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\(^3,4\).

- 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\(^7\).

- The development of fusion proteins with oncogenic potential can result from gene fusion events involving a range of different partner genes\(^7\).

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\(^14,15\).

Abnormal FGFR2 signalling pathway

- Tumour molecular profiling is necessary to identify FGFR2 fusions.\(^5,7\) 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\(^18\).

Figure adapted from Babina IS, Turner NC. 2017;\(^7\) Moeini A, et al. 2015;\(^16\) and Touat M, et al. 2015;\(^16\)

Figure based on Jain A, et al. 2018;\(^5\) Lowery MA, et al. 2018;\(^9\) and Shibata T, et al. 2018\(^10\)
Testing methodologies for the detection of FGFR2 fusions

- A number of methods with varying specificity can be used to detect FGFR2 fusions.

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<tr>
<th>Methodology</th>
<th>Advantages</th>
<th>Challenges</th>
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<tr>
<td>Immunohistochemistry (IHC)</td>
<td>+ Inexpensive process</td>
<td>- Very low sensitivity for identifying rare fusions</td>
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<td>+ Can detect fusions when rearrangements lead to overexpression of the fused protein</td>
<td>- Many IHC approaches use antibodies that cannot distinguish wild-type FGFR2 from fusion proteins</td>
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<td>+ Can provide information about specific fusions depending on protein localisation</td>
<td>- No IHC method has been proven to have sufficient sensitivity and specificity to detect FGFR fusions</td>
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<td>Reverse transcriptase polymerase chain reaction (RT-PCR)</td>
<td>+ Highly sensitive</td>
<td>- Methodology is limited to FGFR2 gene fusions with known fusion partners</td>
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<td>+ Assay can be multiplexed to cover a range of mutations within a single reaction</td>
<td>- Requires prior knowledge of both fusion partners; novel fusion partners cannot be detected</td>
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<td>+ Can easily be performed using clinical formalin-fixed paraffin-embedded samples</td>
<td>- Assay probes have to be designed for each specific fusion combination</td>
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<td>- Sensitive to cross-contamination linked to the carry-over of PCR products</td>
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<td>Fluorescence in situ hybridisation (FISH)</td>
<td>+ Inexpensive process</td>
<td>- Low-resolution method</td>
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<td>+ Well-established methodology and widely available within clinical laboratories</td>
<td>- Mainly restricted to the detection of DNA</td>
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<td>+ Does not require living cells</td>
<td>- Complex rearrangements are usually not easily detectable</td>
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<td></td>
<td>+ Can be easily performed on clinical formalin-fixed paraffin-embedded samples</td>
<td>- Intrachromosomal rearrangements, which account for about 50% of FGFR2 fusions in intrahepatic cholangiocarcinoma, can lead to false-negative results</td>
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<td>+ Break-apart FISH probes can detect unknown fusion partners</td>
<td>- Break-apart FISH probes cannot identify the fusion partner</td>
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<td>+ Relatively fast turnaround time</td>
<td>- Labour intensive and requires experienced pathologists</td>
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<td>Next-generation sequencing (NGS)</td>
<td>+ Multiple targets simultaneously analysed in a single sample</td>
<td>- Slow turnaround time</td>
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<td>+ High sensitivity and specificity</td>
<td>- Not cost effective for small sample numbers</td>
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<td>+ Detects both known and novel fusions, regardless of breakpoints or fusion partners (depending on library prep method)</td>
<td>- Requires bioinformatics and trained personnel</td>
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<td>+ Commercial kits covering gene fusions are available</td>
<td>- DNA-based: detection of novel fusions might be limited, especially when large intronic regions are involved</td>
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<td>+ RNA-based: can distinguish in-frame, transcribed gene fusions versus out-of-frame fusions and avoid difficulties of sequencing large intronic regions</td>
<td>- RNA-based: sensitivity depends on the expression levels of the novel fusion gene; RNA is less stable than DNA</td>
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Least appropriate: RT-PCR, FISH

Most appropriate: IHC, NGS
The European Society for Medical Oncology (ESMO) recommends routine use of NGS to detect *FGFR2* fusions in advanced CCA.

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

1. Patient diagnosed with CCA
2. Acquire patient tumour sample
3. Oncologist to request *FGFR2* fusion test
4. Is in-house *FGFR2* fusion testing available?
   - No: Pathologist to send sample to laboratory with *FGFR2* fusion testing capabilities
   - Yes: Pathologist to perform *FGFR2* fusion testing with an appropriate diagnostic test
5. Pathologist to communicate *FGFR2* fusion status to oncologist
6. Oncologist to consider relevant treatment options for the patient

CCA, cholangiocarcinoma; FGFR2, fibroblast growth factor receptor 2

Visit [www.incyte.com/what-we-do/clinical-trials](http://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\textsuperscript{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:\textsuperscript{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\textsuperscript{31}

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

REFERENCES: