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Cancer Diagnostics and Molecular Pathology

New Routes to Targeted Therapy of Intrahepatic Cholangiocarcinomas Revealed by Next-Generation Sequencing

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

Key Words. Intrahepatic cholangiocarcinoma • Next-generation sequencing • Driver mutations • Targeted therapy

Abstract

Background. Intrahepatic cholangiocarcinoma (ICC) is a subtype of primary liver cancer that is rarely curable by surgery and is rapidly increasing in incidence. Relapsed ICC has a poor prognosis, and current systemic nontargeted therapies are commonly extrapolated from those used in other gastrointestinal malignancies. We hypothesized that genomic profiling of clinical ICC samples would identify genomic alterations that are linked to targeted therapies and that could facilitate a personalized approach to therapy.

Methods. DNA sequencing of hybridization-captured libraries was performed for 3,320 exons of 182 cancer-related genes and 36 introns of 14 genes frequently rearranged in cancer. Sample DNA was isolated from 40 μm of 28 formalin-fixed paraffin-embedded ICC specimens and sequenced to high coverage.

Results. The most commonly observed alterations were within *ARID1A* (36%), *IDH1/2* (36%), and *TP53* (36%) as well as amplification of *MCL1* (21%). Twenty cases (71%) harbored at least one potentially actionable alteration, including *FGFR2* (14%), *KRAS* (11%), *PTEN* (11%), *CDKN2A* (7%), *CDK6* (7%), *ERBB3* (7%), *MET* (7%), *NRAS* (7%), *BRCA1* (4%), *BRCA2* (4%), *NF1* (4%), *PIK3CA* (4%), *PTCH1* (4%), and *TSC1* (4%). Four (14%) of the ICC cases featured novel gene fusions involving the tyrosine kinases FGFR2 and NTRK1 (*FGFR2-KIAA1598, FGFR2-BICC1, FGFR2-TACC3*, and *RABGAP1L-NTRK1*).

Conclusion. Two thirds of patients in this study harbored genomic alterations that are associated with targeted therapies and that have the potential to personalize therapy selection for to individual patients. ***The Oncologist*** 2014; 19:235–242

Implications for Practice: The recent translation of next-generation DNA sequencing technology from the research laboratory to clinical practice has enabled oncologists to personalize therapy decisions for each patient by targeting the genomic alterations driving the disease. For tumors such as primary cholangiocarcinoma of the liver, this new ability to determine all of the major genomic alterations (base substitutions, short insertions and deletions, copy number changes, homozygous deletions, and gene fusions) on very small formalin-fixed paraffin embedded clinical samples holds great promise that less toxic targeted therapies may be available for patients currently being treated with conventional "one size fits all" approaches.

Introduction

Cancer of the bile ducts can arise within the liver as an intrahepatic cholangiocarcinoma (ICC) or originate from extrahepatic bile ducts as a bile duct carcinoma [1–4]. ICC is the second most common primary hepatic malignancy after hepatocellular carcinoma and accounts for 3% of the malignant tumors of the gastrointestinal system and 15% of primary hepatic malignancies [1–4]. In that ICC has a routine histologic appearance of an adenocarcinoma, the diagnosis of ICC on a liver biopsy requires an immunohistochemical study of the tumor and a thorough clinical workup including imaging studies to rule out a metastatic adenocarcinoma to the liver [1–4].

Numerous studies have indicated that the incidence and mortality from ICC are increasing worldwide [1–4]. ICC is associated with primary sclerosing cholangitis, parasitic biliary infection, polycystic disease of the liver, congenital intrahepatic bile duct dilatation (Caroli's disease), congenital hepatic fibrosis, and choledochal cysts [1–4]. Chronic hepatitis C infection is an established cause of ICC, with some studies describing a more than 300-fold increase in ICC incidence in patients with long-standing hepatitis C infections [5]. ICC has also been associated with cigarette smoking, alcohol consumption, and exposure to a variety of toxins and chemical

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carcinogens [1–4]. The initial symptoms of ICC are often vague, typically arise late in the course of the disease, and include abdominal pain, anorexia, and palpable abdominal mass lesions [1–4]. The median survival is less than 6 months for inoperable tumors and only 20%–40% for patients who undergo surgery and achieve clear margins [6, 7].

A series of previously published studies using traditional techniques have described a variety of gene mutations and genomic alterations in ICC, including well-known cancer-related genes such as *KRAS* and *BRAF* [8–10]. Nevertheless, these studies have focused predominantly on the causation and progression of the disease and not on a search for potential actionable genomic alterations that could lead to targeted therapies. Although several oncogenic alterations are known to influence ICC pathogenesis, the percentage of tumors expressing any given alteration remains low, limiting the ability to develop an effective therapy that would be broadly applicable to the treatment of ICC. For each particular tumor, discovering the limited number of targetable alterations will require a sensitive, specific sequencing assay capable of detecting all categories of genomic alterations in a large number of cancer-related genes. In the following study, ICC DNA extracted from clinical cases has been studied in depth by next-generation sequencing to assess how targeted therapies could be used to treat this devastating disease. More than two thirds of tumors were found to have at least one potentially clinically actionable alteration that suggests sensitivity to targeted therapies.

This updated approach to characterizing ICC tumors has also revealed several key concepts with the potential to guide future research and affect the treatment of ICC [7]. First, the prevalence of mutations within the RAS and PI3K pathways strongly suggests that therapies targeting key components of these signal transduction networks would be valuable for many patients with ICC. Second, these results highlight driver mutations that may facilitate the development of novel therapeutic strategies. Receptor tyrosine kinase fusions previously unidentified in ICC indicate that clinically available, targeted inhibitors more commonly used in other tumor types will be relevant for some patients. Furthermore, a host of mutations in proteins related to cell-cycle control suggest that CDK inhibitors under investigation in clinical trials may provide additional treatment options for nearly a quarter of patients with ICC.

Methods

Next-generation sequencing was performed on hybridization-captured, adaptor ligation-based libraries using DNA extracted from four formalin-fixed paraffin-embedded (FFPE) sections cut at 10 μm from 28 ICC that had clinically progressed after either surgical resection and/or conventional chemotherapy. The pathologic diagnosis of each case was confirmed on routine hematoxylin and eosin-stained slides, immunohistochemistry, and clinical/imaging evaluations to rule out the possibility of a nonhepatic primary adenocarcinoma. All samples sent for DNA extraction contained a minimum of 20% nuclei derived from tumor cells. Sequence samples were obtained from liver biopsies in 16 cases (59%), from liver resections in 10 cases (37%), a lymph node metastasis in 1 case (4%), and a lung metastasis in 1 case (4%). DNA sequencing was performed for 3,320 exons of 182 cancer-related genes and 36 introns of 14 genes frequently rearranged in cancer on indexed, adaptor-ligated, hybridization-captured fragments (Agilent SureSelect custom kit; Agilent Technologies, Palo Alto, CA, http://www.agilent.com) using 49-base pair paired reads on the Illumina HiSEquation 2000 (Illumina Inc., San Diego, CA, http://www.illumina.com) at an average sequencing depth of 1,115×. Resulting sequence data were evaluated for genomic alterations including point mutations, insertions, deletions, copy number alterations (amplifications and homozygous gene deletions), and select gene fusions/rearrangements, as described previously [11]. To maximize mutation-detection sensitivity in heterogeneous ICC specimens, the test was validated to detect base substitutions as well as short insertions and deletions at a ≥10% mutant allele frequency with ≥99% sensitivity. Publicly available and custom analysis tools (Foundation Medicine, Inc., Cambridge, MA, http://www.foundationmedicine.com) were used in combination to analyze the data and characterize genomic alterations.

Actionability Classification

The genomic alterations detected were further divided into two main classes of actionability: genomic alterations that predict sensitivity or resistance to approved or standard therapies and genomic alterations that are inclusion or exclusion criteria for specific experimental therapies in National Cancer Institute-registered clinical trials.

Results

A total of 28 patient samples were analyzed, including tumors from 10 male and 18 female patients with a mean age of 55.9 years (range: 23–75 years) (Table 1). Of these, 10 had undergone an attempted curative hepatic resection operation and 18 underwent only a biopsy procedure (16 liver, 1 lymph node, and 1 lung biopsies). Histology analysis showed 18 of the tumors to be intermediate histologic grade 2 and 10 to be high histologic grade 3. Thirteen of the 28 cases were confined to the liver and without vascular invasion (or pathologic stage I), five were stage II tumors (confined to the liver with vascular invasion), five were stage III tumors (local metastasis), and five were stage IV tumors with the tumor disseminated beyond the liver. Immunohistochemical workup of the ICC was available for review in 19 cases (68%) (Table 2). All 19 cases expressed cytokeratin 7 (CK7), and 6 expressed CK19, whereas only 3 expressed CK20. Of the 16 ICCs stained for the CDX2 marker, 5 were immunopositive. Immunostains to rule out nonhepatic primary tumors including TTF1 for non-small cell lung cancer; ER, PR, HER2, mammoglobin, and GCDFP for breast cancer; prostate-specific antigen for prostate cancer; synaptophysin and chromogrannin for neuroendocrine carcinoma; and calretinin for mesothelioma were uniformly negative in all cases when performed. AFP and HEPAR1 immunostaining to rule out primary hepatocellular carcinoma were used in three cases, and all were negative.

A total of 81 genomic alterations were identified in 35 genes with an average of 2.9 alterations per tumor (range: 1–9 alterations) (Fig. 1, supplemental online Table 1). The most common alterations were identified in *ARID1A* (36%), *IDH1/2* (36%), *TP53* (36%), and *MCL1* (21%, all amplifications). In this study, nine (35%) of the ICCs featured mutations in *IDH1* and one (4%) harbored a mutation in *IDH2*. Twenty cases (71%) harbored at least one potentially actionable alteration, with an average of 1.07 actionable alterations per patient including

Table 1. Clinical features of 28 cases of intrahepatic cholangiocarcinoma

Case no.	Patient age	Depth of sequencing coverage	Sex	Tumor grade	Tumor stage
1	64	1,329	F	2	1
2	68	1,348	F	2	1
3	59	793	M	2	1
4	54	351	F	2	2
5	58	1,078	F	2	3
6	59	382	M	2	2
7	47	288	F	2	4
8	48	1,242	F	2	1
9	50	980	M	2	4
10	46	564	M	3	4
11	56	1,370	F	3	2
12	58	1,434	F	2	1
13	49	1,047	F	3	1
14	69	1,318	M	3	1
15	64	174	F	3	2
16	54	1,132	F	2	2
17	50	1,062	F	2	3
18	83	1,273	M	2	3
19	75	1,511	M	2	1
20	76	1,417	F	3	1
21	46	1,447	M	3	1
22	71	1,099	M	2	2
23	65	1,675	M	3	1
24	66	1,184	F	2	4
25	NA	710	F	2	1
26	62	1,086	F	2	1
27	23	1,771	F	2	3
28	44	1,627	F	3	4

Abbreviation: NA, not available.

FGFR2 (14%), *KRAS* (11%), *PTEN* (11%), *CDKN2A* (7%), *ERBB3* (7%), *MET* (7%), *NRAS* (7%), *CDKN2A* (7%), *CDK6* (7%), *BRCA1* (4%), *BRCA2 (4%), NF1* (4%), *PIK3CA* (4%), *PTCH1* (4%), and *TSC1* (4%) (supplemental online Table 1). Of the three *KRAS* mutations identified in this study (12%), there were two G12D mutations and one G12C mutation. The cell-cycle regulation pathway genes *CDKN2A* and *CDK6* were each altered in 7% of the ICCs in this study. Four gene fusions involving protein kinases were identified, including three fusions between *FGFR2* and *BICC1*, *KIAA1598*, or *TACC3* and one fusion between the kinase *NTRK1* and *RABGAP1L*. Two of the three *FGFR2* fusions (Figs. 2, 3) and the one *NTRK1* fusion were novel discoveries. Neither the type nor the frequency of gene alteration was associated with patient age or gender. Two of the *FGFR2* fusions (67%) occurred in female patients and one (33%) occurred in a male. No information was available with regard to whether patients included in this ICC study also suffered from inflammatory bowel disease or with regard to therapy-specific clinical outcome.

The *FGFR2-BICC1* fusion was identified in a grade 2, stage II ICC from a liver biopsy in a 75-year-old man (case 2). This *FGFR2* fusion has been reported previously in cholangiocarcinoma [10]. This tumor also harbored a mutation in *IDH1*. The second *FGFR2* fusion was a novel *FGFR2-KIAA1598* fusion identified as the only mutation in a liver resection specimen of a moderately differentiated stage III ICC (case 27) treated with multiple rounds of chemotherapy, from a 23-year-old female patient. The third *FGFR2* fusion was a novel *FGFR2-TACC3* fusion in a pulmonary metastasis from a grade 3 ICC arising in the liver of a 44-year-old woman. Finally, a third novel fusion, *RABGAP1L-NTRK1*, was identified in the ICC from a 62-year-old woman on liver biopsy.

Two (7%) of the ICCs featured alterations in the mismatch repair genes *MSH2* and *MSH6*. Case 18 is a liver biopsy of a grade 2, stage II ICC in an 83-year-old male patient with a P1087fs*5 *MSH6* mutation associated with additional mutations in *EPHB1*, *EPHA7*, *CDH1*, *PIK3CA*, and *ARID1A*, along with an amplification of *MCL1*. Case 21 is a high-grade advanced stage ICC diagnosed on a lymph node biopsy from a 46-year-old female patient with an *MSH2* homozygous deletion. This tumor also featured mutations in *INHBA*, *BRCA2*, *TSC2*, *PTCH1*, *ERBB3*, *TP53*, and *ARID1A*. The high number of genomic alterations in these two cases are consistent with a hypermutator genotype associated with mismatch repair-deficient tumors.

Discussion

This study identified multiple alterations in *FGFR2*, including three gene fusions, and is the second report of *FGFR2* fusions in primary hepatic cholangiocarcinoma. Amplifications and gain-of-function mutations in *FGFR* genes have been reported in several cancer types and linked to tumor growth, invasion, and angiogenesis [12, 13]. *FGFR2* amplification as seen in case 7 has been reported in several cancer types, most frequently in breast and gastric carcinomas [14, 15]. FGFR2 has been shown to be expressed in cholangiocarcinoma, leading to activation of the MEK1/2 pathway [16]. Amplifications of *FGFR2* have been uniformly linked to FGFR2 protein overexpression [14–16]. In a recent study using whole-exome and whole-transcriptome sequencing, *FGFR2* fusions were identified in two of four cholangiocarcinomas sequenced (50%) [12]. In both of these cases, an *FGFR2-BICC1* fusion was identified [12]. A similar *BICC1-FGFR2* fusion was identified in case 19 of this series. The *FGFR2-BICC1* fusion results in truncation of the 3′UTR of *FGFR2* and likely results in an upregulation of the FGFR2 protein. The *FGFR2-KIAA1598* fusion seen in case 27 of this study results in truncation of the 3′UTR of *FGFR2* and may also result in upregulation of the FGFR2 protein; however, this rearrangement (Figs. 2, 3) has not been reported in cholangiocarcinoma or other cancers, and the functional consequences are uncertain at this time. The *FGFR2-TACC3* fusion found in case 28 of this study is also the first ICC found to harbor this alteration; however, it was recently reported that 3% of glioblastomas feature chromosomal rearrangements that fuse the tyrosine kinase coding domains of *FGFR1* or *FGFR3* in frame to the transforming acidic coiled-coil coding domains of *TACC1* or *TACC3*, respectively [17]. Regorafenib, which inhibits cellular kinases including FGFR2, has been approved for treatment of some metastatic colorectal cancer patients [18], and clinical trials of multiple FGFR inhibitors are currently under way [19]. Finally, the *RABGAP1L-NTRK1* fusion detected in case 2 of this series has not been reported previously (based on database searches of PubMed and COSMIC in January 2013), and *NTRK1*

Table 2. Selected immunohistochemical staining results

Case no.	CK AE1, AE3	CK7	CK19	CK20	CDX2	CAM, 5.2	TTF1	Synaptophysin	HePAR1	ER, GCDFP, Mammoglobin	HER2
1		+		−	−		−			−	
2		+	+	−	−				−	−	
3		+	+	−	−						
4		+		−	+		−				
5		+		+	−		−			−	
6											
7											
8											
9		+	Weak	−	−		−	−			
10											
11											
12		+		+	+	+	−		−	−	
13		+	+	+	−						
14											
15	+	+	+	−	−		−	−	−	−	
16		+			+						
17	+	+		−		+				ER +/−, PR −, GCDFP −	−
18		+		Weak	+		−				
19		+	+	−	−		−				
20		+		Weak					−		
21											
22											
23		+	+	Weak	−		−				
24		+		−	+		−			−	
25											
26											
27		+		Weak			−				
28											

Abbreviations: +, positive; −, negative.

alterations have not been analyzed or studied in cholangiocarcinoma. NTRK1 has been recently considered as a potential target for neuroblastoma and metastatic thyroid cancer [20, 21]. Of potential interest is the recent report of a non-small cell lung cancer patient whose tumor featured an *MPRIP-NTRK1* fusion and who responded to the kinase inhibitor crizotinib [22].

MET amplification has rarely been described in ICC but has been associated with adverse clinical outcome [23–25]. In this study, *MET* amplification of greater than six copies per cell was found in two cases (7%). *MET* amplification may predict sensitivity to MET inhibitors and has been linked to acquired resistance to EGFR and ERBB2 inhibitors [26]. In this study, 12% of the cases featured a mutation or splicing modification of *PTEN*, a tumor suppressor that negatively regulates the PI3K/Akt/mTOR pathway [27]. *PTEN* mutations are rare in ICC (as shown in the COSMIC database in July 2012), although loss of PTEN expression has been associated with increased invasion, advanced tumor stage, and shorter survival [28]. Loss of PTEN may predict sensitivity to inhibitors of PI3K [27], and the mTOR inhibitors temsirolimus and everolimus have been approved for use in some tumor types. Inhibitors of PI3K and Akt are currently in clinical trials in solid tumors, alone or in combination with other therapies.

IDH1 and *IDH2* are highly homologous and have similar functions, and mutation hot spots in both genes are conserved [29]. Alterations in *IDH1* and *IDH2* have been reported previously in cartilagenous tumors, gliomas, and leukemias [29]. Preclinical evidence now suggests that *IDH1* and *IDH2* alterations are actionable [30]. Almost all (99%) of the somatic mutations found in *IDH1* are found at codon R132; this codon is functionally conserved and aligns with R172 of *IDH2* [30]. In this study, eight (89%) of the *IDH1* mutations were at codon 132, with one mutation (11%) at another locus (G97D). A heterozygous mutation at *IDH1* codon R132 alters the activity of the IDH1 enzyme, resulting in a decrease in antioxidant activity in the cell [31–34]. The mutant enzyme promotes the reduction of a-ketoglutarate to 2-hydroxyglutarate, with the coincident conversion of NADPH to NADP+ [30]. Accumulation of d-2-hydroxyglutarate, a potential oncometabolite, has been associated with cancers that possess mutations in the *IDH* genes [32–35]. Mutations at codon 132 have been identified most frequently in gliomas, acute myeloid leukemia, colon cancer, prostate cancer, and chondrosarcomas (as shown in the COSMIC database in June 2012) [31–33].

IDH1 mutations have been identified in 9% (38 of 436) of biliary tract tumors analyzed in the COSMIC database (in May

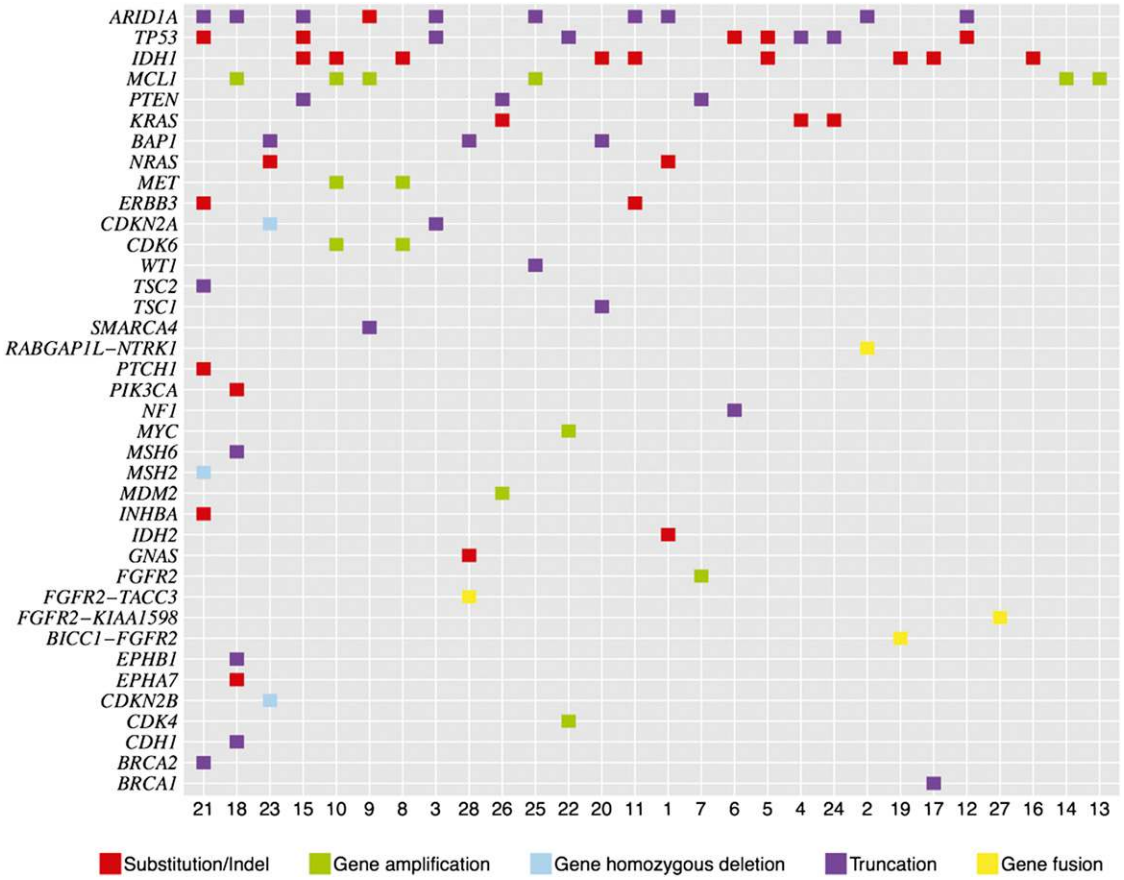

Figure 1. Tile plot of genomic alterations in 28 cases of intrahepatic cholangiocarcinoma.

2013). Recently, *IDH1* and *IDH2* mutations were identified in 34 of 326 ICCs (10%) and associated with longer overall survival for the disease in multivariate analysis [35]. *IDH1* mutations have been identified in 20% of intrahepatic cholangiocarcinomas, and mutation status correlated with increased IDH1 activity [32]. IDH2 amino acid R172 is a proposed substrate binding site and represents one of two genetic hot spots for cancer mutations in this gene [36]. A majority of somatic *IDH2* mutations cluster to two mutation hot spots, R140 and R172, of 841 reported mutations: Mutations at R140 represent 69.9% (588 of 841), and mutations at R172 represent 29.4% (247 of 841) (as shown in the COSMIC database in April 2012). *IDH2* mutations have been found in hematopoietic and lymphoid tissue (6%, 730 of 12,303), bone tumors (4%, 18 of 405), tumors of the central nervous system (2%, 90 of 5,033), and skin tumors (2%, 3 of 127) (as shown in the COSMIC database in December 2012). There are no reports of *IDH2* mutations in biliary tract cancers or other gastrointestinal tumor types in COSMIC (as of May 2013). However, the *IDH2* R172W mutation found in case 1 of this study was also identified in one cholangiocarcinoma of 62 total cases in a previously published study [37]. No therapies targeting this alteration are currently approved, although therapies targeting the altered metabolic pathway resulting from *IDH* mutations are currently in development.

Loss of the chromosomal region containing *CDKN2A* and *CDKN2B* (9p21) has been reported in primary sclerosing cholangitis-associated ICC [38]. Up to 25% of biliary tract tumors harbor *CDKN2A* mutations (as shown in the COSMIC database in November 2012). Tumors with loss of the *CDKN2A/CDKN2B* locus may be sensitive to Cdk4/6 inhibitors, and clinical trials of these agents are currently under way for a variety of solid tumors. Of additional interest is the observation that, given the relatively frequent 21% rate of *MCL1* focal gene amplification in the ICC cohort, CDK inhibitors may function by reducing MCL1 protein levels as their main mechanism of action [39].

Thirty-eight percent of the ICCs sequenced in this study had mutations in the *ARID1A* gene. *ARID1A* encodes the AT-rich interactive domain-containing protein 1A, also known as BAF250a, a member of the SWI/SNF chromatin remodeling complex. ARID1A is believed to function as a tumor suppressor [40]. *ARID1A* mutations have been reported in endometrial cancer (50%, 2 of 4), head and neck squamous cell carcinoma (50%, 3 of 6), ovarian cancer (34%, 97 of 282), skin squamous and basal cell carcinoma (29%, 2 of 7), gastric cancer (11%, 11 of 101), colorectal cancer (9%, 12 of 131), prostate cancer (9%, 2 of 23), pancreatic cancer (8%, 15 of 178), and a small percentage of lung, breast, and kidney carcinomas and gliomas (as shown in the COSMIC database in April 2012) [41]. Mutations span the length of the *ARID1A* gene and include point mutations and small deletions and insertions. There are no reports of *ARID1A* mutation in cholangiocarcinoma (as shown in the COSMIC database in April 2012), and there are no reports of *ARID1A* mutation in cholangiocarcinoma in the literature. Presently, there are no targeted therapies approved that target *ARID1A* mutations.

A variety of "one-off" single gene mutation studies have looked at ICC and concluded that *TP53* and *KRAS* are the most frequently mutated genes found in this tumor type [42, 43].

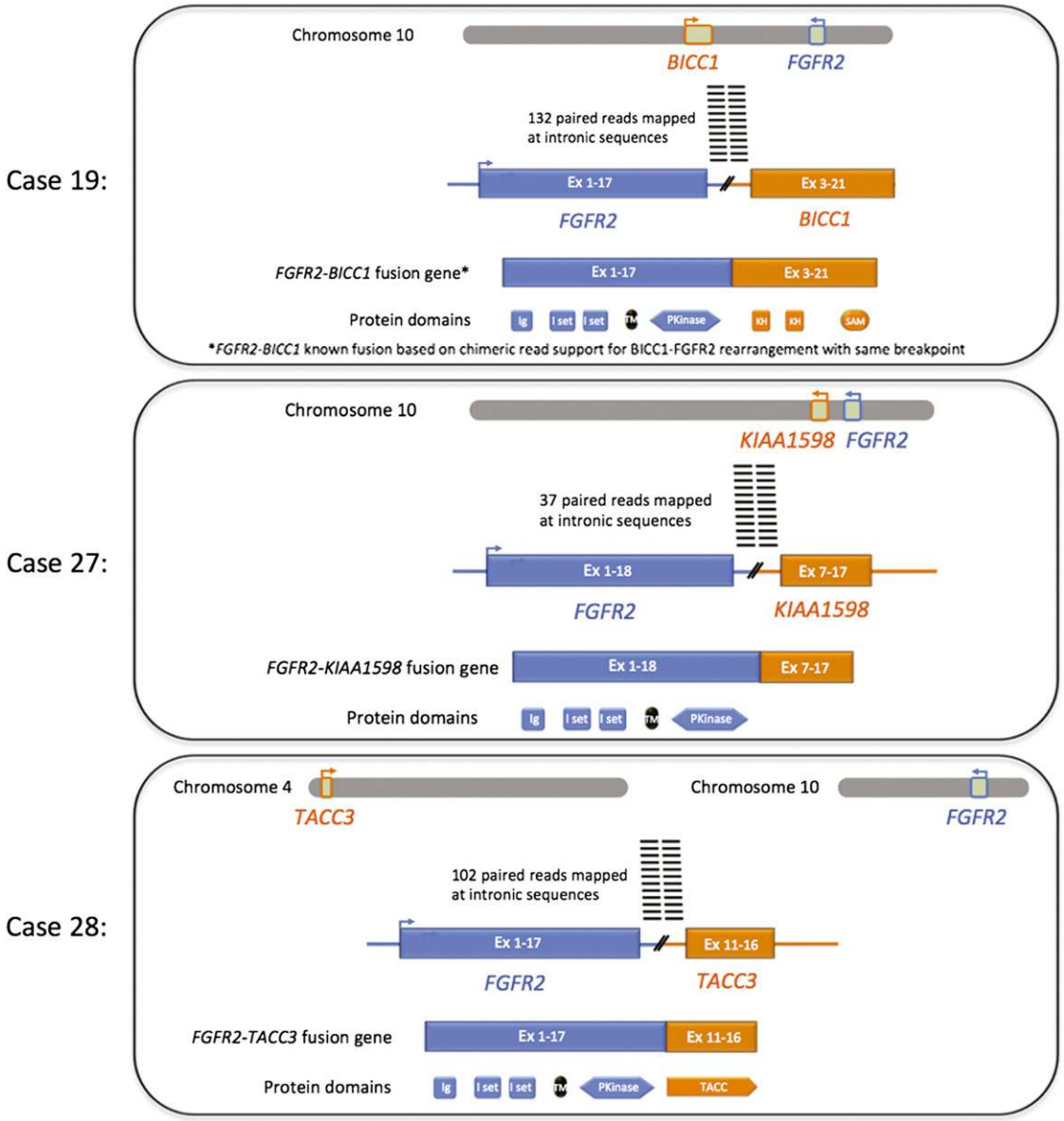

Figure 2. Diagram of *FGFR2* gene fusions in three cases of intrahepatic cholangiocarcinoma.

Activating *KRAS* mutations have been observed in 20%–50% of cholangiocarcinomas (as shown in the COSMIC database in November 2012) [44, 45] and are associated with early recurrence and poor overall survival in ICC [46]. Some investigators have also identified subsets of both extrahepatic cholangiocarcinoma and ICC that appear to be driven by *BRAF* mutations [47–49]. However, this study did not find any *BRAF* mutations in the series of 28 ICCs, a finding also reported by others [50]. *TP53* alterations have been reported in 39% (232 of 596) of biliary tract cancers and specifically in 41% (106 of 259) of bile duct carcinomas (as shown in the COSMIC database in June 2012). Inactivation of *TP53*, through mutation, deletion, or LOH, has been observed in 10%–61% of cholangiocarcinomas [51]. In this study of ICC only, the *TP53* mutation frequency was 36%.

In this study, "actionable" genomic alterations have been defined as those linked to a drug that is approved for the tumor type in question or another tumor type but that targets the identified genomic alteration or pathway or that is mechanistically linked to an agent in an active registered clinical trial. Currently, there are no approved drugs for the treatment of ICC. It should also be noted that some actionable gene alterations actually are negative selectors that suggest lack of benefit of use of the specific drug when the alteration is present. In this approach, practicing oncologists are given information that can guide therapy selection for their patients in an efficient and straightforward manner.

Conclusion

When ICC was comprehensively genomically profiled with a next-generation sequencing-based diagnostic assay, two thirds of patients harbored potentially actionable genomic alterations that have the potential to influence and personalize therapy and guide the selection of targeted therapies approved or in clinical trials. Given the limited treatment options and the poor prognosis of patients with ICC and the diversity of actionable alterations identified in this study, comprehensive genomic profiling has the potential to maximize the identification of new treatment paradigms and to meet an unmet clinical need.

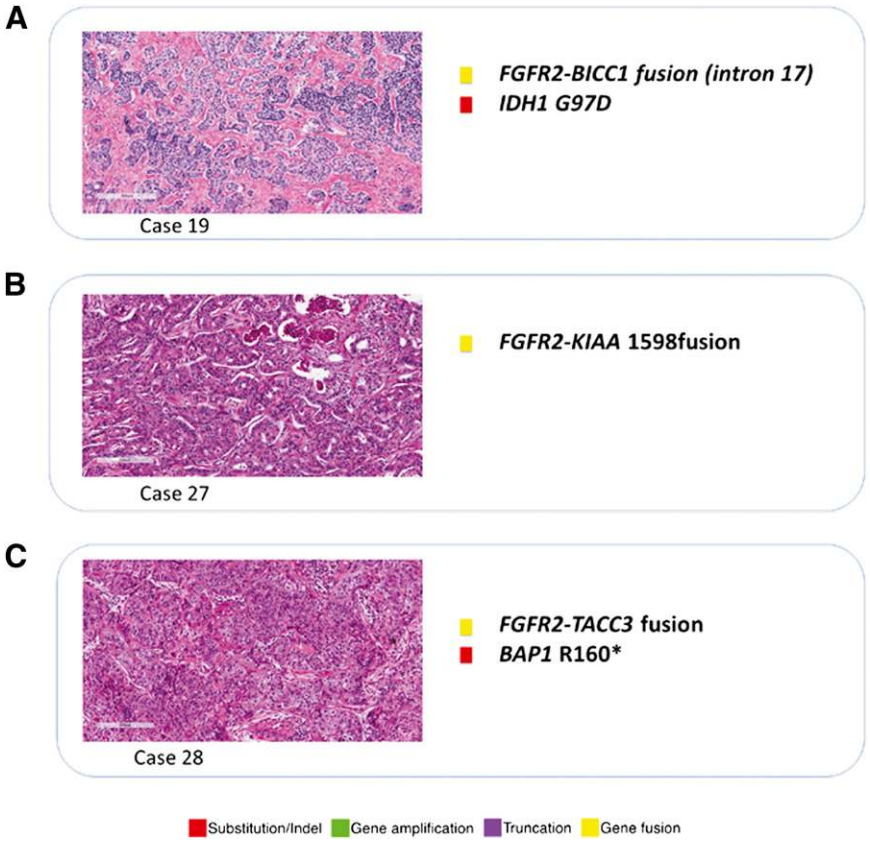

Figure 3. Histology and list of genomic alterations in three cases of intrahepatic cholangiocarcinoma featuring *FGFR2* gene fusions detected by next-generation sequencing.

Acknowledgments

This work was supported by an NIH K12 award (CA139160-01A), a University of Chicago Comprehensive Cancer Center Award in Precision Oncology, and a Live Like Katie Cholangiocarcinoma Foundation Award (to D.V.T.C.).

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Disclosures

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(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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