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Hybrid Capture-Based Tumor Sequencing and Copy Number Analysis to Confirm Origin of Metachronous Metastases in *BRCA1*-Mutant Cholangiocarcinoma Harboring a Novel *YWHAZ-BRAF* Fusion

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Disclosures of potential conflicts of interest may be found at the end of this article.

Abstract

Biliary tract cancers such as cholangiocarcinoma represent a heterogeneous group of cancers that can be difficult to diagnose. Recent comprehensive genomic analyses in large cholangiocarcinoma cohorts have defined important molecular subgroups within cholangiocarcinoma that may relate to anatomic location and etiology [1–4] and may predict responsiveness to targeted therapies in development [5–7]. These emerging data highlight the potential for tumor genomics to inform diagnosis and treatment options in this challenging tumor type. We report the case of a patient with a germline *BRCA1* mutation who presented with a cholangiocarcinoma driven by the novel *YWHAZ-BRAF* fusion. Hybrid capture-based DNA sequencing and copy number analysis performed as part of clinical care demonstrated that two later-occurring tumors were clonally derived from the primary cholangiocarcinoma rather than distinct new primaries, revealing an unusual pattern of late metachronous metastasis. We discuss the clinical significance of these genetic alterations and their relevance to therapeutic strategies. ***The Oncologist*** 2018;23:998–1003

Key Points

- Hybrid capture-based next-generation DNA sequencing assays can provide diagnostic clarity in patients with unusual patterns of metastasis and recurrence in which the pathologic diagnosis is ambiguous.
- To our knowledge, this is the first reported case of a *YWHAZ-BRAF* fusion in pancreaticobiliary cancer, and a very rare case of cholangiocarcinoma in the setting of a germline *BRCA1* mutation.
- The patient's *BRCA1* mutation and *YWHAZ-BRAF* fusion constitute potential targets for future therapy.

Patient Story

A 58-year-old man with chronic hepatitis C virus (HCV) infection developed a palpable abdominal mass in 2011. A computed tomography (CT) scan of the abdomen revealed a 13 × 12 cm left hepatic mass with central necrosis (Fig. 1A) concerning for primary liver malignancy, without any evidence of extrahepatic disease. An extended left hepatectomy was performed. Pathology showed moderately to poorly differentiated adenocarcinoma with immunohistochemistry positive for CK7, CK19, and MOC31 and negative for HepPar1 and arginase, compatible with a diagnosis of intrahepatic cholangiocarcinoma (Fig. 2A). The surgical margins were negative, with no tumor in one lymph node, corresponding to stage pT1cN0M0 cholangiocarcinoma. The patient did not receive any adjuvant therapy in accordance with clinical practice guidelines at that time.

Three years later, in 2014, a surveillance CT scan of the chest, abdomen, and pelvis showed a distal pancreatic duct stricture (Fig. 1B) not present on surveillance imaging 6 months earlier. Esophagogastroduodenoscopy with endoscopic ultrasound was performed, showing a 9 × 9 mm obstructing pancreatic body lesion. Fine needle aspiration of that site identified malignant cells compatible with adenocarcinoma. Multidisciplinary Tumor Board review at that time assessed that the pancreatic lesion most likely represented a second primary malignancy rather than a late metastasis from cholangiocarcinoma, based

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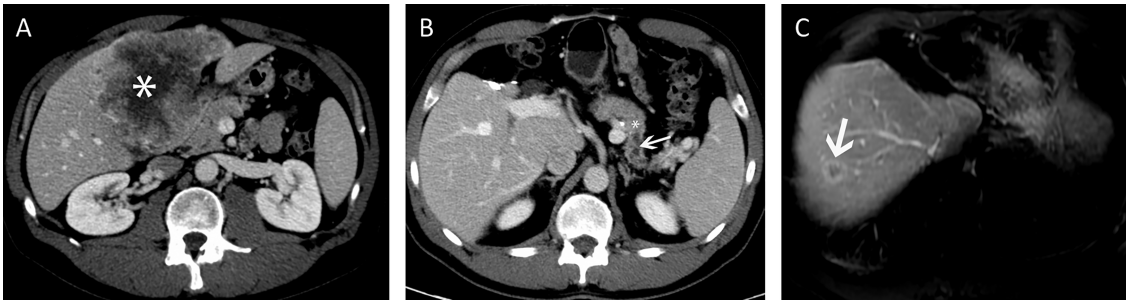

Figure 1. Imaging of primary cholangiocarcinoma and two metachronous tumors. Contrast-enhanced computed tomography **(A, B)** and magnetic resonance imaging **(C)** scans of the patient's abdomen show an intrahepatic cholangiocarcinoma in 2011 (A, white star); a second tumor in the body of the pancreas with focal stricture in 2014 (B, small white arrow); and a third tumor in the remnant right lobe of liver in 2016 (C, larger white arrow).

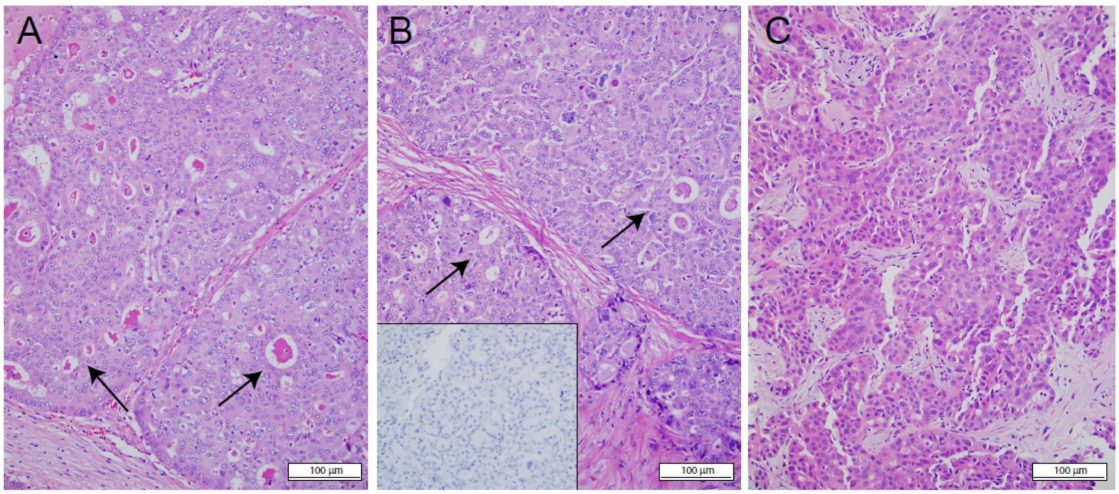

Figure 2. Histology of primary cholangiocarcinoma, pancreatic tumor, and liver tumor. Representative hematoxylin and eosin-stained sections of the liver mass resection from 2011 consistent with intrahepatic cholangiocarcinoma **(A)**, the pancreatic mass resection from 2014 **(B)**, and the liver mass biopsy from 2016 (**C**; all ×200). Trypsin stains were negative on the pancreatic mass (B, inset). All three specimens show similar histology with sheets and large nests of cells with eosinophilic cytoplasm, large nuclei, and single prominent nucleoli. The liver mass (A) and pancreatic mass (B) show areas of cribriform architecture (arrows).

upon the focal stricture presentation, atypical location for metastasis, and relatively late time course. The patient subsequently underwent distal pancreatectomy with splenectomy. Pathology of the resected specimen showed a poorly differentiated carcinoma with extensive pancreatic involvement not visualized on prior imaging (Fig. 2B). The surgical margin was focally positive, but no tumor was detected in 12 lymph nodes, consistent with stage pT3N0 primary pancreatic carcinoma. Immunohistochemistry was strongly positive for CK7 and keratin proteins, although trypsin, chromogranin A, and synaptophysin were negative, excluding neuroendocrine differentiation and atypical for acinar carcinoma. The patient was treated for 6 months with gemcitabine as adjuvant chemotherapy, followed by chemoradiation with capecitabine as a radiosensitizer due to positive margin status and high recurrence risk.

Subsequent surveillance imaging after completion of adjuvant pancreatic cancer therapy showed no evidence of recurrence until 2016, when magnetic resonance imaging of the abdomen and pelvis showed a new 1.5 cm lesion in the right lobe of the liver (Fig. 1C). A core needle biopsy was performed, and pathology showed a poorly differentiated carcinoma similar by histology and immunostaining to the pancreatic tumor from 2014 as well as to the original cholangiocarcinoma from 2011 (Fig. 2C).

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Given the patient's two presumed independent primary cancers and his family history of maternal breast cancer, he was referred for genetic testing (Invitae, Palo Alto, CA). He was found to carry a germline *BRCA1* splice site mutation (c.213-11T>G, ClinVar variation ID: 37449), previously described in association with hereditary breast and ovarian cancer syndrome and, less commonly, with other cancers including pancreaticobiliary tumors [8–11].

Despite the histologic similarities between his three tumors, it was unclear whether they were clonally related or separate primaries, as might be expected in a patient presenting with a germline *BRCA1* mutation and HCV infection. To assess their relationship, hybrid capture-based next-generation DNA sequencing was performed on archival tissue samples from the 2011, 2014, and 2016 tumors using the FoundationOne platform (Foundation Medicine, Cambridge, MA), as has been previously described [12]. Detection of copy number abnormalities and fusion breakpoints was performed routinely as part of FoundationOne testing. Briefly, standard analysis involved a series of algorithms that was used to normalize coverage distribution and allele frequencies against a process-

	Sample 1	Sample 2	Sample 3
Year	2011	2014	2016
Tumor site	Left lobe liver	Pancreatic body mass	Right lobe liver
TMB	6	6	9
Mutations	***BRAF YWHAZ-BRAF* fusion** ***BRCA1* 213-11T>G (48.0%)** ***BRCA1 W1718**** (30.0%) ***PREX2* R115Q (51.0%)** ***TP53* loss (exons 8–11)**	***BRAF YWHAZ-BRAF* fusion** ***BRCA1* 213-11T>G (47.0%)** ***BRCA1 W1718**** (25.0%) ***PREX2* R115Q (46.0%)** ***TP53* loss (exons 8–11)** *FBXW7* E113D (subclonal, 1.0%)	***BRAF YWHAZ-BRAF* fusion** ***BRCA1* 213-11T>G (40.0%)** ***BRCA1 W1718**** (34.0%) ***PREX2* R115Q (48.0%)** ***TP53* loss (exons 7–11)** *MYC* amp (equivocal, 8 copies) *GNAS* amp (equivocal, 8 copies)

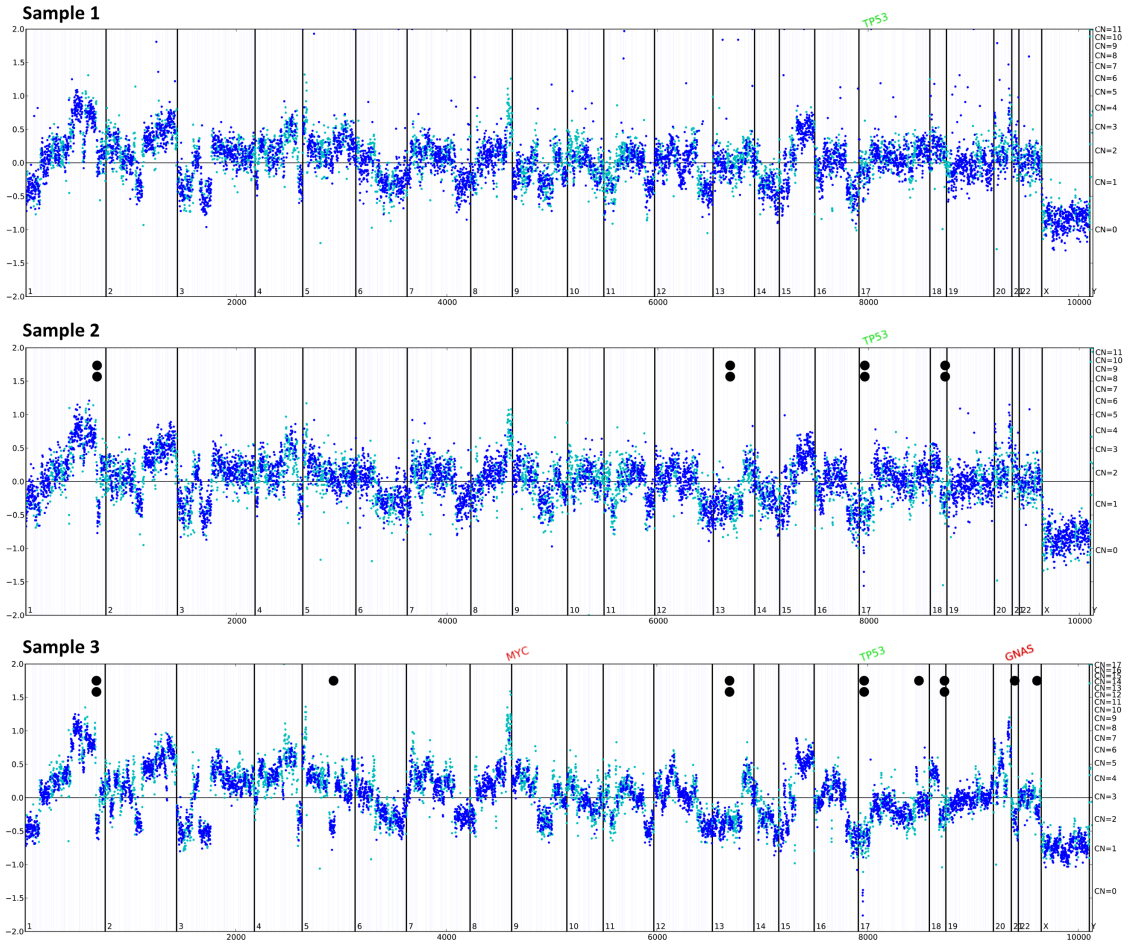

Figure 3. Genomic profiling shows shared mutations and copy number abnormalities across the three tumors. Top: genetic profile of each tumor sample. Shared mutations in all three samples are shown in bold. Mutation allele frequency or copy number is shown next to its respective mutation. Bottom: copy number abnormality plots. Genes listed in the genetic profile as having a copy number loss or amplification are indicated in green or red, respectively, above the corresponding copy number abnormality (CNA). Acquired CNAs common to both samples from 2014 and 2016 are indicated with double dots and CNAs only found in the 2016 sample with single dots above the CNA plot. TMB was comparable in all three samples, although slightly higher in the latest 2016 tumor (top panel).

Abbreviations: amp, amplification; TMB, tumor mutational burden (mutations per megabase).

matched control sample and to plot the normalized data on a logarithmic scale. Clustering of baited targets and minor allele single nucleotide polymorphisms were used to define the boundaries of genomic segments. Using tumor purity and base ploidy, probability matrices were generated and data were fit to a statistical copy number model to detect copy number abnormalities. Genomic rearrangements were detected through clustering of chimeric paired-end reads, and custom algorithms were used to annotate breakpoint fusion sites, as previously described [13].

In addition to the expected germline *BRCA1* mutation, all three tumors were found to share multiple genomic alterations

with similar allele frequencies, including a *BRCA1 W1718** nonsense mutation, *PREX2* R155Q mutation, *TP53* deletion spanning exons 8 to 11, and an in-frame *YWHAZ-BRAF* fusion with identical breakpoints after exon 5 of YWHAZ and before exon 8 of BRAF (Fig. 3, top panel). Furthermore, copy number analysis showed strikingly similar copy number abnormalities (CNAs) among all three tumors, with acquisition of several new copy number aberrations in the 2014 and 2016 samples (Fig. 3, bottom panels).

Collectively, the shared mutations and CNAs, as well as the identical *YWHAZ-BRAF* fusion breakpoints, unambiguously indicated that the three tumors were clonally related. Further, CNAs shared among the two metastases but absent from the primary tumor (such as losses in 1q, 13p, 17p, and 18q) indicated that the 2016 liver metastasis derived from the 2014 pancreatic metastasis, rather than the 2011 primary (Fig. 4).

Functional and Clinical Significance

This case is unique because of the use of molecular profiling to confirm tumor clonality, the rare presentation of cholangiocarcinoma in the setting of a germline *BRCA1* mutation, and the presence of a novel *YWHAZ-BRAF* fusion.

A common challenge in oncology is distinguishing whether a new lesion in a patient with a history of cancer represents metastatic recurrence or a new primary malignancy. When traditional morphologic and immunohistochemical analysis is unable to definitively answer this question, molecular profiling can help—this has been illustrated in non-small cell lung cancer, in which molecular profiling can guide decision-making about the need for surgery or adjuvant chemotherapy [14–16], and other solid tumors [17, 18]. However, caveats exist: Intratumor genetic heterogeneity, tumor molecular evolution over time (particularly after intervening therapies affecting DNA stability), and technical challenges (e.g., related to the poor quality of DNA from archival formalin-fixed, paraffin-embedded tissue or contamination of tumor tissue by stromal cells) can all degrade the utility of molecular profiling data [19, 20]. When the somatic mutation profile by itself is insufficient to clearly distinguish separate primary tumors from metastases, computation of copy number information [21] and identification of passenger mutations can help confirm clonality [22–24]. In the present case, the combination of molecular profiling that identified canonical somatic mutations across the patient's 2011, 2014, and 2016 tumors, combined with highly concordant CNA patterns and matching *YWHAZ-BRAF* fusion breakpoints, unequivocally supported the diagnosis of late metachronous pancreatic and liver metastases.

This is also a rare case of cholangiocarcinoma in the setting of a germline *BRCA1* mutation [8–11]. The *BRCA1* gene, involved in DNA repair by homologous recombination, was discovered in association with hereditary breast and ovarian cancer syndrome [25] and has been shown to confer increased risk for colon, gastric, and pancreatic cancer, although its impact on biliary cancer is not well established [26]. Case reports and series have identified rare cases of cholangiocarcinoma associated with somatic or germline *BRCA1* and *BRCA2* mutations [8–11]. In the largest published series, of 18 cases of mutant *BRCA1*- or *BRCA2*-associated cholangiocarcinoma, the median overall survival for the subset of 11 patients with stage III/IV cholangiocarcinoma at diagnosis was 25 months, much longer

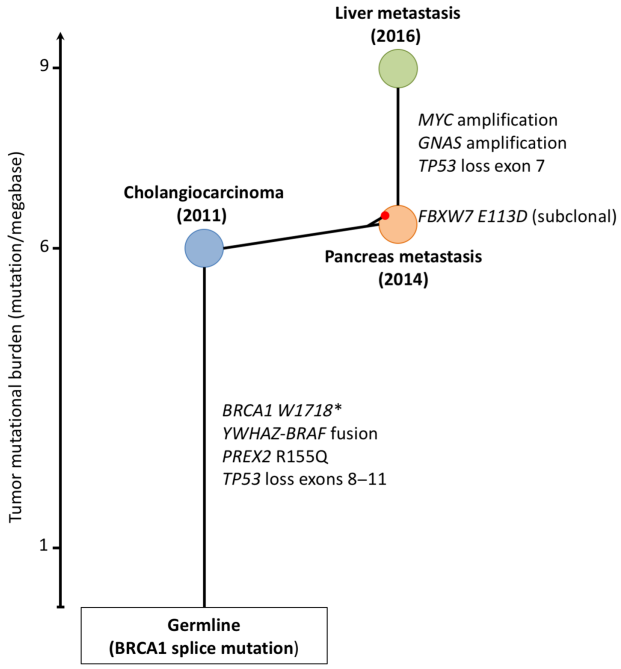

Figure 4. Schematic representation of clonal relationship between the three tumors. Private alterations occurred in the two later-occurring tumors. The pancreas tumor from 2014 showed a subclonal *FBXW7* E113D mutation, and the liver tumor from 2016 harbored *MYC* and *GNAS* amplifications.

than expected for an advanced biliary cancer population. This case builds upon the very limited historical data in demonstrating the potential for an unusual pattern of spread and time course of metastasis.

Finally, this is a rare case of a novel *BRAF* fusion. *BRAF* fusions are themselves rare events that can occur across a wide variety of tumor types. In one retrospective study, genomic profiling of 20,573 solid tumors identified *BRAF* fusions in only 0.3% of cases, with enrichment in certain tumor types including acinar pancreatic cancer [27–29]. However, in the present case, trypsin staining of the 2014 pancreatic resection was negative, arguing against occult acinar carcinoma. *BRAF* rearrangements are also rare among pancreaticobiliary cancers, occurring in 0.5% of 7,035 pancreaticobiliary cancer cases in one clinical sequencing database (Foundation Medicine, unpublished data). To our knowledge, the *YWHAZ-BRAF* fusion is novel among pancreaticobiliary cancers and has not been noted in any other case in the Foundation Medicine database [27]. The resulting fusion transcript expression, driven by the promoter of *YWHAZ*, is predicted to be in-frame and encodes a constitutively active BRAF kinase domain lacking its N-terminal auto-inhibitory domain. The *YWHAZ* gene, normally encoding the 14-3-3ζ protein, is ubiquitously expressed [30], and its transcript is strongly detected in cholangiocarcinoma, hepatocellular carcinoma, and pancreatic adenocarcinoma expression data in The Cancer Genome Atlas (www.cBioPortal.org).

Potential Strategies to Target the Pathway and Implications for Clinical Practice

This patient's *BRCA1* mutation and *YWHAZ-BRAF* fusion constitute potential targets for future therapy. In addition to the

germline *BRCA1* mutation, his tumors harbor a second *BRCA1* alteration in the form of a nonsense mutation. The two alterations are suspected to be in *trans*, leading to biallelic inactivation of *BRCA1*. In the event of future recurrence, he could potentially benefit from treatment with poly adenosine diphosphate ribose polymerase inhibitors or platinum chemotherapy, both of which have been shown to induce synthetic lethality in the context of BRCA deficiency [25]. Indeed, had the clonality of his 2014 metastasis been ascertained at the time of treatment, the treatment recommendation would have been a chemotherapy regimen of gemcitabine plus cisplatin established by the ABC-02 trial for advanced biliary cancer therapy [31].

BRAF fusions result in oncogenic activation of the mitogen-activated protein kinase signaling pathway via dimerization [32], and the patient's *YWHAZ-BRAF* fusion may be a therapeutic target, although clinical data are limited [27]. Although vemurafenib does not demonstrate efficacy in tumors harboring *BRAF* fusions [28, 32, 33], first-generation RAF inhibitors such as sorafenib have produced inconsistent effects, ranging from significant clinical response [27, 28, 34] to tumor growth promotion due to paradoxical activation [27, 35]. Preclinical studies and case reports suggest that MEK inhibition [27, 36] or a novel class of BRAF dimerization inhibitors may be effective in tumors driven by *BRAF* fusions [37]. A clinical trial of the BRAF dimerization inhibitor MLN2480/TAK-580 is ongoing (NCT02327169).

Patient Update

The patient's 2016 right lobe liver metastasis was treated with microwave ablation, and he is currently free of recurrence as of late 2017, over 6 years since the initial diagnosis of intrahepatic cholangiocarcinoma.

Conclusion

We have presented a unique case in which a hybrid capture-based next-generation sequencing assay capable of copy number and fusion breakpoint analyses confirmed the diagnosis of late, metachronous, oligometastatic recurrence of primary intrahepatic cholangiocarcinoma driven by a novel *YWHAZ-BRAF* fusion in a patient with a germline *BRCA1* mutation. This case highlights the utility of comprehensive genomic profiling to assist clinicians, pathologists, radiologists, and molecular medicine experts by reducing diagnostic uncertainty in ambiguous malignancies and complex recurrence patterns, particularly when treatment recommendations could vary depending on the result.

Author Contributions

Conception/design: Huat C. Lim, Meagan Montesion, Thomas Botton, Eric A. Collisson, Sarah E. Umetsu, Spencer C. Behr, John D. Gordan, Phil J. Stephens, Robin K. Kelley

Provision of study material or patients: Sarah E. Umetsu, Spencer C. Behr, John D. Gordan, Robin K. Kelley

Collection and/or assembly of data: Huat C. Lim, Meagan Montesion, Thomas Botton, Eric A. Collisson, Sarah E. Umetsu, Spencer C. Behr, John D. Gordan, Phil J. Stephens, Robin K. Kelley

Data analysis and interpretation: Huat C. Lim, Meagan Montesion, Thomas Botton, Eric A. Collisson, Sarah E. Umetsu, Spencer C. Behr, John D. Gordan, Phil J. Stephens, Robin K. Kelley **Manuscript writing**: Huat C. Lim, Meagan Montesion, Thomas Botton, Eric A. Collisson, Sarah E. Umetsu, Spencer C. Behr, John D. Gordan, Phil J. Stephens, Robin K. Kelley

Final approval of manuscript: Huat C. Lim, Meagan Montesion, Thomas Botton, Eric A. Collisson, Sarah E. Umetsu, Spencer C. Behr, John D. Gordan, Phil J. Stephens, Robin K. Kelley

Disclosures

Meagan Montesion: Foundation Medicine (E, OI); **Phil J. Stephens**: Foundation Medicine (OI). The other authors indicated no financial relationships.

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board

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For Further Reading:
Talia Golan, Maria Raitses-Gurevich, Robin K. Kelley et al. Overall Survival and Clinical Characteristics of BRCA-Associated Cholangiocarcinoma: A Multicenter Retrospective Study. ***The Oncologist*** 2017;22:804-810.

Implications for Practice:
BRCA-associated CCA is uncommon but a very important subtype of hepatic malignancies, due to its rising prevalence. Better clinical characterization of this subtype might allow application of targeted therapy for CCA patients with germline or somatic mutations in *BRCA1/2* genes, especially due to previously reported success of such therapies in other BRCA-associated malignancies. Thus this study, first of its kind, provides a basis for future multi-centered analyses in larger cohorts, as well as clinical trials. Additionally, this study emphasizes the importance of both germline and somatic genotyping for all CCA patients.