Fibroblast growth factor receptor 2 (*FGFR2*) fusions in intrahepatic cholangiocarcinoma (iCCA)

Genomic alterations in fibroblast growth factor receptors (FGFRs)

- FGFRs are a family of receptor tyrosine kinases.[1,2] FGFR signalling pathways play a central role in multiple cellular processes, including cell proliferation, migration and survival[1,2]
- Alterations in *FGFR* genes have emerged as tumourigenic drivers in cancers including iCCA, urothelial carcinoma, myeloid/lymphoid neoplasms and other malignancies[1,3,4]

- *FGFR* amplifications, mutations and fusions have been observed in all *FGFR* subtypes (*FGFR1–4*).[5] Chromosomal rearrangements involving *FGFR2* – resulting in the creation of oncogenic fusion proteins – have frequently been identified in iCCA[6]
  - Gene fusions are a type of genomic alteration where two independent genes or portions of genes are juxtaposed, resulting in a hybrid gene[7,8]
  - The development of fusion proteins with oncogenic potential can result from gene fusion events involving a range of different partner genes[7]

FGFR genomic alterations

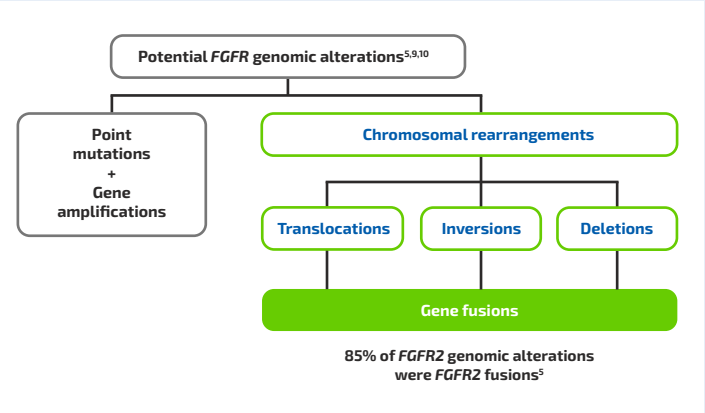


Figure based on Jain A, et al. 2018,[5] Lowery MA, et al. 2018,[9] and Shibata T, et al. 2018[10]

FGFR2 fusions

- *FGFR2* fusions or rearrangements occur in 10–16% of iCCA cases[5,11–13]
- *FGFR2* fusions result in ligand-independent activation of downstream signalling pathways, leading to tumourigenesis[1,14,15]

Abnormal FGFR2 signalling pathway

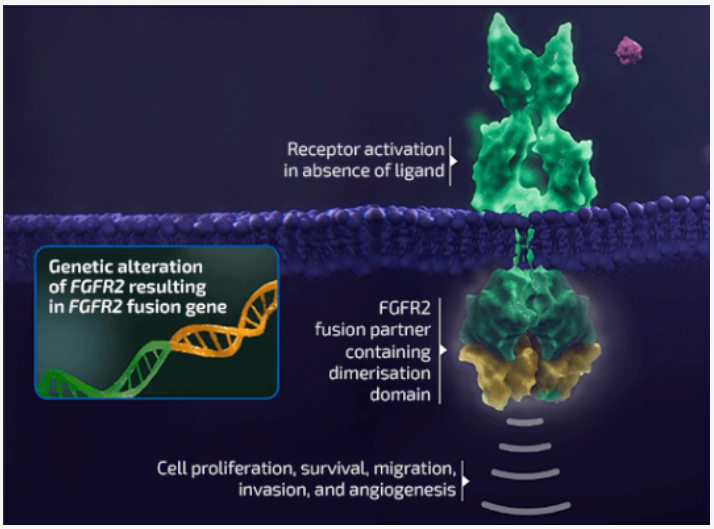


Figure adapted from Babina IS, Turner NC. 2017,[1] Moeini A, et al. 2015,[14] and Touat M, et al. 2015[15]

- Tumour molecular profiling is necessary to identify *FGFR2* fusions.[5,9] Assessment for *FGFR2* fusion positivity should be performed with an appropriate diagnostic test[7]
- *FGFR2* fusions involve a wide range of fusion partners.[9] To identify patients with *FGFR2* fusion-positive cholangiocarcinoma (CCA), it is important to select an assay that:
  - Specifically detects *FGFR2* fusions (distinct from *FGFR2* point mutations)[16,17]
  - Detects *FGFR2* fusions with a wide range of fusion partners[16,17]
- The molecular diversity of CCA supports the use of DNA- or RNA-based next-generation sequencing (NGS) assays as standard to detect both known and novel *FGFR2* fusions or rearrangements[18]

Testing methodologies for the detection of *FGFR2* fusions

- A number of methods with varying specificity can be used to detect *FGFR2* fusions[7]

Immunohistochemistry (IHC)	Reverse transcriptase polymerase chain reaction (RT-PCR)	Fluorescence *in situ* hybridisation (FISH)	Next-generation sequencing (NGS)

Least appropriate[7,19–27] → **Most appropriate**[7,19–27]

Advantages and challenges of different testing methodologies for the detection of *FGFR2* fusions

Immunohistochemistry (IHC)[7,17]

Advantages	Challenges
+ Inexpensive process	– Very low sensitivity for identifying rare fusions
+ Can detect fusions when rearrangements lead to overexpression of the fused protein	– Many IHC approaches use antibodies that cannot distinguish wild-type FGFR2 from fusion proteins
+ Can provide information about specific fusions depending on protein localisation	– No IHC method has been proven to have sufficient sensitivity and specificity to detect FGFR fusions

Reverse transcriptase polymerase chain reaction (RT-PCR)[17,20,21]

Advantages	Challenges
+ Highly sensitive	– Methodology is limited to *FGFR2* gene fusions with known fusion partners
+ Assay can be multiplexed to cover a range of mutations within a single reaction	– Requires prior knowledge of both fusion partners; novel fusion partners cannot be detected
+ Can easily be performed using clinical formalin-fixed paraffin-embedded samples	– Assay probes have to be designed for each specific fusion combination
	– Sensitive to cross-contamination linked to the carry-over of PCR products

Fluorescence *in situ* hybridisation (FISH)[7,22-25]

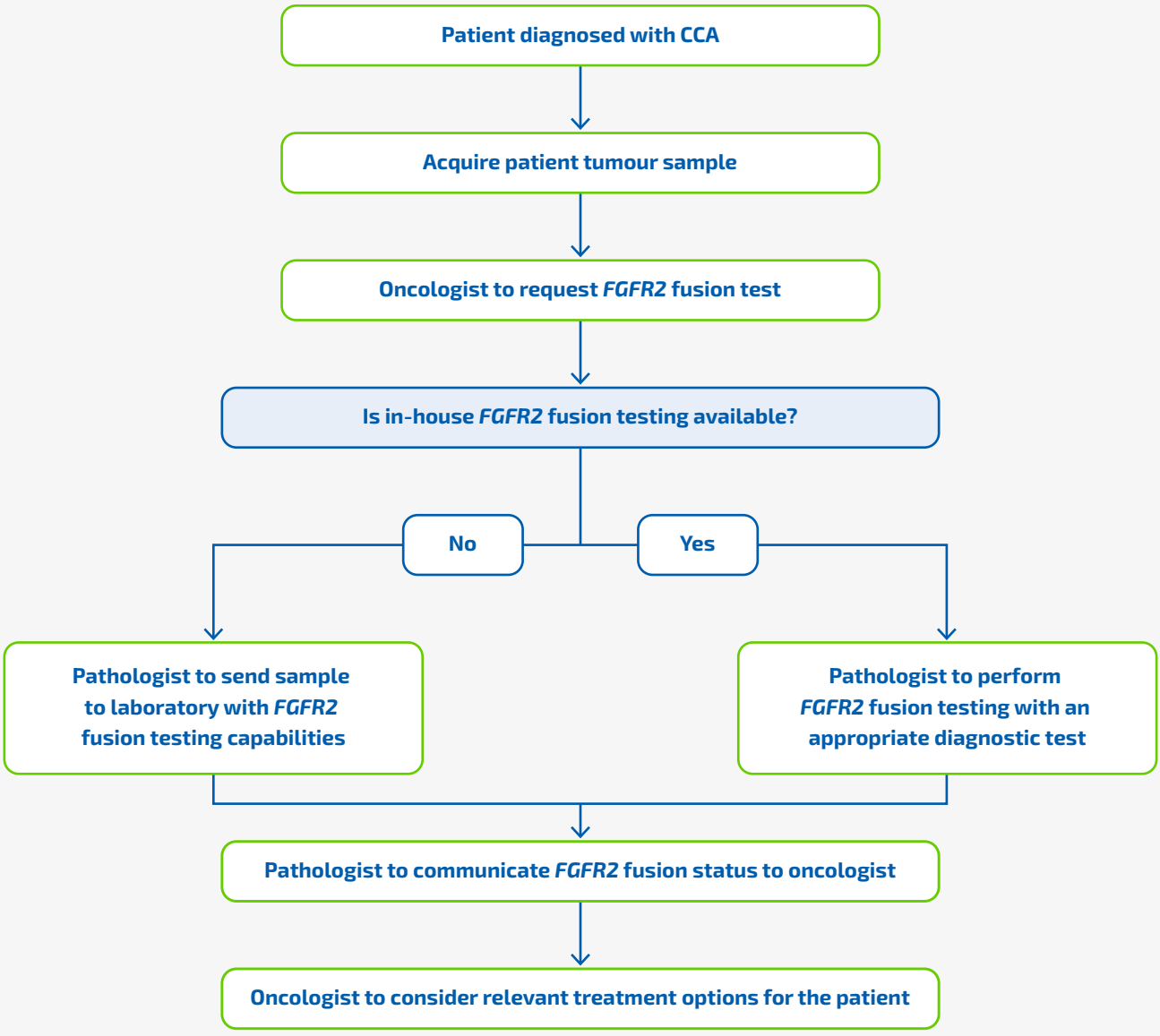
Advantages	Challenges
+ Inexpensive process	– Low-resolution method
+ Well-established methodology and widely available within clinical laboratories	– Mainly restricted to the detection of DNA
+ Does not require living cells	– Complex rearrangements are usually not easily detectable
+ Can be easily performed on clinical formalin-fixed paraffin-embedded samples	– Intrachromosomal rearrangements, which account for about 50% of *FGFR2* fusions in intrahepatic cholangiocarcinoma, can lead to false-negative results
+ Break-apart FISH probes can detect unknown fusion partners	– Break-apart FISH probes cannot identify the fusion partner
+ Relatively fast turnaround time	– Labour intensive and requires experienced pathologists

Next-generation sequencing (NGS)[7,22,23,26,27]

Advantages	Challenges
+ Multiple targets simultaneously analysed in a single sample	– Slow turnaround time
+ High sensitivity and specificity	– Not cost effective for small sample numbers
+ Detects both known and novel fusions, regardless of breakpoints or fusion partners (depending on library prep method)	– Requires bioinformatics and trained personnel
+ Commercial kits covering gene fusions are available	– **DNA-based:** detection of novel fusions might be limited, especially when large intronic regions are involved
+ **RNA-based:** can distinguish in-frame, transcribed gene fusions versus out-of-frame fusions and avoid difficulties of sequencing large intronic regions	– **RNA-based:** sensitivity depends on the expression levels of the novel fusion gene; RNA is less stable than DNA

The European Society for Medical Oncology (ESMO) recommends routine use of NGS to detect *FGFR2* fusions in advanced CCA[28]

Proposed algorithm of how *FGFR2* fusion testing can be incorporated into a diagnostic work-up



CCA, cholangiocarcinoma; FGFR2, fibroblast growth factor receptor 2

Visit www.incyte.com/what-we-do/clinical-trials to learn more about Incyte-sponsored clinical trials for patients with *FGFR2* fusion- or rearrangement-positive CCA

A multidisciplinary team (MDT) approach is crucial to optimise patient care in iCCA[29]

- As part of this MDT approach, a tumour molecular profiling plan should be considered early in your patient's treatment journey

- Key considerations for molecular profiling:[30]
  - Determining which clinically relevant genes to test for
  - Understanding test sample requirements (quantity and quality)
  - Understanding strengths and limitations of different testing methodologies
  - Understanding turnaround times
  - Understanding clinical implications of test results

External quality assurance programmes are essential to ensure accurate and reliable clinical biomarker testing[31]

Visit www.iqnpath.org to learn more about external quality assurance schemes for molecular testing in Europe

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