

Molecular Characterisation and Therapeutic Implications of FGFR2 Fusions in Tissue and Circulating Tumor DNA from Patients with Intrahepatic Cholangiocarcinoma: A Review

Alessandro Ricci^{1*}, Hannah Weiss² and Vikram Nair³

¹Department of Hepatobiliary Oncology, University of Bologna, Bologna, Italy

²Department of Molecular Pathology, University of Heidelberg, Heidelberg, Germany

³Department of Medical Oncology, Tata Memorial Centre, Mumbai, India

*Correspondence to: Alessandro Ricci, Department of Hepatobiliary Oncology, University of Bologna, Bologna, Italy

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Abstract

FGFR2 fusion is the one alteration in intrahepatic cholangiocarcinoma that reliably changes what a patient is offered. Finding it is harder than the literature implies, because the fusion partner varies from case to case and most assays are built around partners they already know.

Objectives: To review the published evidence on FGFR2 fusions in intrahepatic cholangiocarcinoma: how often they occur, how reliably the available assays detect them in tissue and in plasma, what response and survival have been reported with FGFR inhibition, and how acquired resistance emerges and is detected.

Material and Methods: Narrative review of discovery studies, diagnostic accuracy and proficiency studies, prospective trials and correlative sequencing analyses indexed in PubMed and the major oncology and pathology journals. Studies were eligible if they reported the prevalence or architecture of FGFR2 fusions, the performance of a detection assay in tissue or plasma, outcomes with FGFR-directed therapy, or mechanisms of acquired resistance. No new patient data were generated; every frequency, accuracy estimate and survival figure quoted is one published by the original investigators.

Results: FGFR2 fusions occur in roughly 10 to 15 per cent of intrahepatic cholangiocarcinomas. In a series of 226 cases assayed three ways, detection rates were 10.2 per cent by break-apart FISH, 9.7 per cent by RNA-based sequencing and 7.1 per cent by DNA-based sequencing, and only 57.7 per cent of the cases called positive by any method were positive by all three. Against RNA sequencing as reference, FISH showed 95.2 per cent sensitivity and DNA sequencing 71.4 per cent. Plasma performs worse still with standard panels: among patients with a tissue-confirmed fusion, cell-free DNA recovered the fusion in 18 per cent overall, 58 per cent where the partner was BICC1 and 2 per cent where it was not. A custom panel tiling the FGFR2 intron recovered fusions in 88.9 per cent. Objective response with FGFR inhibition ranged from 23.1 per cent with infigratinib to 46.5 per cent with tirafugratinib, and median overall survival reached 17.5 months with pemigatinib and 21.7 months with futibatinib.

Conclusion: The limiting factor in this disease is assay design rather than biology. A negative FGFR2 result carries a materially different weight depending on which test produced it, and a negative plasma result from a standard panel is close to uninformative. Plasma earns its place in serial monitoring, where it detects the polyclonal resistance mutations that tissue rebiopsy cannot practically capture.

Abbreviations: iCCA – Intrahepatic Cholangiocarcinoma, CCA – Cholangiocarcinoma, FGFR – Fibroblast Growth Factor Receptor, FGFRi – FGFR Inhibitor, NGS – Next-Generation Sequencing, FISH – Fluorescence In Situ Hybridisation, IHC – Immunohistochemistry, cfDNA – Cell-Free DNA, ctDNA – Circulating Tumour DNA, FFPE – Formalin-Fixed Paraffin-Embedded, ORR – Objective Response Rate, DCR – Disease Control Rate, PFS – Progression-Free Survival, OS – Overall Survival, VAF – Variant Allele Frequency.

Keywords: FGFR2 Fusion; Intrahepatic Cholangiocarcinoma; Liquid Biopsy; Molecular Diagnostics; Acquired Resistance

Introduction

Cholangiocarcinoma is a heterogeneous group of biliary tract tumours classified by anatomical origin, of which the intrahepatic subtype accounts for 10 to 20 per cent [1,2] and is defined by its origin proximal to the second-order bile ducts [29]. It has historically been a disease of limited therapeutic options, and until the past

decade the molecular characterisation that transformed treatment in lung and colorectal cancer had produced nothing comparable here.

That changed with the identification of recurrent FGFR2 rearrangements. Whole transcriptome sequencing of cholangiocarcinoma specimens without KRAS, BRAF or ROS1 alterations identified two fusion kinase genes, FGFR2-AHCYL1 and FGFR2-BICC1, following earlier surveys of tyrosine kinase

signalling that had shown the disease to carry recurrent kinase rearrangements [28]. Screening then found the fusion in 13.6 per cent of intrahepatic cases and almost nowhere else: rarely in colorectal and hepatocellular carcinoma and not at all in a gastric series [3]. Independent work reported translocations at comparable frequency and confirmed their restriction to the intrahepatic subtype [4], and further sequencing uncovered additional partners including PPHLN1 [5].

The alterations are mutually exclusive with KRAS and BRAF mutation, they activate MAPK signalling. These receptors carry an extracellular ligand-binding region, solved structurally in complex with ligand and heparin [32], and an intracellular kinase domain that the fusions retain; their transforming capacity is attributable to kinase activity and suppressed by FGFR inhibition in preclinical models [3]. That combination of features, a driver present in a defined minority and druggable by an available class of agent, is what made FGFR2 the first genuinely actionable target in this disease [6,7].

Two selective inhibitors now carry regulatory approval on the strength of single-arm trials [8,9,10,11,33,34], and the practical question has shifted from whether to target the fusion to how reliably it can be found. That question turns out to be less settled than the approvals suggest. FGFR2 fuses to a large and growing list of partners, the genomic breakpoints fall in a region that is technically awkward to sequence, and the assays in routine use differ substantially in what they can see [12,13,14].

The aim of this review is to set out what has been published on the characterisation of FGFR2 fusions in tissue and in circulating tumour DNA, what each compartment reliably detects, what treatment has achieved, and how resistance emerges once treatment begins.

Material and Methods

Search strategy: PubMed and the archives of the major oncology, hepatology and pathology journals were searched for studies of FGFR2 alterations in cholangiocarcinoma. Search terms combined FGFR2, fibroblast growth factor receptor 2 and FGFR inhibitor with intrahepatic cholangiocarcinoma, gene fusion, rearrangement, fusion partner, fluorescence in situ hybridisation, RNA sequencing, circulating tumour DNA, liquid biopsy and acquired resistance. Reference lists of retrieved trials, reviews and diagnostic studies were screened by hand for further primary sources.

Eligibility: Studies were included if they reported the prevalence or genomic architecture of FGFR2 fusions in a defined cholangiocarcinoma population; the analytical or clinical performance of a detection assay in tissue or plasma; clinical outcomes with FGFR-directed therapy in fusion-positive disease; or mechanisms of acquired resistance. Prospective trials and their prespecified correlative analyses were included preferentially. Preclinical studies are cited where they establish the biological basis of an observed clinical finding.

Data extraction and handling: Design, sample size, assay platform, reference standard, reported detection rates, accuracy estimates and efficacy outcomes with their confidence intervals were extracted as published. No pooling, re-analysis or recalculation was performed and no patient-level data were obtained. Detection rates are reported alongside the assay and the reference standard used, because in this setting the two cannot be separated. Figures 1 to 5 and Tables 1 and 2 reproduce published values only. This is a narrative synthesis and was not registered as a systematic review.

Results

Prevalence and clinicopathological setting: Reported frequency of FGFR2 fusions in intrahepatic cholangiocarcinoma clusters between 10 and 15 per cent, with individual series ranging somewhat wider depending on assay and population [3,4,15,16]. The original transcriptome screen reported 13.6 per cent [3]. Comprehensive genomic profiling series have reported figures in the same region and have consistently shown the alteration to be near-absent in extrahepatic disease [15,16,17,27]. Eastern and western cohorts differ modestly in the broader mutational landscape of the disease but not dramatically in FGFR2 fusion frequency [18]. Transcriptomic subtyping has separately identified stem-like classes within intrahepatic disease [35], while IDH mutation defines a further group that blocks hepatocyte differentiation and promotes biliary tumorigenesis [36].

Fusion-positive tumours are mutually exclusive with KRAS and BRAF mutation and tend to occur in younger patients [3,8]. In the pivotal pemigatinib trial, the fusion-positive cohort had a median age of 55.5 years, was 61 per cent female, and 99 per cent had intrahepatic primaries against 65 and 59 per cent in the comparison cohorts [8]. The interval from diagnosis to starting FGFR-directed treatment in that cohort was a median of 1.3 years, which is longer than would be expected in unselected advanced disease and suggests a somewhat more indolent natural history [8].

Three assays, three answers: The most instructive diagnostic evidence comes from a series in which 226 intrahepatic cholangiocarcinomas were assayed in parallel by RNA-based sequencing, DNA-based sequencing and break-apart FISH. Detection rates were 10.2 per cent by FISH, 9.7 per cent by RNA sequencing and 7.1 per cent by DNA sequencing (Figure 1). Of the 26 cases called positive by any method, only 15, or 57.7 per cent, were positive by all three [12].

Taking RNA sequencing as the reference standard for an oncogenic fusion, FISH achieved 95.2 per cent sensitivity and 98.5 per cent specificity, while DNA sequencing achieved 71.4 per cent sensitivity and 99.5 per cent specificity (Figure 2) [12]. DNA sequencing did, however, identify four cases carrying FGFR2 point mutations that the other assays would not have reported, including an oncogenic extracellular activating mutation for which FGFR inhibition has produced responses [12]. Each assay therefore misses a different subset, and the choice between them is not a matter of one being uniformly better.

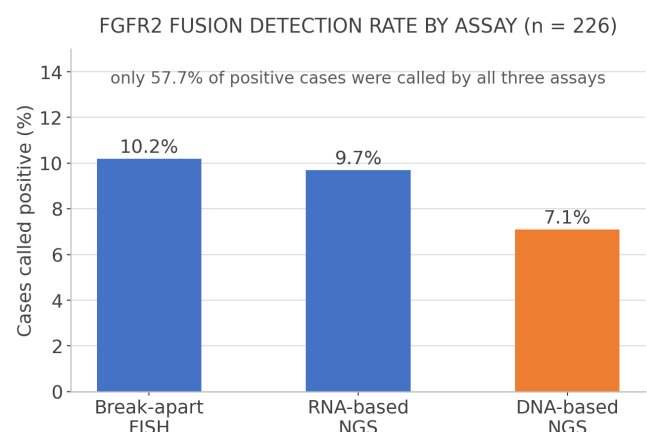


Figure 1: FGFR2 fusion detection rate by assay in 226 intrahepatic cholangiocarcinomas tested in parallel [12]

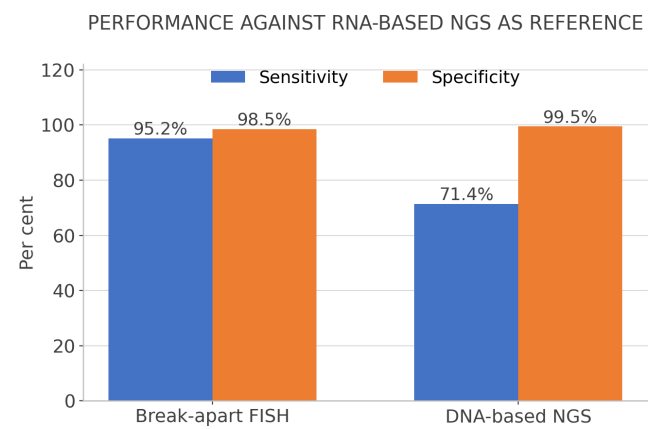


Figure 2: Sensitivity and specificity of FISH and DNA-based sequencing against RNA-based sequencing as the reference standard [12]

Why detection is difficult: The explanation lies in the architecture of the fusions themselves. More than fifty partner genes have been described, BICC1 is the most common but accounts for a minority, and in one series only 35 per cent of partners were located on chromosome 10 with the remainder distributed across the genome [12,13,19,30]. An assay designed around a fixed list of partners will therefore miss a substantial share of true positives.

A multi-centre proficiency exercise made the practical consequences explicit. Ten cholangiocarcinomas, four fusion-positive and six negative, were distributed to participating laboratories. Thirteen of sixteen centres, 81 per cent, passed the RNA-sequencing round, and three of five, 60 per cent, passed the combined sequencing and FISH round. The obstacles identified were bioinformatic pipelines not optimised for FGFR2 fusions and assays incapable of detecting unknown partners. The authors concluded that fusion-partner-agnostic, open methodological approaches are superior [14]. A further technical difficulty is that detecting full-length transcripts by RNA sequencing in formalin-fixed material is compromised by RNA degradation [14].

Study	n	Material	Principal reported finding
Arai et al. 2014 [3]	102	Tissue	FGFR2 fusion in 13.6% of intrahepatic cases; defines a subtype
Cao et al. 2024 [12]	226	Tissue	Only 57.7% of positives called by all three assays
Neumann et al. 2023 [14]	21 centres	Tissue	81% of centres passed NGS proficiency testing
Berchuck et al. 2022 [20]	1,671	Plasma	cfDNA sensitivity for a known fusion only 18%
González-Medina et al. 2024 [21]	18	Tissue + plasma	Custom intron-tiled panel recovered 88.9% of fusions
Abou-Alfa et al. 2020 [8]	147	Trial	Pemigatinib ORR 35.5% in fusion-positive cohort
Goyal et al. 2023 [11]	103	Trial	Futibatinib ORR 42%; median OS 21.7 months

Table 1: Principal studies reviewed, with the material examined and the finding as reported by the original investigators

What plasma recovers, and what it does not: The largest analysis of cell-free DNA in biliary tract cancer covered 1,671 patients, with an intra-patient concordance analysis in 194 of them, 67 of whom had a tissue-proven FGFR2 fusion. Among those patients, cell-free DNA profiling recovered the fusion in only 18 per cent. Broken down by partner, sensitivity was 58 per cent for FGFR2-BICC1

fusions and 2 per cent for everything else, and the authors identified partner diversity as the factor hampering detection (Figure 3) [20]. That figure is not a property of plasma but of the panels being used on it. A study of 18 patients with tissue-confirmed fusions applied a custom hybrid capture panel in which probes were tiled across intron 17 of FGFR2, the region in which the breakpoints fall, rather than across a list of known partners. Sixteen patients, 88.9 per cent, were positive for the fusion in plasma [21]. The gap between 18 and 88.9 per cent is a design difference, not a biological one.

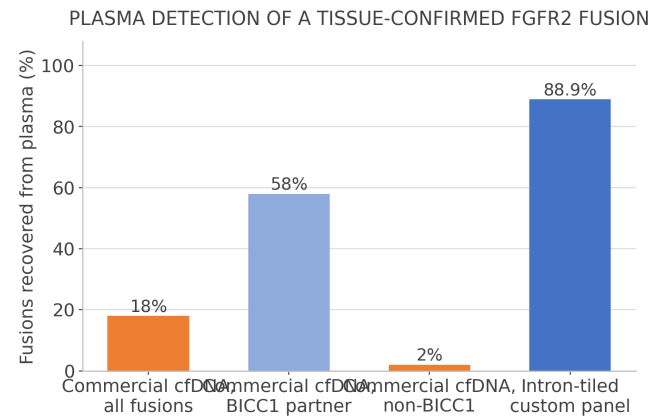


Figure 3: Recovery of a tissue-confirmed FGFR2 fusion from plasma, by assay design and fusion partner [20,21]

Approach	Detects	Principal limitation
Break-apart FISH	Rearrangement at the locus, partner-agnostic	Does not identify the partner or confirm an in-frame oncogenic fusion [12]
RNA-based NGS	Expressed in-frame fusion transcript	RNA degradation in FFPE material; pipeline dependent [12,14]
DNA-based NGS	Breakpoints plus point mutations and amplification	Sensitivity 71.4% for fusions against RNA sequencing [12]
Plasma, standard panel	Known partners, chiefly BICC1	2% sensitivity for non-BICC1 fusions [20]
Plasma, intron-tiled panel	Breakpoints regardless of partner	Not yet widely available; small validation series [21]

Table 2: Detection approaches for FGFR2 fusions, what each recovers and the limitation reported for it

What finding the fusion delivers: Four selective FGFR inhibitors, drawn from a class developed across several tumour types [31], have reported results in fusion-positive disease. Infigratinib produced an objective response rate of 23.1 per cent [9,10]. Pemigatinib produced 35.5 per cent in the primary analysis of its pivotal trial, revised to 37.0 per cent in the final analysis after a median 45.4 months of follow-up, with three complete and thirty-seven partial responses among 108 patients [8]. Futibatinib, a covalent irreversible inhibitor, produced 42 per cent among 103 patients with a disease control rate of 83 per cent [11]. Lirafugratinib, an FGFR2-selective irreversible agent, reported 46.5 per cent by independent review in 114 previously treated inhibitor-naïve patients (Figure 4) [22,23].

Survival figures follow the same ordering but with narrower separation. Final analysis of the pemigatinib trial reported median progression-free survival of 7.0 months and median overall survival of 17.5 months, with median duration of response 9.1 months [8]. Futibatinib reported median progression-free survival of 9.0 months, median overall survival of 21.7 months and median duration of response 9.7 months (Figure 5) [11]. These are single-arm results in a population with no established comparator, so cross-trial

comparison should be read cautiously; the cohorts differ in prior therapy and in the proportion with intrahepatic primaries.

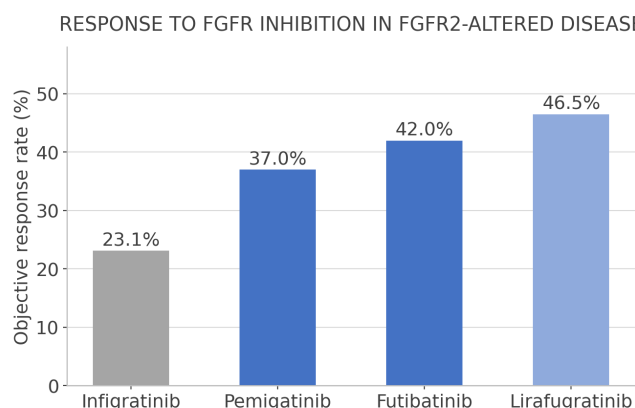


Figure 4: Objective response rate reported for four selective FGFR inhibitors in FGFR2-altered cholangiocarcinoma [8,9,10,11,22,23]

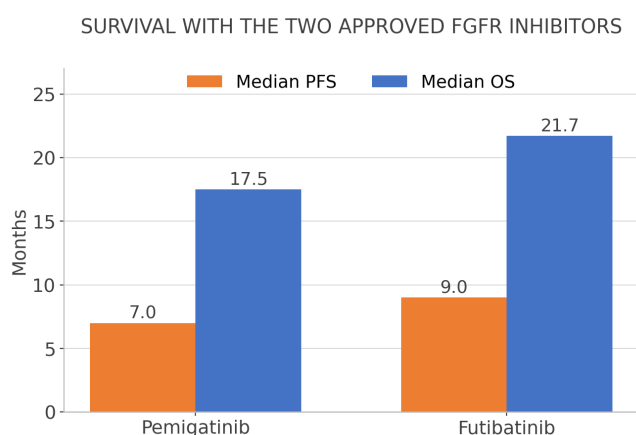


Figure 5: Median progression-free and overall survival reported for the two approved agents in fusion-positive disease [8,11]

Resistance is polyclonal and emerges in plasma: Response to FGFR inhibition is not durable in most patients, and the mechanism was established through integrative analysis of cell-free DNA, primary tumour and metastases in patients progressing on infigratinib. Serial cell-free DNA revealed multiple point mutations in the FGFR2 kinase domain at progression, with the p.V564F gatekeeper mutation, which sterically hinders drug binding, common to the patients studied and accompanied by further substitutions including N549H, E565A, K641R, L617V and K659M [24].

A rapid autopsy in one patient demonstrated why single-site rebiopsy would have been misleading: genomic characterisation of twelve metastatic lesions revealed marked inter-lesional and intra-lesional heterogeneity, with different FGFR2 mutations in individual resistant clones [24]. Molecular modelling and in vitro work indicated that each mutation conferred resistance that was surmountable by structurally distinct inhibitors, which is the rationale for the covalent agents [24,25]. A clinicogenomic analysis of the pemigatinib trial population reached compatible conclusions, identifying correlates of response alongside kinase domain mutations at progression [26].

Serial plasma has since been shown to do more than characterise resistance after the fact. In the eighteen-patient series above, resistance mutations were detected at progression in 82 per cent of patients with an evaluable sample, and nineteen distinct FGFR2 kinase domain mutations were found across the cohort. The number differed by prior agent, with a mean of two after futibatinib against nine after pemigatinib. Increases in circulating tumour fraction and

the emergence of resistance mutations preceded radiological progression by around three months [21].

Discussion

Two conclusions follow from this evidence, and they point in different directions for the two compartments. For finding the fusion in the first place, tissue remains the reference and the choice of assay matters more than is generally acknowledged. A negative FGFR2 result from DNA-based sequencing carries roughly a one-in-four chance of being wrong against RNA sequencing [12], and a negative result from a panel built around known partners carries no useful information at all in a case whose partner is not on the list [14,20].

For plasma, the headline figure of 18 per cent sensitivity has been widely cited as evidence that liquid biopsy cannot find these fusions [20]. The more accurate reading is that panels designed for point mutations cannot find them. Where probes are tiled across the intron in which the breakpoints fall, plasma recovers close to nine fusions in ten [21]. The constraint is engineering rather than biology, and it is one that has already been solved in a research setting.

Implications for testing

Three practical points follow. First, where tissue is adequate, an RNA-based or otherwise partner-agnostic method should be preferred for fusion detection, with DNA sequencing retained for the point mutations and amplifications it uniquely detects [12]. Second, a negative plasma result from a standard commercial panel should not be treated as excluding a fusion, and where clinical suspicion is high the appropriate response is tissue testing rather than reassurance [20]. Third, laboratories reporting these fusions should be aware that a fifth of participating centres failed a structured proficiency exercise, and that bioinformatic pipeline design was a principal reason [14].

Where plasma is unequivocally useful is after treatment starts. Serial sampling detects the polyclonal kinase domain mutations that drive resistance, captures clones distributed across anatomically separate lesions that no single rebiopsy would reach, and signals progression ahead of imaging [21,24]. That is a different clinical question from initial detection, and plasma answers it better than tissue does.

Study limitations

The limitations belong to the underlying literature. The diagnostic comparison data derive from single-centre retrospective series in which the reference standard, RNA sequencing, is itself imperfect on formalin-fixed material [12,14]. The plasma studies are smaller still: the custom panel result rests on eighteen patients from one institution, and requires independent validation before its performance can be assumed to generalise [21]. Efficacy data come entirely from single-arm trials with no randomised comparator, in a population whose natural history appears more indolent than unselected advanced disease, so the contribution of selection to the reported survival cannot be separated from the contribution of treatment [8,11]. Cross-trial comparison of response rates is confounded by differences in prior lines, in the proportion of intrahepatic primaries and in whether response was assessed centrally [8,9,11].

Two gaps deserve naming. No study has prospectively compared partner-agnostic plasma testing against tissue testing as the primary route to treatment eligibility, which is the design that would establish whether plasma can replace biopsy for this indication. And the resistance literature, though mechanistically clear, has not yet shown

that acting on a plasma-detected resistance mutation before radiological progression improves any patient outcome [21,24].

Conclusion

FGFR2 fusions define a distinct and treatable subtype of intrahepatic cholangiocarcinoma, present in roughly one patient in eight, with response rates to selective inhibition between 23 and 47 per cent and median survival that now exceeds eighteen months in trial populations. The constraint on translating that into practice is detection. Assays differ sharply in what they recover, agreement between them is only moderate, and in plasma the difference between a standard panel and one designed around the FGFR2 breakpoint region is the difference between finding one fusion in five and finding nine in ten. Partner-agnostic assay design in both compartments, and serial plasma sampling once treatment has begun, are what the published evidence supports.

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Conflict of Interest

The authors identify no competing interest in this work.

Ethics Statement

Only published studies are examined in this article. No new patient data were assembled and nobody was recruited to take part, so the work sat outside the scope of ethical approval.

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