title page

### Title

Resistance mechanism to fibroblast growth factor receptor (FGFR) inhibitors in cholangiocarcinoma

### Short title

Resistance mechanism to FGFRi in CCA

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### Statements

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### Abstract

Precision medicine is a major achievement that has impacted on management of patients diagnosed with advanced cholangiocarcinoma (CCA) over the last decade. Molecular profiling of CCA has identified targetable alterations, such as fibroblast growth factor receptor-2 (*FGFR-2*) fusions, and has thus led to the development of a wide spectrum of compounds. Despite favourable response rates, especially with the latest generation FGFRi, there are still a proportion of patients who will not achieve a radiological response to treatment, or who will have disease progression as the best response. In addition, for patients who do respond to treatment, secondary resistance frequently develops and mechanisms of such resistance are not fully understood. This review will summarise the current state of development of FGFR inhibitors in CCA, their mechanism of action, activity, and the hypothesised mechanisms of resistance.

## Keywords

Cholangiocarcinoma, FGFR, fusion, FGFR inhibitor, resistance, primary, secondary

## Manuscript text

### Introduction: current management of CCA

Cholangiocarcinomas (CCAs) are subdivided into intrahepatic cholangiocarcinoma (iCCA) and extrahepatic cholangiocarcinoma (eCCA) (further divided into perihilar (pCCA) and distal (dCCA)) based on their location.

The majority of patients diagnosed with CCA are diagnosed at advanced non-curable stages and are therefore managed with palliative intent (1). For years, treatment in the advanced setting was limited to cytotoxic chemotherapy, with cisplatin and gemcitabine (CisGem) (2). Recently, second-line chemotherapy with 5-FU and oxaliplatin (FOLFOX) (3) or liposomal irinotecan (nal-IRI) and 5-FU have been added as post first-line treatment options (4–6).

Two main therapeutics are changing the treatment paradigm for CCA (7): immunotherapy and targeted therapies. The addition of checkpoint inhibitors such as durvalumab to CisGem has shown improvement in overall survival (OS), progression-free survival (PFS) and objective response rate (ORR), over CisGem, for treatment-naïve biliary tract tumours (including CCA) (8). Molecular profiling of CCA has identified targetable alterations, such as isocitrate dehydrogenase-1 (*IDH-1*) mutations, fibroblast growth factor receptor-2 (*FGFR-2*) fusions, Human Epidermal Growth Factor-2 (HER-2), B-Raf proto-oncogene serine/threonine kinase (*BRAF*) V600E mutations and neurotrophic tyrosine receptor kinase (*NTRK*) fusions), among others (9,10).

Of all these potentially targetable alterations, the *FGFR-2* fusion-positive CCA population has been the target of multiple phase II studies, with the development of a wide spectrum of compounds. This review will summarise these compounds, their mechanism of action, activity and the hypothesised mechanism of resistance.

### Role of FGFR in cancer and its relevance as a target

The FGF-FGFR pathway consists of five different receptors *FGFR1*, *FGFR2*, *FGFR3*, *FGFR4*, and *FGFR5* (11,12). The first four are subtypes of transmembrane receptors with intracellular tyrosine kinase domains (RTKs), *FGFR1–4* (13), while *FGFR5* is a structurally-related protein without tyrosine kinase activity which appears to function as a co-receptor for *FGFR1* (12). The extracellular domain of FGFRs can bind 22 different FGFs (*FGF1–14*, *FGF16–23*; there is no human

FGF15) (13,14). The binding of FGF ligands to their respective receptors at the cell surface results in receptor dimerisation and transphosphorylation of tyrosine residues on their cytoplasmic tails (13), leading to activation of several downstream intracellular signalling pathways, including Ras-Raf-MAPK, PI3K-AKT-mTOR, JAK-STAT, and phospholipase C (PLC) (13–15), and gene transcription that activates several intracellular survival and proliferative pathways.

Physiological activation of the FGF/FGFR pathway occurs in embryogenesis, morphogenesis, organogenesis, tissue repair, proliferation, and tumour angiogenesis, metabolic homeostasis, and neuroendocrine balance, playing an important role in a variety of key biological processes such as apoptosis, cellular migration, mitogenesis, and cell differentiation (15–18). Dysregulation and hyperactivation of FGFR signalling cascade has been implicated in oncogenesis and tumour progression (including urothelial carcinoma, intrahepatic cholangiocarcinoma, breast carcinoma, non-small cell lung carcinoma, endometrial carcinoma, and head and neck squamous cell carcinoma), drug resistance to anticancer therapy, immune evasion, and angiogenesis (19,20). The expanding knowledge on the biological activity of FGFs uncovered their role in glucose metabolism, bile acid, and phosphorous homeostasis, mediated by binding to FGF receptor (FGFR) via klotho co-receptor or heparin cofactor (21,22).

Wu and colleagues first described FGFR2 fusions in patients with iCCA in 2013 (23). FGFR2–BICC1 (Bicaudal family RNA binding protein 1) fusion is the most common FGFR aberration (24,25). Many other fusion partners beyond BICC1 have been described up to now in iCCA including FGFR2-PPHLN1 (Periphilin 1), FGFR2-AHCYL1 (Adenosylhomocysteinase Like 1), FGFR2-PARK2 (Parkin RBR E3 ubiquitin protein ligase), FGFR2-MGEA5 (Meningioma Expressed Antigen 5), FGFR2-TACC3 (Transforming Acidic Coiled-Coil Containing Protein 3), FGFR2-CCDC186 (Coiled-Coil Domain Containing 186), FGFR2-NOL4 (Nucleolar Protein 4) and FGFR2-KIAA1598 (Shootin 1)(25,26). Current evidence supports that it is the presence of the FGFR2 fusion itself which entitles the sensitivity to FGFRi, regardless of the fusion partner identified (27). FGFR2 fusions are typically identified along with tumour suppressor gene alterations (BAP1 and CDKN2A), while associated presence of mutations in other cancer drivers such as TP53, IDH1, BRAF, HER2, SMAD4 or KRAS are less frequent (27).

The presence of FGFR2 fusions is now expected to be around 9% of iCCA (28) (previously reported as higher (15-20%), most likely due to selection bias of the studies available (10)). Its presence seem to be associated with a certain clinical phenotype in iCCA, with a more favourable natural history (29,30), with a higher rate of young and female patients, typically diagnosed with bone metastases and normal tumour markers (29,31,32). The benefit from cytotoxic chemotherapy seems to be similar compared with iCCA without FGFR2 fusions (33), even though some research groups suggest this to be the case in the first-line rather than the second-line setting only (34).

#### FGFRi in CCA: Clinical evidence to support their use

Since the identification of FGFR2 as a potential target, multiple FGFR inhibitors have been developed (10,35). These FGFR inhibitors have different characteristics in their structure,

mechanisms of target engagement, and specificities for FGFR1, 2, 3 and 4 and other related kinases. According to their mechanism of action and FGFR specificity, they can be classified into three groups: 1) small-molecule Tyrosine Kinase Inhibitors (TKIs); 2) FGF ligand traps; and 3) FGFR-directed monoclonal antibodies (**Figure 1**). **Table 1** provides a summary of their mechanism of action and reported activity.

Three of these compounds have been approved by regulatory agencies: pemigatinib (Food and Drug Administration (FDA) and European Medicines Agency (EMA) approved in 2020 and 2021, respectively), infigratinib (FDA approved in 2021, FDA withdrawn in 2022) and futibatinib (FDA approved in 2022, EMA approved in 2023) (7).

### Small-molecule Tyrosine Kinase Inhibitors (TKIs)

The first generation of TKIs (e.g. lenvatinib, regorafenib, pazopanib, lucitanib, dovitinib) target several tumorigenic receptors such as VEGFRs, PDGFRs, RET, KIT, and FGFRs, due to the similarity at the ATP binding site of the intracellular kinase domains (36,37). Thus, they are characterised by not being FGFR specific. First generation TKIs showed limited antitumor activity in solid tumours with FGFR2 alterations and greater toxic effects due to low specificity, such as cardiovascular and liver toxicities, proteinuria, and hypertension (38).

A second generation of TKIs, characterised by selective, reversible, and ATP-competitive binding to FGFR kinase domain, emerged with the aim of improving clinical outcomes and decreased adverse events (39). These have been widely explored in iCCA with FGFR alterations.

*Derazantinib* (ARQ 087) is an oral, non-selective TKI with strong inhibition of FGFR1-4 (40,41). The phase I clinical trial reported an overall response rate (ORR), disease control rate (DCR) and estimated median progression-free survival (mPFS) of 20.7%, 82.8% and 5.7 months (95% CI 4.0-9.2), respectively (42). Based on these results, derazantinib is being assessed in the phase II FIDES-01 trial in pretreated patients with iCCA with FGFR2 fusions and mutations or amplifications (NCT03230318)(43). Updated data on 28 patients with iCCA and FGFR2 mutations and amplifications treated with derazantinib within this study reported an ORR of 8.7% and median PFS of 7.3 months (44).

*Erdafitinib* (JNJ-42756493) is an oral, reversible, potent TKI with activity against FGFR1-4 and other kinases (45,46). The phase II LUC2001 trial with pre-treated CCA with FGFR alterations, including mutations and fusions, reported an ORR of 40.9%, mPFS of 5.6 months (95% CI: 3.6-12.7), and a median OS of 40.2 months (95% CI: 9.9-NR) (47); in patients with FGFR2 alterations, ORR was 60.0%; DCR 100%; and mPFS was 12.35 months (95% CI: 3.15, 19.38) (48). Activity and safety profile seems comparable between Asian population and patients for Europe and United

States (49). Erdafitinib has been tested in a patients with solid tumours and a wide spectrum of FGFR alterations (including iCCA) in the RAGNAR study, with an ORR of 30% (50).

*Pemigatinib* (INCB054828) is an oral, highly selective, reversible, inhibitor against FGFR1–3. The phase II FIGHT-202 trial, including advanced refractory or metastatic cholangiocarcinoma with FGFR2 fusions, other alterations in *FGF/FGFR* or no alterations in these genes, reported an ORR of 35.5% with complete response (CR) of 2.8% in those with FGFR2 fusions; no responses were reported in the other groups(51). The median PFS and OS in the FGFR2 group was 6.9 and 21.1 months, respectively(51,52). In the case of FGF/FGFR alterations or no aberrations, the mPFS was 2.1 months and 1.7 months, respectively, and the median OS was 6.7 months and 4.0 months, respectively(51,52). Updated long follow-up data with a median follow-up of 42.9 months confirmed an ORR of 37% with median PFS and OS of 7 and 17.5 months, respectively (53). The phase III, FIGHT-302 study (NCT03656536) is ongoing, comparing the efficacy of pemigatinib versus CisGem as first-line treatment for unresectable or metastatic cholangiocarcinoma with FGFR2 alterations.

*Infigratinib* (BGJ398) is an oral, reversible, selective TKI against FGFR1-3 (54). The phase II study included patients with advanced refractory or metastatic CCA with chimeric *FGFR* fusions or rearrangements(55). In the group with FGFR2 aberrations, the ORR was 23.1% (95% CI 15.6–32.2), with a median duration of response of 5.0 months and mPFS of 7.3 months (95% CI 5.6–7.6 months)(54,56). Infigratinib was under evaluation in the phase III PROOF trial (NCT03773302) as first-line treatment for patients with cholangiocarcinoma harbouring FGFR2 gene translocations. Unfortunately, this trial is now close (“active, not recruiting” as of December 2022) due to challenges in recruitment, which highlights an ongoing issue in the delivery of randomised phase III for infrequent molecular alterations in rare malignancies, especially with ongoing competing studies running concomitantly worldwide.

*Debio 1347* (CH5183284), is an oral, reversible, highly selective FGFR1-3 inhibitor (57). The phase I dose-escalation trial, including patients with advanced pretreated solid tumours with FGFR1-3 gene fusions, reported antitumour activity in several tumour types, including iCCA (58,59). In the iCCA group, there was partial response (PR) in 22.2% and 44.4% had stable disease (SD), and the median time on treatment was 24 weeks (range, 4–57 weeks). All patients with iCCA that had a response had FGFR2 alterations (60). Currently, a phase II, multicentre, open-label FUZE (NCT03834220) trial is exploring the efficacy of Debio 1347 in pretreated malignancies with FGFR1-3 fusions, including CCAs, urothelial carcinomas, and other solid tumours (61).



Although second-generation FGFR inhibitors showed favourable antitumor activity in iCCA, a third generation of FGFR inhibitors with covalent and irreversible binding to the ATP site, is emerging (62).

*Futibatinib* (TAS- 120) is an oral, irreversible, highly selective FGFR1-4 inhibitor (63,64), with efficacy in patients with FGFR2 fusions and progression on prior FGFR inhibitors(65–67). The phase II FOENIX-CCA2 trial, which enrolled advanced and metastatic iCCA with FGFR2 gene fusions/rearrangements after progression on standard treatment, after a median follow-up of 17.1 months, reported an ORR of 42%, median PFS of 9.0 months and median OS of 21.7 months (68). In addition to the radiological response reported, there has been an improvement in symptoms and tolerable toxicity, with maintained quality of life reported for this compound (69). Currently, activity of this compound is being confirmed within the phase II FOENIX-CCA4 trial (NCT05727176) evaluating futibatinib two different doses of after progression to chemotherapy patients with iCCA with FGFR2 gene fusions/rearrangements..

*RLY-4008* (lirafugratinib) is an oral, irreversible, highly selective FGFR2 inhibitor designed to target both driver alterations and FGFR2 resistance mutations (70). This seems to be the first potent highly-specific FGFR2 inhibitor in the field, which may provide significant clinical advantages such as avoiding off-target FGFR-related dose limiting toxicities, maximising activity and overcoming the mechanism of primary and secondary resistance to other FGFR inhibitors (**Table 2**). A Phase I/II study (NCT04526106), enrolling patients with CCA with FGFR2 fusion/rearrangements, reported an ORR of 88% (data on 17 patients reported), DCR 100%, with a median duration of treatment of 6 months (<0.1 - 18.5 months) (71). Data from the phase I trial showed a ORR of 29% in patients with CCA and FGFR2 mutations, and an ORR of 21% (at the RP2D) for patients with FGFR2 fusions who had already progressed to prior FGFR inhibitors (72). Despite this promising activity, longer follow-up and larger number of patients is required to confirm these results. Its activity continues to be explored in other FGFR2 alterations (mutations/amplifications) and also on progression to other FGFR inhibitors (NCT04526106).

Many new FGFR inhibitors are currently under development. KIN-3248 is a selective, irreversible pan-FGFR inhibitor able to inhibit secondary kinase domain mutations associated with progression to FGFR inhibitors (73) and is currently being tested in a phase I clinical trial (KN-4802 trial; NCT05242822). Tinengotinib (TT-00420) reported a ORR of 16.7% in 42 evaluable patients (3 out of 7 partial responses were reported in patients diagnosed with CCA(74). This compound is being tested in an ongoing phase III clinical trial in CCA (FIRST-308 trial; NCT05948475). Many other FGFR inhibitors are being developed.

#### Toxicity profile of FGFRi TKIs

Toxicity profile from FGFRi can be classified as 1) class-specific (on-target): hyperphosphataemia, stomatitis (20–40%), alopecia (24–46%), hyponatraemia, arthralgia, nail toxicity (5–17%), and ophthalmologic toxicity [dry eye (19–21%), retinal toxicities and central serous retinopathy (9%)] (75,76); and 2) non-specific (off-target): fatigue (32–71%), diarrhoea (15% to 60%) anorexia, fever and liver toxicity (10,75,76). The most frequently reported toxicities associated with FGFR inhibitors are quite comparable, even though toxicity profile is more “on-target” with the more “specific” FGFR2 inhibitors being developed. It is of importance to identify and adequately identify and manage FGFRi-associated toxicities, which in many occasions may need from treatment break and dose reduction (77).

#### FGF ligand trap and FGFR-targeted monoclonal antibodies:

FGF ligand trap and FGFRs monoclonal antibodies are two additional strategies to inhibit FGFR signalling, by interfering with ligand-binding or receptor dimerization (10,78).

FGFR-targeted monoclonal antibodies can be helpful in patients with tumours harbouring FGFR overexpression or amplification. Its development has been challenging, with toxicity issues before reaching active dose being an issue in some occasions (79,80). The most promising FGFR2 monoclonal antibody currently in development is bemarituzumab (FPA144), which specifically targets FGFR2-IIIb with favourable safety and activity observed in gastric cancer overexpressing FGFR2 (81). This compound is currently being tested in the FORTITUDE-301 clinical trial recruiting patients with solid tumours with FGFR2 overexpression, including CCA (NCT05325866).

The FGF ligand trap is an interesting strategy to block the interaction between FGF ligands and FGFRs impeding FGFR activation. By the development of decoy receptors that lack the transmembrane and cytoplasmic domains, but maintain the extracellular FGFR domain, the interaction and subsequent sequestration of FGF ligands can be achieved (10,79). Activity of FP-1039/GSK3052230 in patients diagnosed with endometrial cancer harbouring FGFR2 mutations was being tested (NCT01244438), unfortunately the study closed to recruitment due to challenges lack of eligible patients being identified after screening of 70 patients. No studies seem to be currently planned in CCA with FG ligand trap compounds.

#### Resistance mechanisms to FGFR TKIs in CCA

Understanding mechanism of resistance to FGFRi in CCA is of significant relevance (82), since it could inform design of future studies for better patient selection (primary resistance) and allow development of strategies to overcome acquired secondary resistance (7). Overview provided in **Figure 2**.

#### Primary resistance to FGFRi in CCA

When reflecting on the activity data, it is evident that the presence of the FGFR2 fusion alone does not secure a response to treatment, and that a proportion of patients will have primary resistance to treatment, with progression being the best response achieved. This phenomenon is present in around 5% of patients across all studies exploring activity of FGFR inhibitors in CCA.

The only compound reporting a DCR of 100% is RLY4008, when the recommended phase II dose (RP2D) cohort is analysed. This, however, could be explained by the limited number of patients included in this analysis (17 patients) and larger cohorts would be required to confirm this.

In addition, a significant proportion of patients will have stable disease as best response, which has triggered post-hoc analyses of the available studies looking into potential factors associated with radiological response. The main factors evaluated have been the FGFR2 fusion partners and other concomitant molecular alterations. Data from the FIGHT-202 study (pemigatinib) was analysed to identify factors that may impact response to treatment (83). Despite identification of multiple fusions partners, BICC was the most common fusion partner. Neither the fusion partner nor the presence of rearrangements/fusions impacted outcome. None of the molecular co-alterations explored were associated with differences in response rate. In contrast, patients harbouring alterations in tumour suppressor genes, CDKN2A/B, TP53 or PBRM1 had a shorter mPFS.

Some preclinical studies have suggested the presence of KRAS-activated mitogen-activated protein kinase signaling as a cause of primary resistance to FGFRi (84). In addition, the type of FGFR2 fusion (break point) and the resultant fusion protein may also predict the sensitivity to FGFRi. Pu and colleagues described that the protein products of FGFR2 fusions could be classified into three subtypes: classical fusions (retain the tyrosine kinase and the immunoglobulin-like domains); sub-classical fusions (retain only the tyrosine kinase domain) and non-classical fusions (lack both tyrosine kinase and immunoglobulin-like domains). The classical and sub-classical fusions were associated with greater sensitivity to FGFR inhibitors, with subsequent suppression of the MAPK/ERK and AKT/PI3K activities (85). In this regard, research exploring potential roles of targeting the FGFR2 fusion proteins has suggested that upfront concomitant inhibition of HSP90 with FGFRi may enhance activity of FGFRi by inducing rapid degradation of these fusion proteins (86). Other groups have suggested the potential clinical utility of dual FGFR2-MEK1/2 blockade, blocking this downstream effector (87).

#### FGFRi mechanism of secondary resistance in CCA

Despite initial response to treatment, patients end up developing resistance to therapies. In some occasions, this secondary resistance may be developed relatively quickly, with median duration of response (DOR) ranging between 5 and 9 months with most of FGFR inhibitors currently available (largest reported was with erdafitinib, DOR of 12 months) (**Table 1**). No DOR data is as yet available for RLY4008.

Development of resistance mutations had been previously described as mechanism of resistance to FGFRi (79,88). Translational research within the FIGHT-202 clinical trial with pemigatinib explored mechanisms of secondary resistance in 8 patients with initial tumour shrinkage (83). This work identified development of resistance mutations in the FGFR2 kinase domain as the predominant mechanism of secondary resistance. Polyclonal resistance (the presence of multiple acquired alterations in the same patient), was identified in 3 patients, while single mutations were observed in others: FGFR2 p.N549K/H was observed in 4 patients, whereas FGFR2 p.E565A, p.K659M, p.L617V, and p.K641R were each observed in 2 patients. These mutations were

creating either less favourable pemigatinib-binding conditions or a conformational shift and constitutive kinase activation.

Interestingly, some patients with acquired resistance to a prior second generation FGFR inhibitor also experienced responses with futibatinib (one of the third generation TKIs available) (67,89). Goyal and colleagues identified polyclonal secondary FGFR2 mutations which drove acquired resistance to FGFR inhibition, based on serial analysis of cell free DNA that demonstrated multiple recurrent point mutations in the FGFR2 kinase domain at progression, and how futibatinib was able to overcome resistance to second generation FGFRi (90).

Alternative ways of overcoming these secondary resistance mechanisms are being explored. The development of FGFR2 kinase domain p.E565A and p.L617M single-nucleotide variants were identified following progression on infigratinib, and were associated with upregulation of the PI3K/AKT/mTOR (91); based on this data, the use of mTOR inhibitors as potential ways to overcome resistance to FGFR inhibition, specific to infigratinib may be an option to explore. In addition, new molecules such as proteolysis targeting chimeras (PROTACs)(92) and LY2874455 are emerging as promising options that have demonstrated activity against FGFR gatekeeper mutations in vitro (93).

Changes in Epithelial to mesenchymal transition have also been suggested as potential mechanism of secondary resistance, but little data is available in iCCA (82).

### Future steps

Precision medicine is rapidly evolving in CCA, with FGFR2 fusions being one of the most attractive targets. Despite promising response rates (greater than those seen with chemotherapy), especially with the latest generation FGFRi, there is still a proportion of patients who will not achieve a radiological response to treatment, or who will have progression as best response. Understanding the mechanism of resistance is therefore of major importance, first, to improve patient outcome, second, to improve patient selection for future studies, and third, to design rational combinations which, given upfront, could overcome such primary resistance. In addition, for patients with responses to treatment, secondary resistance frequently develops. The mechanisms of resistance are not fully understood, and translational research associated with ongoing clinical trials is required to identify ways of overcoming resistance and extending the benefit that may be derived from these treatments.

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Tables and figure

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Figure 1: summary of current clinical relevance of FGFR2 fusion and its potential role as treatment target in iCCA

iCCA: intrahepatic cholangiocarcinoma, +Ve: positive, TKI: tyrosine kinase inhibitor,

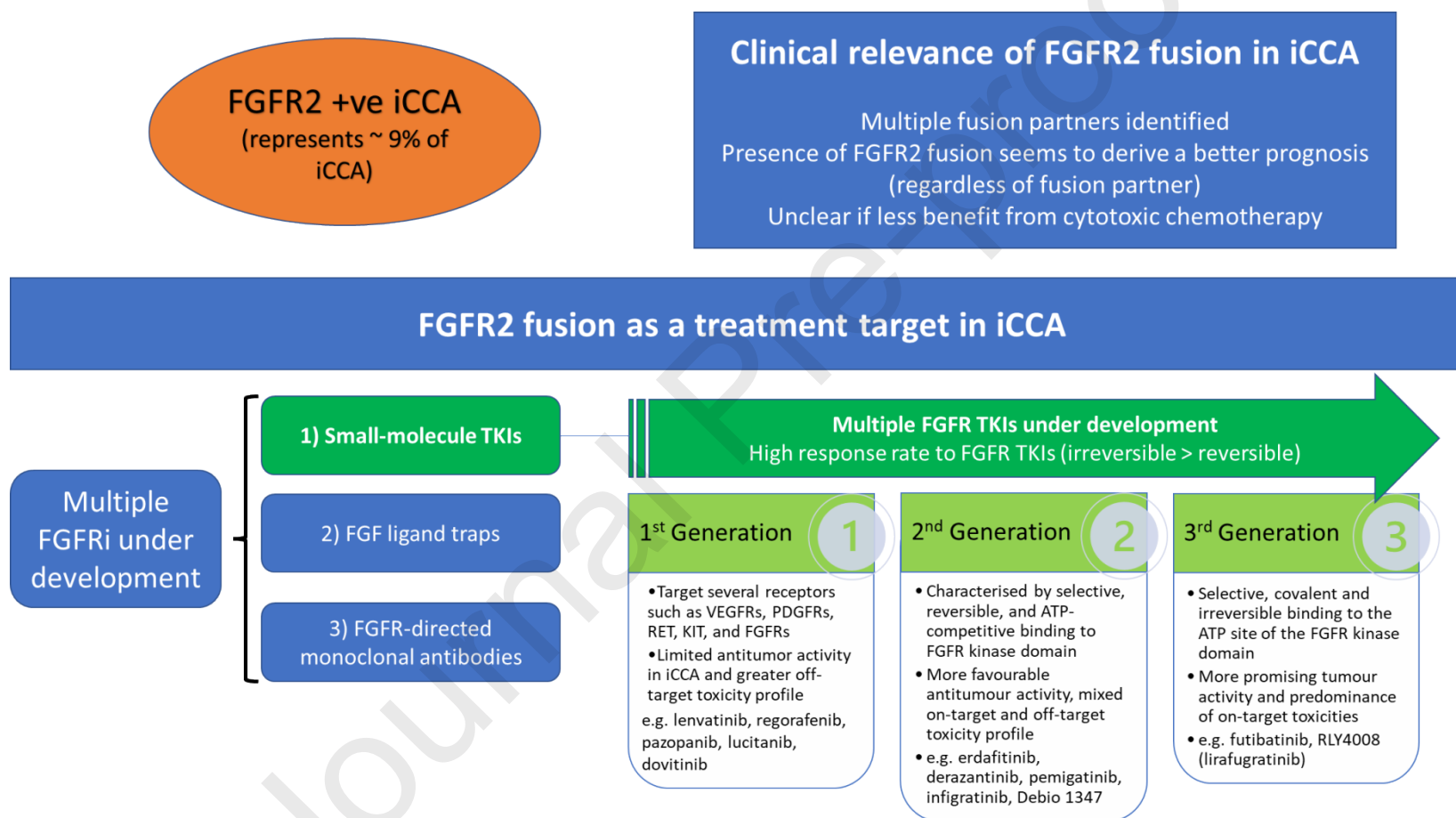


Table 1. Status of development of FGFR2 inhibitors in iCCA.



Drug	Target	Status of drug development	Primary endpoint	Outcome
<b>Second-generation inhibitors:</b> Selective oral TKI, reversible, ATP-competitive mechanism				
Derazantinib-ARQ087(40,42–44)  300 mg daily	FGFR1-3	Phase I/II ARQ 087-101 (NCT01752920)  - Advanced solid tumours, including iCCA, for which standard therapy failed - FGFR genetic alterations including FGFR2 gene fusion - Status: Completed	Safety and tolerability	FGFR2 fusions: - ORR 20.7%; mPFS 5.7 months (95% CI 4.0–9.2)  FGFR2 mutations/amplifications: - ORR 0%; mPFS 6.7 months (95% CI 1.0–14.7)  No FGFR2 alterations: - ORR 0%; mPFS 1.5 months (95% CI 0.7–N)
		Phase II FIDES-01 (NCT03230318)  - iCCA with previous systemic therapy or ineligible for first-line chemotherapy - FGFR2 gene fusion, mutation, or amplification - Status: Active, not recruiting	ORR  PFS	FGFR2 fusions: - ORR 21.4% (95% CI 13.9, 30.5); DCR 75.7% (95% CI 66.3, 83.6) - mPFS 8.0 months (95% CI 5.5, 8.3); mOS 17.2 months (95% CI 12.5, 22.4).  FGFR2 mutations/amplifications: - ORR was 6.5% (95% CI 0.8, 21.4); DCR 58.1% (95% CI 39.1, 75.5) - mPFS 8.3 months (95% CI 1.9, 16.7); mOS 15.9 months (95% CI 8.4, NE)  Updated data on FGFR2 mutations/amplifications

				<p>- ORR was 8.7%</p> <p>- mPFS 7.3 months</p>
<p>Pemigatinib- INCB054828 (52)</p> <p>13.5 mg daily</p> <p>2-week on/1-week off</p>	<p>FGFR 1-3 VEGFR2</p>	<p>Phase II FIGHT-202 (NCT02924376)</p> <p>- CCA which failed previous therapy</p> <p>- FGFR genetic alterations including FGFR2 translocations</p> <p>- Status: Completed</p>	<p>ORR</p>	<p>FGFR2 translocations:</p> <p>- ORR: 35.5%; mPFS: 6.9 months (95% CI 6.2–9.6); mOS: 21.1 months</p> <p>Other FGF/FGFR alterations:</p> <p>- ORR: 0%; mPFS: 2.1 months (95% CI 1.2–4.9); mOS 6.7 months</p> <p>No FGF/FGFR alterations:</p> <p>- ORR: 0%; mPFS: 1.7 months (95% CI 1.3–1.8); mOS 4 months</p>
		<p>Phase III FIGHT-302 (NCT03656536)</p> <p>- Advanced CCA first-line treatment</p> <p>- FGFR2 Rearrangement</p> <p>- Status: Recruiting</p>	<p>PFS</p>	<p>N/A</p>
<p>Infigratinib- BGJ398(56)</p> <p>125 mg daily</p>	<p>FGFR 1-3</p>	<p>Phase II (NCT02150967)</p> <p>- CCA which failed or are intolerant to platinum-based chemotherapy</p> <p>- FGFR genetic alterations including FGFR2 gene fusion</p>	<p>ORR</p>	<p>FGFR2 fusions:</p> <p>- ORR 23.1% (95% CI 15.6–32.2); mDOR 5.0 months</p> <p>- mPFS 7.3 months (95% CI 5.6–7.6)</p>

3-week on/1-week off		<ul style="list-style-type: none"> <li>- Status: Completed</li> </ul>		<ul style="list-style-type: none"> <li>FGFR2 mutation: 0% PR</li> <li>FGFR2 amplification: 0% PR</li> </ul>
<p>Debio1347- CH5183284(60)</p> <p>80 mg daily</p>	<p>FGFR 1-3</p>	<p>Phase III PROOF (NCT03773302)</p> <ul style="list-style-type: none"> <li>- Advanced CCA first-line treatment</li> <li>- FGFR2 gene fusions or translocations</li> <li>- Status: Interrupted</li> </ul>	<p>PFS</p>	<p>N/A</p>
		<p>Phase I (NCT1948297)</p> <ul style="list-style-type: none"> <li>- Advanced solid malignances</li> <li>- FGFR1-3 genes alterations</li> <li>- Status: Completed</li> </ul>	<p>Safety and tolerability</p>	<p>iCCA with FGFR2 deletion:</p> <ul style="list-style-type: none"> <li>- ORR 22%; DCR 62.5%</li> </ul>
		<p>Phase II FUZE (NCT03834220)</p> <ul style="list-style-type: none"> <li>- Advanced solid malignances</li> <li>- FGFR1-3 fusions</li> </ul>	<p>ORR</p>	<p>N/A</p>

		-Status: Terminated (Due to lower antitumor activity than expected)		
<p>Erdafitinib</p> <p>JNJ42756493(47,50,94)</p> <p>8 mg QD</p> <p>To be escalated to 9 mg daily at day 14 if plasma phosphate levels &lt; 5.5 mg/dL</p>	<p>FGFR 1-4</p>	<p>Phase I (NCT01703481)</p> <p>- Advanced solid tumours or Lymphoma for which standard therapy failed</p> <p>- FGFR status not specified</p> <p>- Status: Completed</p>	<p>MTD</p>	<p>CCA patients with FGFR mutation/fusion:</p> <p>- ORR 27.3% (95% CI 6-61); mDOR 12.9 months; DCR: 55%</p> <p>- mPFS 5.1 months (95% CI 1.6-16.4)</p>
		<p>Phase II LUC2001 (NCT02699606)</p> <p>- Asian patients with advanced solid tumor including CCA</p> <p>- FGFR status not specified</p> <p>- Status: Active, not recruiting</p>	<p>ORR</p>	<p>CCA patients with FGFR alterations:</p> <p>- ORR 40.9% (95% CI, 20.7%–63.6%); mDOR 7.3 months</p> <p>- mPFS 5.6 months (95% CI, 3.6–12.7); mOS 40.2 months (95% CI: 9.9–NR)</p>
		<p>Phase II RAGNAR trial (NCT04083976)</p> <p>- Solid tumours</p> <p>- FGFR alterations</p>	<p>ORR</p>	<p>Solid tumours:</p> <p>- ORR of 30%</p>
<p><b>Third-generation inhibitors:</b> highly selective oral TKI, irreversible, ATP-covalent mechanism</p>				
<p>Futibatinib (68,89)</p> <p>TAS120</p>	<p>FGFR 1-4*</p> <p>*Inhibits mutant and wild type</p>	<p>Phase I/II FOENIX-101 (NCT02052778)</p>	<p>RP2D</p> <p>Safety and tolerability</p>	<p>FGFR2 gene fusion:</p>

20 mg daily	FGFR2	<ul style="list-style-type: none"> <li>- Advanced solid tumors which failed to standard therapy</li> <li>- FGFR fusion or activating mutation or amplification</li> <li>- Status: Active, not recruiting</li> </ul>	ORR	<p>ORR 25%</p> <p>FGFR other alterations:</p> <p>ORR 17.6%</p> <p>Patients who had previously been treated with other FGFR inhibitors:</p> <p>ORR 30,8%</p>
		<p>Phase II FOENIX-CCA2 (NCT02052778)</p> <ul style="list-style-type: none"> <li>- Advanced iCCA patients one prior systemic therapy, no prior FGFR inhibitor.</li> <li>- FGFR2 fusions, activating mutation or amplification</li> <li>- Status: Active, not recruiting</li> </ul>	ORR	<p>iCCA patients with FGFR2 alterations:</p> <ul style="list-style-type: none"> <li>- ORR 41.7%; DCR 82.5%; mDOR 9.5 months</li> <li>- mPFS 8.9 months; mOS was 20.0 months</li> </ul>
		<p>Phase II FOENIX-CCA4 (NCT05727176)</p> <ul style="list-style-type: none"> <li>- Advanced CCA first-line treatment</li> <li>- FGFR2 gene rearrangements</li> <li>- Status: Recruiting</li> </ul>	PFS	N/A

RLY4008 (71,72)  70 mg qd	highly selective FGFR2 inhibitor	Phase I/II ReFocus (NCT04526106 )  - Advanced CCA (and other solid tumors)  - FGFR2 gene rearrangements (and other alterations)  - Status: Recruiting	RP2D  Safety and tolerability	CCA patients with FGFR2 fusions:  - Across all doses: ORR 63.2%, DCR 94.7%  - On the RP2D: ORR 88.1%, DCR 100%  Phase I trial  - CCA FGFR2 mutation: ORR 29% (all doses)  - CCA FGFR2 fusion, FGFR pre-treated: ORR 21% (RP2D)
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CCA, cholangiocarcinoma; CI, confidence interval; DCR, disease control rate; FGFR, fibroblast growth factor receptor; iCCA, intrahepatic cholangiocarcinoma; mDOR, median duration of response; mPFS, median progression-free survival;

ORR, objective response rate; PR, partial response; RP2D, recommended phase 2 dose; TKI, tyrosine kinase inhibitor; VEGFR, vascular endothelial growth factor receptor.

**Table 2: Summary of the design and pre-clinical development of RLY-4008**

Extracted from (70).

Conclusion	Supporting data
<b>RLY-4008 is a selective irreversible inhibitor of FGFR2</b>	Authors assessed differences in conformational dynamics between FGFR2 and other FGFRs to enable the design of RLY-4008 as an FGFR2-selective inhibitor that covalently binds to Cys491. This covalent binding and inhibition of FGFR2 by RLY-4008 was characterized by both biophysical and biochemical techniques, including mass spectrometry and liquid chromatography-mass spectrometry. In terms of the rate of covalent labelling of FGFR2 (vs FGFR1) by RLY-4008, it was seen how FGFR2 was labelled rapidly ( $t_{1/2} = 8$ s), while labelling of FGFR1 was delayed ( $t_{1/2} = 351$ s), confirming that the RLY-4008

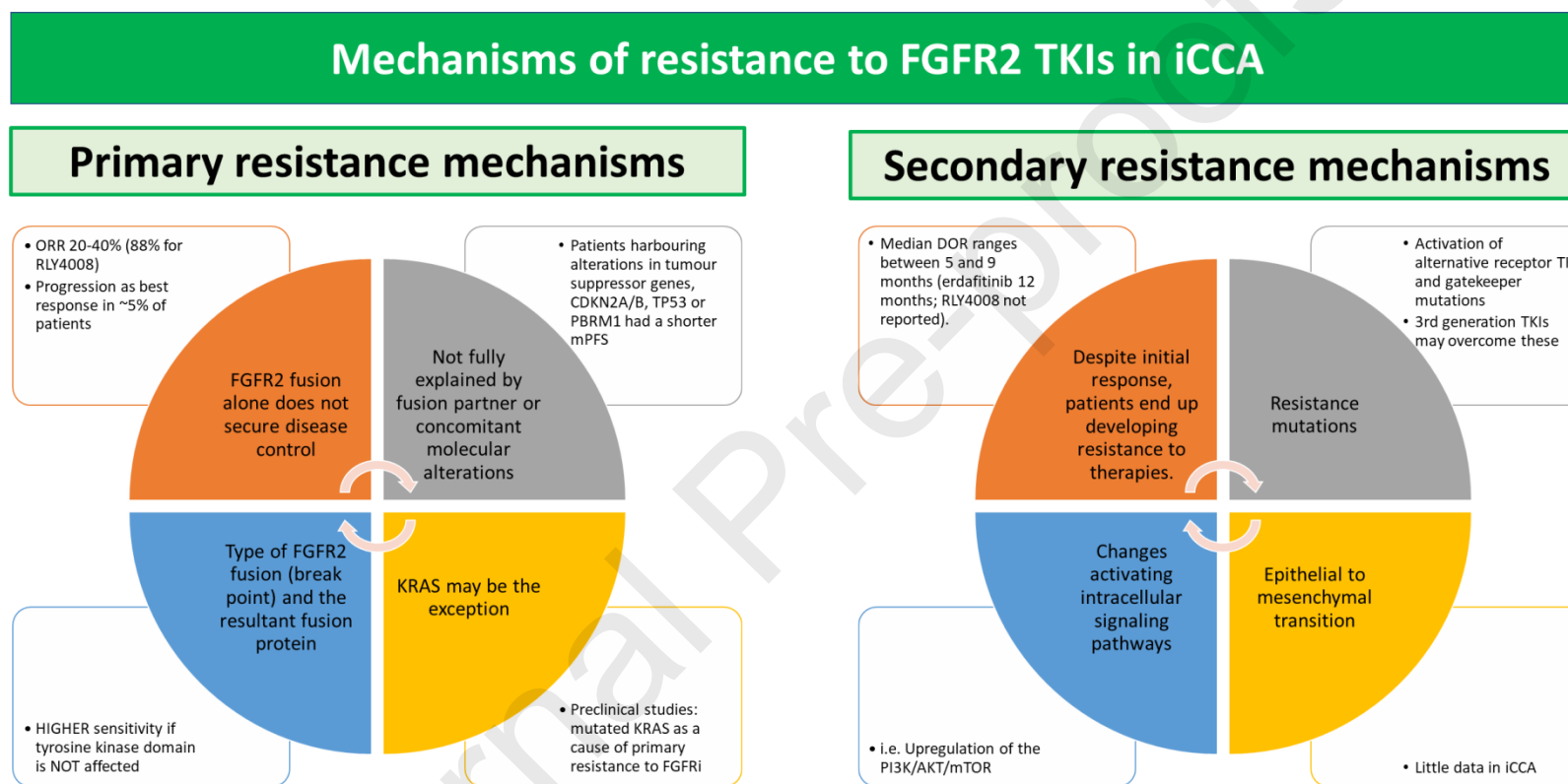
	<p>induced a conformation in FGFR1 that was, unlike in FGFR2, not favourable for covalent bond formation. When these experiments were replicated with futibatinib, it covalently labelled both FGFR1 and FGFR2 at a similar rate (FGFR1 t<sub>1/2</sub> = 8 s; FGFR2 t<sub>1/2</sub> = 13 s), consistent with its pan-FGFR activity.</p>
<p><b>RLY-4008 is a potent and selective inhibitor of FGFR2</b></p>	<p>Inhibition potency (expressed by the ratio of the inactivation rate constant (k<sub>inact</sub>) to the binding constant (K<sub>i</sub>) of RLY-4008 was higher for FGFR2 (3.45 × 10<sup>-2</sup> s/μM) compared to FGFR1 (3.79 × 10<sup>-4</sup> s/μM/L). In addition, RLY-4008 demonstrated potent selectivity of FGFR2 enzyme activity with IC<sub>50</sub> of 3 nM for FGFR 2, and weaker inhibition of other FGFRs (all above IC<sub>50</sub> 270nM) and other 468 kinases that were tested. Other pan-FGFR inhibitors demonstrated limited selectivity for FGFR2 when experiments were replicated.</p>
<p><b>RLY-4008 inhibits FGFR2 in vitro in FGFR2-driven cancer cell lines</b></p>	<p>RLY-4008 demonstrated dose-dependent reduction of phosphorylation of FGFR2 signaling pathway, including FRS2, AKT and ERK and early markers of apoptosis such as cleavage of caspase 3 and poly (ADP-ribose) polymerase (PARP). Notably, RLY-4008 did not inhibit FGFR2 phosphorylation in the presence of a Cys491Ser mutation (present in the FGFR2 fusion-positive (FGFR2-OPTN) intrahepatic CCA (iCCA) cell line ICC13-7), confirming the on-target nature of RLY-4008 cellular activity. RLY-4008 inhibited cellular proliferation with IC<sub>50</sub> &lt;14 nM in FGFR2-dependent cell lines including those derived from solid tumours with FGFR2-amplification, FGFR2 fusion and FGFR2-mutations.</p>
<p><b>RLY-4008 demonstrates antitumor activity in FGFR2-altered cancer in vivo</b></p>	<p>The activity of RLY-4008 in subcutaneous xenograft mouse models harbouring different FGFR2 alterations (mutations/amplifications/fusions) was tested. RLY-4008 was administered orally, twice daily from 1 to 30 mg/kg, and it was well tolerated at all doses. It exhibited dose-dependent antitumor activity with tumor regression in all models. In comparison, pemigatinib and futibatinib seemed to be less efficacious than RLY-4008. Activity was supported by pharmacokinetic and pharmacodynamic (PK/PD) analyses of plasma exposure and target engagement in tumour samples in all models. At a dose of 30 mg/kg twice daily, RLY-4008 achieved a ≥ 90% pFGFR2 inhibition that was sustained throughout the 12 h dosing interval and resulted in tumour regression. Interestingly, in the FGFR2 fusion-positive iCCA model, &lt; 90% sustained pFGFR2 inhibition also induced tumour regression, maybe</p>

	<p>suggesting that fusion-positive tumours have a greater dependency (and are more sensitive to FGFR2 inhibition) on FGFR2 signaling compared to FGFR2 mutated/amplified. Activity of futibatinib, pemigatinib, erdafitinib and infigratinib were also tested; all drugs caused tumour regression. Hyperphosphatemia (32-47% over vehicle; <math>P &lt; 0.0001</math>, one-way ANOVA) was also identified (this was not seen with RLY-4008 (<math>P &gt; 0.2</math>, one-way ANOVA)), thus, supporting in vivo the FGFR1 inhibition activity of these compounds and the lack of FGFR2 selectivity.</p>
<p><b>RLY-4008 is active in the presence of FGFR2 resistance mutations</b></p>	<p>activity of RLY-4008 and other FGFR inhibitors was tested in cell lines harbouring two of the best described mutations of resistance detected in FGFR2 fusion-positive CCA at progression on FGFR inhibitors: the FGFR2 V564F 'gatekeeper' mutation and the FGFR2 N549K 'molecular brake' mutation. The data presented confirmed that only RLY-4008 remained active in both scenarios. While the other FGFR inhibitors lack activity, the only exception was futibatinib, which retained similar activity to the one seen for RLY-4008 in the presence of FGFR2 N549K mutation. Experiments in xenografts confirmed these findings.</p>



Figure 2: Primary and secondary resistance mechanisms to FGFR tyrosine kinase domain inhibition in CCA

TKI: tyrosine kinase inhibitor; ORR: overall response rate, DOR: duration of response



Dr Angela Lamarca and Dr Lorena Ostios prepared the first draft of this manuscript

All the authors reviewed the draft and approved the final version

Molecular profiling of CCA has identified targetable alterations

*FGFR-2* fusions are targeted by a variety of new compounds.

Primary resistance is still an issue for a proportion of patients

Mechanism of secondary resistance are being defined and better understood

## Conflict of interest

Dr Angela Lamarca declares travel and educational support from Ipsen, Pfizer, Bayer, AAA, SirtEx, Novartis, Mylan and Delcath; speaker honoraria from Merck, Pfizer, Ipsen, Incyte, AAA, QED, Servier, Astra Zeneca, EISAI, Roche and Advanz Pharma; advisory and consultancy honoraria from EISAI, Nutricia Ipsen, QED, Roche, Servier, Boston Scientific, Albireo Pharma, AstraZeneca, Boehringer Ingelheim, GENFIT, TransThera Biosciences and Taiho; she is also a member of the Knowledge Network and NETConnect Initiatives funded by Ipsen.

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Menarini, Merck, Merus, Millennium, MSD, Nanobiotix, Nektar, Novartis, Odonate Therapeutics, Pfizer, Pharma Mar, PharmaMar, Principia, PsiOxus, Puma, Regeneron, Rigontec, Roche, Sanofi, Sierra Oncology, Synthon, Taiho, Takeda, Tesaro, Transgene, Turning Point Therapeutics, Upshersmith.

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