This article, sponsored by Incyte Corporation, is based on a paid interview with Ghassan Abou-Alfa, MD, a medical oncologist at Memorial Sloan Kettering Cancer Center, conducted on November 12, 2021.

The Importance of Molecular Profiling in Cholangiocarcinoma

Because of its relatively low incidence and challenges in diagnosis, cholangiocarcinoma (CCA) has traditionally been poorly understood.[1] In recent years, however, our understanding of CCA has greatly improved, largely due to significant leaps in the genomic characterization of this cancer.

Dr Ghassan K. Abou-Alfa, MD
Medical Oncology
Memorial Sloan Kettering Cancer Center

CCA exhibits several characteristic genomic alterations, some of which are targetable with either existing therapies or those currently in development.[2-5] But to make use of these newer therapies, we must have a clear understanding of each patient's individual tumor and which genomic alterations it carries. Because of this, molecular profiling in CCA is a critical step in not only the diagnosis of the condition, but in the development of an appropriate treatment plan as well. Timing of testing, ideally at diagnosis, is also important.

FGFR2 fusions and rearrangements, in particular, are a common and important target in patients with intrahepatic CCA (iCCA).[2,5-7] Because of the nature of genomic alterations, selection of an appropriate molecular profiling assay is important to ensure that potential *FGFR2* fusions and rearrangements are not missed. Next-generation sequencing (NGS) is currently the technique that offers the greatest opportunity to identify patients with FGFR fusions regardless of fusion partner.[8]

In this paper, we will cover the following topics related to iCCA:

- Genomic alterations in iCCA
- *FGFR2* fusions and rearrangements
- Molecular profiling in iCCA
- Counseling patients
- Establishing a molecular profiling plan

> **Identification of genomic alterations is now an essential part of a definitive diagnosis in intrahepatic cholangiocarcinoma.**

Evolving Landscape of iCCA

Major gains in the genetic characterization of iCCA have been made over the past seven or eight years. One such important concept we developed at Memorial Sloan Kettering was that certain genetic alterations do not coexist in iCCA, a phenomenon we named mutual exclusivity of genetic alterations (MEGA). However, there are genetic alterations that can and do coexist in iCCA.[5]

Another was looking across cancer types to better understand tumor genomics. In the case of iCCA, learnings from neuro-oncology helped to further understanding of iCCA. In particular, the presence of *IDH* mutations in glioblastoma multiforme informed work on this genomic alteration in iCCA.[9]

As the genomic profile of iCCA has become clearer, actionable alterations that are amenable to treatment with either existing agents or those in development have come into focus. Based on the literature, actionable genomic alterations have been identified in up to 50% of patients with iCCA, although this percentage may vary in actual practice.[2-5] Alterations may include point mutations, gene amplifications, and chromosomal rearrangements that may result in fusion proteins.[5,10,11]

> **It is critical to know the exact type of genomic alteration to help set expectations for how the disease may progress or respond to treatment.**

Molecular profiling is necessary to detect actionable genomic alterations, but there are additional benefits. Molecular profiling data may help in identifying clinical trials for which the patient may be eligible, helps add the totality of our knowledge about iCCA and, moving forward, may help us to better understand the relationship between specific alterations and prognosis.[12]

Common actionable genomic alterations in iCCA.[2,5-7]

Selected Actionable Genomic Alterations in iCCA	
Type of Genomic Alteration	Prevalence in iCCA, %
IDH mutation[6,13]	20–25
FGFR2 fusion[2,6,7]	10–16
KRAS mutation[14]	9–24
dMMR[15]	<4
MSI-H status[5,16]	<1–2.5

IDH mutations are the most common type of actionable genomic alteration found in iCCA, having been identified in up to 20% to 25% of patients.[6,13] These mutations lead to accumulation of 2-hydroxyglutarate (2-HG), which may promote oncogenesis.[17,18] Elevated 2-HG levels have been demonstrated in CCA.[17]

FGFR has also emerged as an important tumorigenic driver in various tumor types, including iCCA.[19-21] *FRGR2* fusions are among the most common actionable genomic alterations, having been identified in up to 10% to 16% of patients with iCCA.[2,6,7] When *FGFR2* fusions occur, they cause constitutive *FGFR2* signaling, which contributes to a variety of tumorigenic processes including cell proliferation, survival, migration, invasion, and angiogenesis.[19,22]

> **Before they come to the oncology clinic, patients need to have next generation sequencing performed. Whether it's the gastroenterologist, hepatologist, or even surgeon, the first touchpoint for these patients should already be aware that they need NGS testing. The earlier the better, from day zero.**

Molecular profiling is necessary to identify actionable genomic alterations in iCCA

Routine molecular profiling is important in identifying actionable genomic alterations in patients with iCCA.

A variety of molecular profiling methods are now available, including next generation sequencing (NGS), fluorescence in situ hybridization (FISH), and liquid biopsy. However, this latter technique is not yet sufficiently refined for routine clinical use in iCCA.

NGS

- Allows the opportunity, in a molecular target-rich disease like CCA, to analyze a tissue sample for multiple alterations at the same time
- While specimen size for NGS is initially larger and turnaround time may be longer, NGS may preclude the need for repeat biopsy to obtain additional tissue for molecular profiling[23]

FISH

- Conducted to identify one specific, predetermined alteration at a time
- Carries potential risk of missing other alterations[24]

Liquid biopsy

- Less invasive than tissue biopsy and easily repeatable
- Lacks clinical validation
- Not sufficiently developed for use in iCCA at present[25]

Of note, *FGFR2* fusions may require special consideration for NGS testing. *FGFR2* fusions have a wide range of fusion partners, which may be a consideration for assay selection. In an analysis of genomic alterations in a large clinical trial in cholangiocarcinoma, *BICC1* was the most common fusion partner, occurring in about 30% of patients. However, there were numerous other fusion partners, including partners occurring in a small percentage of patients all the way down to "N-of-One" alterations that were unique to individual patients.[8]

A comprehensive NGS test should be able to detect all *FGFR2* fusions, including those with known (frequently occurring) and unknown (rare or patient-specific) fusion partners.[5,10,26] Effective testing should also distinguish *FGFR2* fusions from point mutations.[5,8]

FGFR2 fusions have a wide range of fusion partners.[1] Therefore, to identify patients with _FGFR2_ fusions, it is important to select an assay that:

- Can detect all *FGFR2* fusions, including those with known or unknown fusion partners[1,3,6]
- Reports *FGFR2* fusions (vs *FGFR2* mutations)[1,2]

Over time, we may find that location of a tumor, for example, more toward the hepatic periphery, may be associated with specific genomic alterations. There are also data suggesting that artificial intelligence applications may be useful for analyzing tumors to identify "signatures" of specific genomic alterations.[27] However, these types of techniques are far from fully developed, and we need to await further work before these types of diagnostic modalities will be ready for use in the clinic.

Practical considerations for molecular profiling

There are a number of practical considerations to take into account with molecular profiling. The first consideration is timing. It is important to perform molecular profiling at diagnosis.[28] Getting the results of molecular profiling may take some time, so testing at diagnosis can help avoid unnecessary delays.

Biopsy technique is also an important consideration for NGS testing in patients with iCCA. Additional tissue may be required to satisfy NGS testing in addition to the pathological diagnosis, so the initial biopsy technique used should account for this. In my institution's experience, about 20%-30% of patients may not have adequate tissue taken at the time of initial biopsy. Though the specimen size for NGS is initially larger and the turnaround time may be longer, it may preclude the need for further biopsies for purposes of molecular profiling.[23]

Although more invasive than fine-needle aspiration, core-needle biopsy typically yields enough tissue to allow for comprehensive molecular profiling as well as other examinations.[29,30] Obtaining an adequate biopsy specimen at the time of initial biopsy can help to prevent the need for rebiopsy later, when the patient's condition—including issues like the potential need to interrupt therapy and the need to improve blood counts—may pose challenges.

Even if some time has passed since diagnosis, it may not be too late to perform NGS testing. In some cases, tissue from the initial biopsy may be usable for NGS testing. However, if it is not, patients may often be willing to undergo repeat biopsy if it will have an impact on treatment decisions.

While liquid biopsy is an exciting prospect in many tumor types, it is too early to rely on liquid biopsy in iCCA. It is unclear how liquid biopsy would be best used in iCCA and how results should be interpreted with reference to clinical outcomes and decision-making as well as how results of liquid biopsy correlate with tissue biopsy. Further research and refinement of liquid biopsy holds promise for the future.

Counseling patients about molecular profiling

As with other aspects of diagnosis and treatment, educating patients about the role molecular profiling plays in the care of iCCA is critical. Rather than focusing on the more technical aspects of NGS testing, helping patients to gain a high-level understanding of the role of molecular profiling in their care tends to be more helpful. Patients may easily grasp that having a better understanding of the genomic alterations in their tumor can help open up additional treatment options.

It is important, however, to place molecular profiling in a realistic context for the patient. Because we can't know prospectively what the results of NGS will be, it is important to counsel patients that the results may not return an actionable alteration.

Additionally, the patient should understand that if the results of NGS do identify actionable alterations, this result will be combined with multiple other considerations when developing the overarching treatment plan. For example, if I have a patient that is doing well on a current course of chemotherapy, I won't interrupt or change that therapy solely on the basis of the results of molecular profiling.

Finally, we need to set realistic expectations for the results of molecular profiling. We should be very careful not to lead the patient to believe that certain genomic alterations are associated with better outcomes than others based on our current knowledge of iCCA. Further data are needed about how specific genomic alterations may affect outcomes.

Making molecular profiling a standard part of care of iCCA

Given the importance of molecular profiling in iCCA, having a standard plan for molecular profiling may help to facilitate testing at diagnosis. Note that molecular profiling may involve a team-based, multispecialty approach.

Some questions to consider in establishing a molecular profiling plan include:
Who at my institution is responsible for ordering molecular profiling?
What molecular profiling technique is used?
What other specialties do I need to interact with to make sure molecular profiling is carried out successfully?
How can I help ensure that an adequate tissue sample—one that will provide tissue for pathological diagnosis as well as molecular profiling—is obtained at biopsy?
What can I do to help expedite the biopsy process and the sending of tissue for testing?

Putting it all together

Advances in the understanding of the genomics of iCCA make it possible for you to offer additional treatment options for your patients, potentially impacting clinical outcomes.

> **There's no doubt having a standard molecular profiling plan will make a big difference for patients with iCCA.**

Taking Action For Your Patients With iCCA

- Our understanding of the genomics of iCCA has evolved greatly over time and genomic characterization of iCCA is essential
- Many genomic alterations in iCCA are now actionable[2-5]
- In order to identify genomic alterations, molecular profiling must be performed
- Molecular profiling should be performed at diagnosis using a comprehensive NGS assay that can identify both known (frequently occurring) and unknown (rare or patient-specific) fusions and rearrangements[5,10,26]

Having a clear plan for how your practice carries out molecular profiling at diagnosis is critical to allow patients to take full advantage of targeted treatment options

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