Published OnlineFirst May 30, 2018; DOI: 10.1158/1078-0432.CCR-18-0078

Precision Medicine and Imaging

Clinical Cancer Research

Comprehensive Molecular Profiling of Intrahepatic and Extrahepatic Cholangiocarcinomas: Potential Targets for Intervention

Maeve A. Lowery[1], Ryan Ptashkin[2], Emmet Jordan[2], Michael F. Berger[2], Ahmet Zehir[2], Marinela Capanu[2], Nancy E. Kemeny[2,3], Eileen M. O'Reilly[2,3], Imane El-Dika[2], William R. Jarnagin[2,3], James J. Harding[2,3], Michael I. D'Angelica[2,3], Andrea Cercek[2,3], Jaclyn F. Hechtman[2], David B. Solit[2,3], Nikolaus Schultz[2], David M. Hyman[2,3], David S. Klimstra[2,3], Leonard B. Saltz[2,3], and Ghassan K. Abou-Alfa[2,3]

Abstract

Purpose: Various genetic driver aberrations have been identified among distinct anatomic and clinical subtypes of intrahepatic and extrahepatic cholangiocarcinoma, and these molecular alterations may be prognostic biomarkers and/or predictive of drug response.

Experimental Design: Tumor samples from patients with cholangiocarcinoma who consented prospectively were analyzed using the MSK-IMPACT platform, a targeted next-generation sequencing assay that analyzes all exons and selected introns of 410 cancer-associated genes. Fisher exact tests were performed to identify associations between clinical characteristics and genetic alterations.

Results: A total of 195 patients were studied: 78% intrahepatic and 22% extrahepatic cholangiocarcinoma. The most commonly altered genes in intrahepatic cholangiocarcinoma were *IDH1* (30%), *ARID1A* (23%), *BAP1* (20%), *TP53* (20%), and *FGFR2* gene fusions (14%). A tendency toward mutual exclusivity was seen between multiple genes in intrahepatic cholangiocarcinoma including *TP53:IDH1*, *IDH1:KRAS*, *TP53:BAP1*, and *IDH1:FGFR2*. Alterations in CDKN2A/B and ERBB2 were associated with reduced survival and time to progression on chemotherapy in patients with locally advanced or metastatic disease. Genetic alterations with potential therapeutic implications were identified in 47% of patients, leading to biomarker-directed therapy or clinical trial enrollment in 16% of patients.

Conclusions: Cholangiocarcinoma is a genetically diverse cancer. Alterations in *CDKN2A/B* and *ERBB2* are associated with negative prognostic implications in patients with advanced disease. Somatic alterations with therapeutic implications were identified in almost half of patients. These prospective data provide a contemporary benchmark for guiding the development of targeted therapies in molecularly profiled cholangiocarcinoma, and support to the use of molecular profiling to guide therapy selection in patients with advanced biliary cancers. *Clin Cancer Res; 24(17); 4154–61. ©2018 AACR.*

Introduction

Cholangiocarcinoma, a primary malignancy of the biliary tract, is characterized by late presentation and aggressive clinical course, and few treatment options exist for patients with advanced disease (1, 2). Biliary tract malignancies, excluding gallbladder cancer, are traditionally subdivided according to site of origin in the biliary tree: intrahepatic versus extrahepatic cholangiocarcinoma. However, it is increasingly evident that patients with cholangiocarcinoma may be additionally categorized based upon their molecular profiles (3, 4). Large-scale sequencing studies of cholangiocarcinoma have identified multiple recurrent driver alterations with complex interactions (5, 6). However, the etiologic factors leading to these diverse molecular phenotypes are as yet poorly understood as are the prognostic implications of individual somatic alterations (7, 8). Importantly and in contrast to other upper gastrointestinal malignancies, multiple potentially targetable genetic alterations have been identified in biliary tumors, and ongoing prospective studies are evaluating the activity of targeted therapies including agents that target fibroblast growth factor receptor 2 (*FGFR2*), *IDH1*, *HER2*, and *NTRK* fusions in genetically selected populations (9–14). The purpose of this study was to assess the feasibility and utility of prospective next-generation sequencing (NGS) in patients with cholangiocarcinoma, to identify novel therapeutic targets and prognostic biomarkers of treatment response.

Materials and Methods

Patients

Patients were identified over a 2-year period starting in July 2014 and were eligible for the study if they had a confirmed histologic diagnosis of cholangiocarcinoma. Informed consent for tumor profiling was obtained under protocol NCT01775072

[1]Trinity College Dublin, Dublin, Ireland. [2]Memorial Sloan Kettering Cancer Center, New York, New York. [3]Weill Cornell Medical College, New York, New York.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Corresponding Author: Ghassan K. Abou-Alfa, Memorial Sloan Kettering Cancer Center, 300 East 66th Street, New York, NY 10065. Phone: 646-888-4184; Fax: 646-888-4255; E-mail: abou-alg@mskcc.org

doi: 10.1158/1078-0432.CCR-18-0078

Published OnlineFirst May 30, 2018; DOI: 10.1158/1078-0432.CCR-18-0078

Translational Relevance

This report evaluates the prognostic and therapeutic implications of comprehensive genetic analysis of patients with advanced cholangiocarcinoma. Through targeted deep sequencing of all exons and selected introns of 410 key cancer-associated genes, we identified genetic alterations with potential therapeutic implications in 47% of patients, leading to biomarker-directed therapy or clinical trial enrolment in 16% of patients. Correlation of genetic alterations with clinical outcomes demonstrated that alterations in *CDKN2A/B* and *ERBB2* were associated with reduced overall survival and shorter time to progression on first-line chemotherapy. These findings indicate that molecular profiling can facilitate enrollment of patients with cholangiocarcinoma to biomarker-selected clinical trials and that specific genotypes may have prognostic implications in terms of clinical outcomes.

"Tumor Genomic Profiling in Patients Evaluated for Targeted Cancer Therapy." The protocol was approved by the Institutional Review Board at Memorial Sloan Kettering Cancer Center, and the study was conducted in accordance with the Good Clinical Practice guidelines and the Declaration of Helsinki. Written consent was obtained from every patient.

Results from 195 patients with cholangiocarcinoma who had consented to the study were available at the time of analysis. Clinical data were collected including demographics (age, sex, race, and prior viral hepatitis B/C exposure), family and personal history of malignancy, overall and disease-free survival, treatments delivered, and therapeutic response.

Sample preparation

A pathologist reviewed all tumor samples, and macrodissection was performed as needed to enrich for tumor content. Previously collected samples (e.g., archival tissue from prior resection or biopsy) were used in all cases. Macrodissection was performed in selected cases. Samples with estimated tumor purity < 10% based on histopathologic assessment were deemed insufficient for sequencing. The standard input of DNA was 250 ng, and minimum input was 50 ng in cases where DNA quantity was limited. Matched germline DNA from prospectively collected blood samples was analyzed in all patients. Although paired germline sequencing was used for somatic mutation calling, we did not analyze samples for pathogenic germline mutations in this study.

Genetic analysis

Tumors were profiled for somatic genomic alterations using MSK-IMPACT, an in-house, deep sequencing assay (15). Custom DNA probes were designed to capture all exons and selected introns of 341 ($n = 20$) or 410 ($n = 318$) oncogenes, tumor-suppressor genes, and members of pathways deemed potentially actionable by targeted therapies. Genomic DNA from tumor and patient-matched normal samples was analyzed as previously described (6, 7, 15, 16). Somatic copy-number alterations (CNA) were identified by comparing sequence coverage of targeted regions in the tumor sample relative to standard diploid normal as previously described (6). The resulting high confidence single-nucleotide variants (SNV), indels, somatic CNAs, and structural variants as detected by MSK-IMPACT were used to produce a binary alteration matrix across all altered genes and samples.

Genetic alterations were classified as actionable using a scale of 1–4, where levels 1-2A alterations indicated standard therapeutic interventions, likely to be covered by insurance, and levels 2B-4 included investigational therapeutic alterations, which may direct a patient toward a clinical trial relevant to that biomarker (17, 18). Classification was performed using the OncoKB knowledge database, which integrates biological, clinical, and therapeutic information curated from multiple resources, including recommendations derived from FDA labeling, National Comprehensive Cancer Network (NCCN) guidelines, and the medical literature (17).

Statistical analysis

Fisher exact tests were performed to identify significant differences in gene alterations (mutations and CNAs) between patient groups sharing a particular clinical feature. We calculated the OR and FDR-corrected P value for each gene alteration. Overall survival and progression-free survival (PFS) were calculated using the Kaplan–Meier method; the χ^2 test was used to compare PFS and overall survival between patients with and without mutations/CNA in all genes tested and in pairs of genetic alterations. We investigated associations between somatic alterations and PFS for the 158 patients in the cohort treated at MSKCC with first-line chemotherapy who had follow-up data at the time of analysis. To assess survival, a Cox proportional hazards model was fitted to the data. Here, the covariates of age at diagnosis, sex, sample type (primary vs. metastasis), and genes with somatic alterations were each assessed through both univariate and multivariate Cox regression. Covariates significant in univariate analysis were applied to the multivariate model to calculate HRs and 95% confidence intervals (CI).

Results

Out of 214 samples attempted, the success rate was 91% ($n = 195$); samples from 195 individual patients were analyzed, see Table 1 for patient and sample characteristics. One hundred and fifty-eight cases (81%) were intrahepatic cholangiocarcinoma, and 37 (19%) were extrahepatic cholangiocarcinoma. The majority of patients (89%) were Caucasian with a slight male predominance. Twenty-four (12%) of patients had hepatitis B. Seventy-one patients underwent surgical resection for localized disease, of whom 42 had recurred at the time of analysis. A total of

Table 1. Clinical characteristics ($n = 195$ patients)

Clinical characteristics	Number (%)
Sex	
Male	101 (51.8)
Female	94 (49.2)
Anatomic location	
Intrahepatic cholangiocarcinoma	158 (81)
Extrahepatic cholangiocarcinoma	37 (19)
Ethnicity	
Caucasian	174 (89.2)
Asian	14 (7.1)
African American	7 (3.6)
Age	
Median (range)	62 (24–86)
Sample analyzed	
Primary tumor biopsy or resection	141 (72%)
Biopsy of metastatic site	54 (27%)

Published OnlineFirst May 30, 2018; DOI: 10.1158/1078-0432.CCR-18-0078

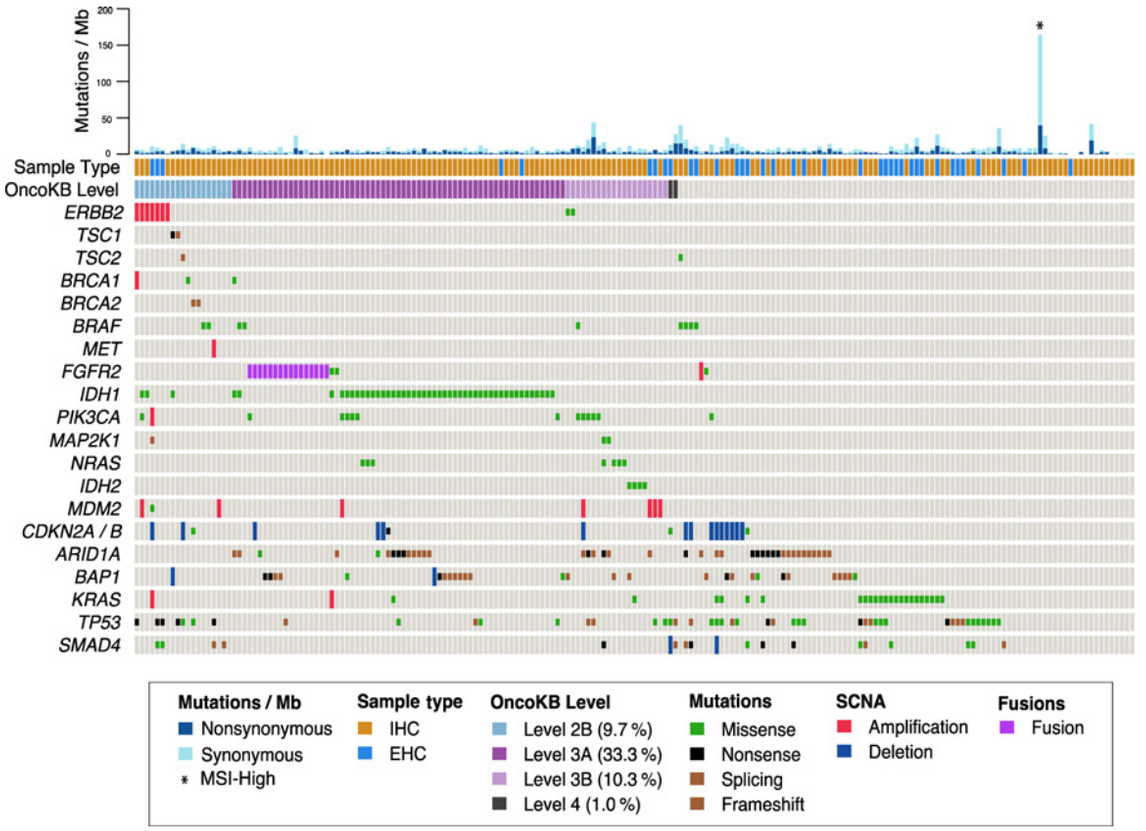

Figure 1.

Common mutations and OncoKB annotation. *KRAS, SMAD4*, and STK11 alterations were more commonly seen in extrahepatic cholangiocarcinoma, whereas mutations in *IDH1, BAP1, TP53*, and *FGFR2* fusions occurred with greater frequency in intrahepatic cases.

775 genetic alterations were identified among 189 of the 195 samples. Six patients had no somatic genetic alterations identified. The median number of mutations per samples was 3 (see Fig. 1). Median sample coverage was 759X. The most commonly mutated genes were *IDH1* (25%), *TP53* (24%), *ARID1A* (21%), *BAP1* (15%), *KRAS* (13%), *PBRM1* (12%), *SMAD4* (9%), and *ATM* (8%). Potentially oncogenic focal CNAs were noted in multiple genes including *CDKN2A* deletions (8%) and *MDM2* (4%), *ERBB2* (4%), and *MCL1* (4%) amplifications. Thirty-eight structural alterations were identified in samples from 35 patients (18%), of which the majority were in-frame fusion events predicted to result in FGFR2 activation. Multiple fusion partners with FGFR2 were identified, the most frequent being BICC1 and KIAA1217 (see Fig. 2). Three patients had multiple samples sequenced: 2 patients with both a primary and metastatic site sequenced. For both metastatic samples, variants observed in primary sample were observed plus additional subclonal variants. In 1 patient with two metastatic samples sequenced, the results were concordant.

One tumor (0.5%) had a signature of microsatellite instability (MSI-H, MSIsensor score of 35.1). This tumor was hypermutated (48 somatic mutations), and loss of MLH1 and MSH6 protein expression was present on immunohistochemistry analysis. This patient was a 57-year-old man with a history of a choledochal cyst after choledochoduodenostomy and was diagnosed with cholangiocarcinoma with intestinal features. Germline genetic testing was not performed, and he did not have a family history strongly suggestive of Lynch syndrome [one second-degree relative with bladder cancer and a first-degree relative with renal cell carcinoma (RCC)]. This patient was treated with several lines of chemotherapy but did not receive immunotherapy.

Distinct patterns of genetic alterations between intrahepatic and extrahepatic cholangiocarcinoma were identified. *KRAS, SMAD4*, and STK11 alterations were more commonly seen in extrahepatic cholangiocarcinoma, whereas mutations in *IDH1*, *BAP1*, *TP53*, and *FGFR2* fusions occurred with greater frequency in intrahepatic cases (Fig. 1 and Table 2); *BAP1* mutations and *FGFR2* gene fusions were identified exclusively in patients with intrahepatic cholangiocarcinoma. We also observed mutual exclusivity between commonly altered genes beyond that explained by anatomic location including *IDH1:TP53*, *TP53:BAP1*, and *IDH1:SMAD4*. A tendency toward cooccurrence was also seen with multiple genes including *TP53:CDKN2A*, *SMAD4:KRAS*, and *TP53:CDKN2B* (Table 3).

We analyzed the cohort to identify individual genes that were enriched in the 54 metastatic versus 141 primary tumor samples. However, no genetic alterations occurred with a significantly different frequency in metastatic versus primary tumor samples (Table 4).

Published OnlineFirst May 30, 2018; DOI: 10.1158/1078-0432.CCR-18-0078

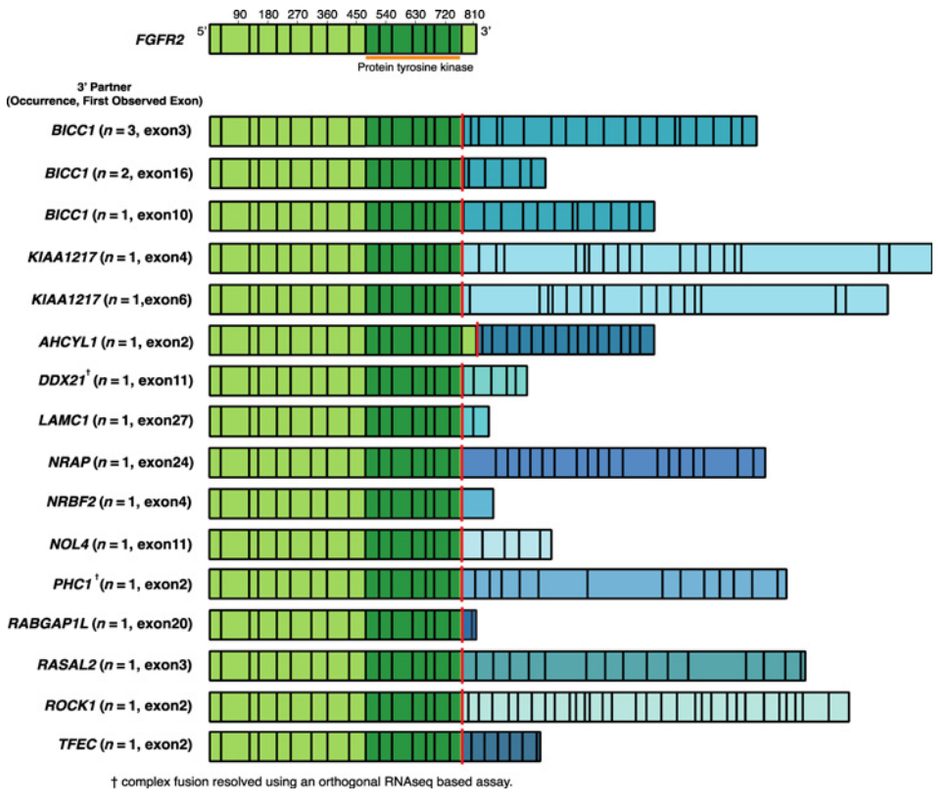

Figure 2.
FGFR2 gene fusions. Multiple fusion partners with FGFR2 were identified, the most frequent being BICC1 and KIAA1217.

Molecular predictors of clinical outcome in patients treated with cytotoxic chemotherapy

One hundred and fifty-eight patients (81%) received first-line chemotherapy for advanced disease (127 patients: 80% gemcitabine/platinum), with a median time to progression of 8.8 months. Additional regimens used in the first-line setting included FOLFOX, capecitabine, and gemcitabine/nab-paclitaxel. Patients with alterations in *CDKN2A/B* ($n = 15$, $P = 0.002$), *ERBB2* ($n = 8$, $P = 0.028$), and *MDM2* ($n = 7$, $P = 0.026$) had significantly shorter time to progression on first-line chemotherapy. No significant difference in time to progression was noted with other commonly altered genes including *IDH1*, *FGFR2*, *BAP1*, *ATM*, *ARID1A*, and *TP53* (Fig. 3; Supplementary Table S1). Overall survival from date of diagnosis with locally advanced or metastatic disease was calculated for the 178 patients with stage IV disease either at diagnosis or who recurred following surgery, and was significantly shorter in patients with alterations in *CDKN2A/B* ($n = 18$, $P = 0.0015$), *ERBB2* ($n = 9$, $P = 0.0015$), and *KRAS* ($n = 21$, $P = 0.026$; Supplementary Fig. S1).

Table 2. (a) Genes significantly altered in IHC relative to EHC; (b) Genes significantly altered in EHC relative to IHC

Gene	IHC mutated ($n = 158$)	EHC mutated ($n = 37$)	OR	*P* value	*q* value
(a)					
IDH1	46	2	7.137	0.001	0.011
BAP1	30	0	Inf	0.002	0.013
FGFR2	20	0	Inf	0.016	0.087
(b)					
KRAS	11	14	0.125	0.000	0.000
SMAD4	8	11	0.128	0.000	0.001
TP53	28	18	0.230	0.000	0.007
STK11	1	4	0.054	0.005	0.031

Abbreviations: EHC, extrahepatic cholangiocarcinoma; IHC, intrahepatic cholangiocarcinoma; Inf, infinite.

Potentially actionable genetic alterations

Ninety-three patients (47.6%) had at least one actionable finding, defined as a somatic genetic alteration classified as level 3B or higher using the OncoKB classification (17). Several patients had more than one potentially actionable genetic alteration with 4 patients having 4 actionable findings, 5 patients with 3, and 15 patients with 2 genetic alterations for which targeted inhibitors have demonstrated compelling clinical activity in cholangiocarcinoma or other cancer types. As there are no standard-of-care targeted agents for patients with cholangiocarcinoma, no patients had a level 1 or 2A alteration. Sixteen patients (8%) had at least one somatic alteration that was classified as level 2B, defined as an FDA-approved biomarker in another cancer indication, but not FDA or NCCN-compendium listed for cholangiocarcinoma. These included *ERBB2* amplification (6 patients), likely pathogenic somatic alterations in *TSC1/2* (3) or *BRCA1/2* (2 patients), BRAF V600E mutation (1 patient), and MET amplification (1 patient). Seventy-seven patients (39%) had a level 3 alteration as their highest level actionable gene. Level 3 includes those for which clinical evidence links the biomarker to drug response in patients, but use of the biomarker is not currently a standard of care in any cancer type. Level 3 genetic alterations consisted mainly of known oncogenic mutations in IDH1 (43 patients) and fusion events involving *FGFR2* (17 patients). Additional potentially actionable mutations present at low frequency

Published OnlineFirst May 30, 2018; DOI: 10.1158/1078-0432.CCR-18-0078

Table 3. Genes with tendency toward mutual exclusivity (OR < 0.5)

Gene1	Gene2	Both genes altered (*n*)	Gene2 altered (*n*)	Gene1 altered (*n*)	Neither gene altered (*n*)	OR	*P* value	*q* value
IDH1	TP53	3	43	45	104	0.162	0.001	0.488
IDH1	SMAD4	0	19	48	128	0.000	0.004	1
TP53	BAP1	1	29	45	120	0.093	0.004	1
BAP1	KRAS	0	25	30	140	0.000	0.017	1
BAP1	SMAD4	0	19	30	144	0.000	0.029	1
IDH1	FGFR2	1	19	47	128	0.144	0.030	1
IDH1	KRAS	2	23	46	124	0.236	0.046	1
TP53	FGFR2	1	19	45	130	0.153	0.049	1
PBRM1	KRAS	0	25	23	147	0.000	0.049	1

included *PIK3CA* ($n = 6$), *NRAS* ($n = 4$), and *ERBB2* ($n = 2$) hotspot mutations.

Twenty-five patients (16% of those patients with advanced disease) received matched therapy based on the molecular profiling results, including 13 patients treated with *IDH1* inhibitor, 6 patients with *FGFR* inhibitors, 2 with *HER2*-directed therapy, 1 each with *EZH2* and *ERK* inhibitors, and 1 patient who received the multitargeted kinase inhibitor sorafenib. Sixteen of the 25 patients (64%) treated with targeted therapy had evidence of response or clinical benefit to treatment.

Discussion

Recent large-scale sequencing efforts in cholangiocarcinoma have identified a wealth of diverse and potentially actionably somatic genomic alterations. In this study, we demonstrated the feasibility of performing prospective targeted sequencing of cancer-associated genes in 195 patients with cholangiocarcinoma. Archival formalin-fixed, paraffin-embedded (FFPE) tissue obtained from core biopsy or resected specimen of primary or metastatic sites of disease was used for genomic profiling. We identified at least one actionable genetic alteration in almost 50% of patients with cholangiocarcinoma.

As we used archival FFPE samples for analysis, it is unknown whether there was significant evolution of genetic changes from the time the sample was collected to the use of the genomic data to guide treatment selection. We did not, however, observe any significant differences in the prevalence of actionable alterations between the 54 metastatic versus 141 primary tumor samples analyzed. For molecular analysis, we utilized a targeted NGS platform, which captures all exons and select intronic regions of several hundred cancer-associated genes. A more comprehensive analysis using whole-exome/genome sequencing and/or transcriptome analysis may have identified additional potentially actionable genomic alterations or gene signatures but would not have been feasible in all patients, due to cost and the availability of only limited FFPE tissue for many of the patients. Broader analysis would have also prolonged the real-time turnaround of genomic information needed to inform clinical care decisions in a prospective clinical setting, an important consideration in a highly aggressive and fatal disease such as cholangiocarcinoma.

Table 4. Genes altered in samples from primary versus metastatic sites

Gene	Mutated in primary ($n = 141$)	Mutated in metastasis ($n = 54$)	OR	*P* value	*q* value
IDH1	36	12	1.199	0.712	0.979
TP53	29	17	0.565	0.132	0.647
ARID1A	32	8	1.684	0.243	0.647
BAP1	25	5	2.105	0.185	0.647
KRAS	18	7	0.983	1.000	1.000
PBRM1	19	4	1.941	0.324	0.647
FGFR2	14	6	0.882	0.796	0.979
CDKN2A/B	11	8	0.489	0.176	0.647
SMAD4	10	9	0.384	0.058	0.647

The most common actionable findings observed were known hotspot gain-of-function mutations in *IDH1*, and rearrangements in *FGFR2*, which result in constitutive activation of the *FGFR2* receptor. Notably, alterations in these genes were mutually exclusive, suggesting that such alterations identify biologically distinct molecular cholangiocarcinoma subtypes. This is consistent with findings from prior studies of whole-genome and targeted exon sequencing of intrahepatic cholangiocarcinoma (6, 19). Our study confirmed that unlike other gastrointestinal tumors, cholangiocarcinomas often harbor potentially actionable genetic rearrangements, most commonly in FGFR2. Fusions involving *NTRK1/3* and ROS1 have also previously been identified in patients with cholangiocarcinoma, although we did not observe any in this cohort. We identified multiple fusion partners with FGFR2 and a wide variation in break points, lending support to the use of NGS as molecular prescreening platform to identify patients for *FGFR* inhibitor therapy. Several selective inhibitors of the FGF receptors (FGFR1–4) are being tested in molecularly selected population of patients with biliary cancer, and activity with these agents has been most notable in tumors that harbor FGFR2 gene fusions, as opposed to other *FGFR* alterations such as gene amplification or mutation (9, 20). An ongoing randomized phase III study is evaluating the activity of IDH1 inhibitor, AG-120, in patients with advanced cholangiocarcinoma that had progressed on prior chemotherapy. Additional inhibitors of IDH1 and IDH2 are also in phase I clinical trials (21). Preliminary results from phase I/II studies of agents targeting FGFR2 alterations and IDH1 mutations indicate that these agents have activity in molecularly selected populations. Data from 73 patients with IDH1-mutant cholangiocarcinoma treated on a phase 1 study of AG-120, an orally active IDH1 inhibitor in a heavily pretreated patient population, demonstrated that 28 patients (38.5%) were progression free at 6 months and 15 patients (21%) were progression free at 12 months (PFS 12; ref. 22). Correlative studies indicated that AG-120 treatment inhibited plasma levels of the oncometabolite 2-hydroxyglutarate (2-HG) produced by mutant IDH1 to within levels found in healthy volunteers, and also reduced 2-HG in tumor biopsies, demonstrating an on-target effect of the inhibitor. An ongoing randomized phase III study is evaluating the activity of AG-120, in patients with advanced IDH1-mutant cholangiocarcinoma that has progressed on prior chemotherapy; additional inhibitors of IDH1 and IDH2 are also in phase I clinical

Published OnlineFirst May 30, 2018; DOI: 10.1158/1078-0432.CCR-18-0078

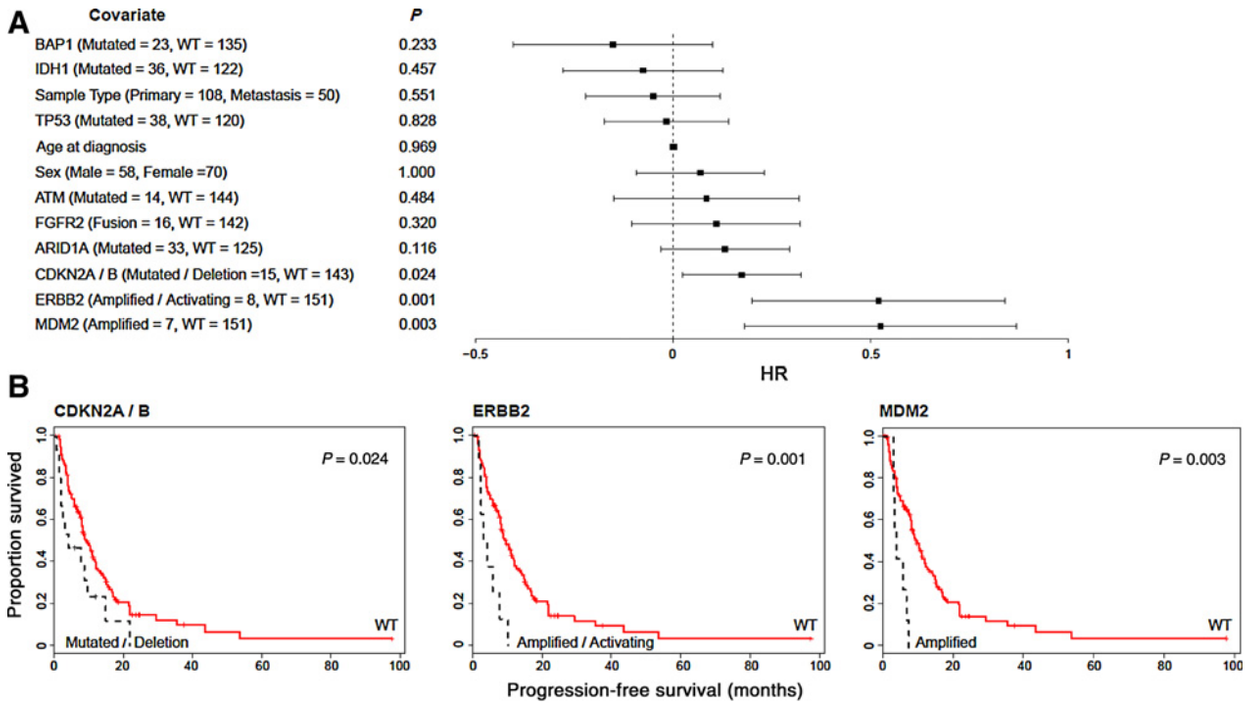

Figure 3.

PFS on first-line chemotherapy for advanced disease. Patients with alterations in *CDKN2A/B*, *ERBB2*, and *MDM2* had significantly shorter time to progression on first-line chemotherapy.

trials (23). A phase II study of the selective pan-*FGFR* inhibitor BGJ398 in patients with advanced *FGFR*-altered cholangiocarcinoma reported a disease control rate of 75.4% with response rate of 14.8% and median-free survival was 5.8 months (95% CI, 4.3–7.6 months; ref. 19). As has been observed in other cancer types, intrinsic and acquired resistance limit the efficacy of targeted therapies in patients with cholangiocarcinoma with secondary FGFR2 kinase mutations shown to confer resistance to *FGFR* inhibition have been observed in a minority of patients who had sequencing of tumor tissue or cfDNA following progression of disease on study treatment (23). Other pan-*FGFR* inhibitors have demonstrated similar activity to BGJ398 including ARQ087, which was evaluated in a phase I study that included 35 patients with biliary tract cancer. In this trial, a response rate of 20% was reported in cholangiocarcinoma patients, with a disease control rate of 76% and median time on treatment of 183 days (24). Our results support the use of molecular profiling in patients with advanced biliary cancer to identify targetable genetic alterations and thereby facilitate enrollment to clinical trials of molecularly targeted agents with realistic potential for clinical benefit.

Notably, we identified a signature of mismatch repair deficiency in just 1 patient (0.5%), a prevalence much lower than that observed in prior limited series (25, 26). However, the available literature on mismatch repair (MMR) deficiency in biliary cancers is limited to small retrospective studies performed in limited patient populations and utilizing varying microsatellite markers, antibody panels, and definitions for MSI-H (25). In contrast, this study represented a prospective and otherwise unselected patient population of patients with recurrent/metastatic disease that may be more reflective of the population of patients in need of novel systemic therapies. However, although our data suggest that MMR deficiency may be a less common occurrence in patients with recurrent/metastatic cholangiocarcinoma than previous reports had suggested, our data confirm that a signature of MSI can be identified using clinical NGS in cholangiocarcinoma patients in need of systemic therapy (27). Given the recent FDA approval of immune checkpoint blockade for such patients, such a finding could have significant standard therapeutic implications; the 1 patient identified with MMR deficiency in this study died before this FDA approval.

A challenge to the design of prospective studies in rare cancers such as cholangiocarcinoma is that the predictive and prognostic implications of commonly identified genetic alterations remain unclear. Understanding clinical outcome differences among molecular subtypes can thus inform the design of future clinical trials of targeted and immunotherapies. Although our ability to definitively define prognostic implications of particular genetic alterations was limited by the clinical and molecular heterogeneity of the population analyzed, alterations in *CDKN2A/B* and *ERBB2* were consistently associated with shorter overall survival from diagnosis with advanced disease and time to progression on first-line chemotherapy. The negative prognostic implications of *CDKN2A/B* alterations are consistent with findings by Javle and colleagues where results from targeted exon sequencing of 321 biliary tract cancer samples were correlated with clinical outcomes (28). They additionally found a negative prognostic implication of KRAS and TP53 mutations in biliary tract cancers and longer overall survival in patients whose tumor harbored an FGFR2 alteration;

Published OnlineFirst May 30, 2018; DOI: 10.1158/1078-0432.CCR-18-0078

ERBB2 was not included in the analysis of survival. The analysis differs from our study in that we performed survival analysis calculated from date of diagnosis with advanced or metastatic disease, whereas they calculated overall survival from date of diagnosis at any stage (28). In addition, our patient cohort included a significant minority of patients who had undergone prior surgical resection, and it is possible that an earlier stage at presentation may have impact on overall prognosis and/or sensitivity to chemotherapy in the advanced disease setting outside of the genomic profile. These findings require validation in a prospective study, but imply that the clinical phenotype associated with commonly identified targetable alterations such as those described above may vary depending on the presence of comutations in additional genes.

In summary, we identified multiple potentially actionable genetic alterations in a prospective cohort of patients with cholangiocarcinoma. The availability of the NGS data in a clinically meaningful timeframe facilitated the enrollment of 16% of patients in this cohort onto clinical trials of molecularly selected therapies. Given the promising early data with *FGFR* and IDH1 inhibitors in patients with cholangiocarcinoma, the recent FDA approval of pembrolizumab for patients with MSI-H tumors independent of site of tumor origin, and the recent profound clinical response observed in patients with NTRK fusions, we believe that molecular profiling of patients with advanced cholangiocarcinoma should be considered for all patients with a sufficient level of clinical well-being to be potential clinical trial candidates. The identification of potentially predictive biomarkers to targeted therapy in almost half of patients with cholangiocarcinoma suggests that prospective molecular characterization could accelerate clinical trials in this population and lead to a paradigm change in the management of this rare but highly fatal cancer type in the near future. Given the rarity and the clinical and genomic heterogeneity of this disease, the efficient development of targeted therapies for patients with cholangiocarcinoma will require cooperation between industry and academic centers to harmonize the efforts for companion diagnostic development and minimize duplication of testing in view of the mutual exclusivity of key driver genetic alterations. Finally, as we anticipate the development of targeted agents in combination with and/or compared with standard chemotherapy, the natural history of specific genotypes in terms of clinical outcomes will be crucial to informing study design in the first-line setting.

Disclosure of Potential Conflicts of Interest

M.A. Lowery is a consultant/advisory board member for Agios Pharmaceuticals and Roche. D.B. Solit is a consultant/advisory board member for Loxo Oncology and Pfizer. D.M. Hyman is a consultant/advisory board member for ArQule, AstraZeneca, Atara Biotherapeutics, Bayer, Boehringer Ingelheim, Chugai Pharma, Cytom Therapeutics, Debiopharm Group, Genentech and Pfizer, and reports receiving commercial research grants from AstraZeneca, Loxo Oncology and Puma Biotechnology. G.K. Abou-Alfa is a consultant/advisory board member for Agios and Incyte. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: M.A. Lowery, R. Ptashkin, N.E. Kemeny, E.M. O'Reilly, D.B. Solit, D.S. Klimstra, L.B. Saltz, G.K. Abou-Alfa

Development of methodology: M.A. Lowery, R. Ptashkin, A. Zehir, D.B. Solit, G.K. Abou-Alfa

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M.A. Lowery, E. Jordan, A. Zehir, N.E. Kemeny, E.M. O'Reilly, W.R. Jarnagin, J.J. Harding, D.B. Solit, N. Schultz, D.M. Hyman, D.S. Klimstra, L.B. Saltz, G.K. Abou-Alfa

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M.A. Lowery, R. Ptashkin, M.F. Berger, A. Zehir, M. Capanu, E.M. O'Reilly, W.R. Jarnagin, M.I. D'Angelica, J.F. Hechtman, D.B. Solit, N. Schultz, D.S. Klimstra, L.B. Saltz, G.K. Abou-Alfa

Writing, review, and/or revision of the manuscript: M.A. Lowery, R. Ptashkin, A. Zehir, M. Capanu, N.E. Kemeny, E.M. O'Reilly, I. El-Dika, W.R. Jarnagin, J.J. Harding, M.I. D'Angelica, A. Cercek, J.F. Hechtman, D.B. Solit, D.M. Hyman, D.S. Klimstra, L.B. Saltz, G.K. Abou-Alfa

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M.A. Lowery, R. Ptashkin, E. Jordan, W.R. Jarnagin, D.B. Solit, D.M. Hyman, G.K. Abou-Alfa

Study supervision: M.A. Lowery, D.B. Solit, L.B. Saltz, G.K. Abou-Alfa

Acknowledgments

This work was funded in part by Cycle for Survival, the Marie-Josée and Henry R. Kravis Center for Molecular Oncology, and the National Cancer Institute Cancer Center Core Grant No. P30-CA008748.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received January 16, 2018; revised May 4, 2018; accepted May 18, 2018; published first May 30, 2018.

References

1. Kim Y, Moris DP, Zhang XF, Bagante F, Spolverato G, Schmidt C, Dilhoff M, et al. Evaluation of the 8th edition American Joint Commission on Cancer (AJCC) staging system for patients with intrahepatic cholangiocarcinoma: a surveillance, epidemiology, and end results (SEER) analysis. J Surg Oncol 2017;116:643–50.
2. Razumilava N, Gores GJ. Cholangiocarcinoma. Lancet 383:2168–79.
3. Churi CR, Shroff R, Wang Y, Rashid A, Kang HC, Weatherly J, et al. Mutation profiling in cholangiocarcinoma: prognostic and therapeutic implications. PLoS ONE 9:e115383, 2014.
4. Jiao Y, Pawlik TM, Anders RA, Selaru FM, Streppel MM, Lucas DJ, et al. Exome sequencing identifies frequent inactivating mutations in BAP1, ARID1A and PBRM1 in intrahepatic cholangiocarcinomas. Nat Genet 2013;45:1470–3.
5. Andersen JB, Spee B, Blechacz BR, Avital I, Komuta M, Barbour A, et al. Genomic and genetic characterization of cholangiocarcinoma identifies therapeutic targets for tyrosine kinase inhibitors. Gastroenterology 2012; 142:1021–31.e15.
6. Nakamura H, Arai Y, Totoki Y, Shirota T, Elzawahry A, Kato M, et al. Genomic spectra of biliary tract cancer. Nat Genet 2015;47:1003–10.
7. Ong CK, Subimerb C, Pairojkul C, Wongkham S, Cutcutache I, Yu W, et al. Exome sequencing of liver fluke-associated cholangiocarcinoma. Nat Genet 2012;44:690–3.
8. Tyson GL, El-Serag HB. Risk factors of cholangiocarcinoma. Hepatology 2011;54:173–84.
9. Javle MM, Shroff RT, Zhu A, Sadeghi S, Choo SP, Borad MJ, et al. A phase 2 study of BGJ398 in patients (pts) with advanced or metastatic FGFR-altered cholangiocarcinoma (CCA) who failed or are intolerant to platinum-based chemotherapy. J Clin Oncol 2016;34:(suppl 4S; abstr 335).
10. Javle M, Churi C, Kang HC, Shroff R, Janku F, Surapaneni R, et al. HER2/neu-directed therapy for biliary tract cancer. J Hematol Oncol 2015;8:58.
11. Rougier P, Riess H, Manges R, Karasek P, Humblet Y, Barone C, et al. Randomised, placebo-controlled, double-blind, parallel-group phase III study evaluating aflibercept in patients receiving first-line treatment with gemcitabine for metastatic pancreatic cancer. Eur J Cancer 2013;49:2633–42.

12. Mahaseth H, Brutcher E, Kauh J, Hawk N, Kim S, Chen Z, et al. Modified FOLFIRINOX regimen with improved safety and maintained efficacy in pancreatic adenocarcinoma. Pancreas 2013;42:1311–5.
13. Ghorani E, Wong HH, Hewitt C, Calder J, Corrie P, Basu B. Safety and efficacy of modified FOLFIRINOX for advanced pancreatic adenocarcinoma: a UK single-centre experience. Oncology 2015;89:281–7.
14. Portal A, Pernot S, Tougeron D, Arbaud C, Bidault AT, de la Fouchardière C, et al. Nab paclitaxel plus gemcitabine for metastatic pancreatic adenocarcinoma after failure of folfirinox: Results of an AGEO multicenter prospective cohort. J Clin Oncol 2015;33:(Meeting abstracts).
15. Cheng DT, Mitchell TN, Zehir A, Shah RH, Benayed R, Syed A, et al. Memorial sloan kettering-integrated mutation profiling of actionable cancer targets (MSK-IMPACT): a hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. J Mol Diagnos 2015;17:251–64.
16. Won HH, Scott SN, Brannon AR, Shah RH, Berger MF, et al. Detecting somatic genetic alterations in tumor specimens by exon capture and massively parallel sequencing. J Vis Exp 2013:e50710.
17. Chakravarty D, Gao J, Phillips SM, Kundra R, Zhang H, Wang J, et al. OncoKB: a precision oncology knowledge base. JCO Precision Oncol 2017:1–16.
18. Yu KH, Ricigliano M, Hidalgo M, Abou-Alfa GK, Lowery MA, Saltz LB, et al. Pharmacogenomic modeling of circulating tumor and invasive cells for prediction of chemotherapy response and resistance in pancreatic cancer. Clin Cancer Res 2014;20:5281–9.
19. Farshidfar F, Zheng S, Gingras MC, Newton Y, Shih J, Robertson AG, et al. Integrative genomic analysis of cholangiocarcinoma identifies distinct IDH-mutant molecular profiles. Cell Rep 2017;18:2780–94.
20. Mazzaferro V, El-Rayes BF, Cotsoglou C, Harris WP, Damjanov N, Masi G. ARQ 087, an oral pan-fibroblast growth factor receptor (FGFR) inhibitor, in patients (pts) with advanced intrahepatic cholangiocarcinoma (iCCA) with FGFR2 genetic aberrations. J Clin Oncol 2017;35:(suppl; abstr 4017).
21. Lowery MA, Abou-Alfa GK, Valle JW, Kelley RK, Goyal L, Shroff RT. ClarIDHy: a phase 3, multicenter, randomized, double-blind study of AG-120 vs placebo in patients with an advanced cholangiocarcinoma with an IDH1 mutation. J Clin Oncol 2017;35:(suppl; abstr TPS4142).
22. Lowery AM, Abou-Alfa GK, Burris HA, Janku F, Shroff RT, Cleary JM, et al. Phase I study of AG-120, an IDH1 mutant enzyme inhibitor: results from the cholangiocarcinoma dose escalation and expansion cohorts. J Clin Oncol 201735:(suppl; abstr 4015).
23. Goyal L, Saha SK, Liu LY, Siravegna G, Leshchiner I, Ahronian LG, et al. Polyclonal secondary *FGFR2* mutations drive acquired resistance to FGFR inhibition in patients with FGFR2 fusion–positive cholangiocarcinoma. Cancer Discov 2017;7:252–63.
24. Mazzaferro V, El-Rayes BF, Cotsoglou C, Harris WP, Damjanov N, Masi G, et al. ARQ 087, an oral pan-fibroblast growth factor receptor (FGFR) inhibitor, in patients (pts) with advanced intrahepatic cholangiocarcinoma (iCCA) with FGFR2 genetic aberrations. J Clin Oncol 2017;35:(suppl; abstr 4017).
25. Silva VWK, Askan G, Daniel TD, Lowery M, Klimstra DS, Abou-Alfa GK, et al. Biliary carcinomas: pathology and the role of DNA mismatch repair deficiency. Chinese Clin Oncol 2016;5:62.
26. Suto T, Habano W, Sugai T, Uesugi N, Kanno S, Saito K, et al. Infrequent microsatellite instability in biliary tract cancer. J Surg Oncol 2001;76:121–6.
27. Stadler ZK, Battaglin F, Middha S, Hechtman JF, Tran C, Cercek A, et al. Reliable detection of mismatch repair deficiency in colorectal cancers using mutational load in next-generation sequencing panels. J Clin Oncol 2016;34:2141–7.
28. Javle M, Bekaii-Saab T, Jain A, Wang Y, Kelley RK, Wang K, et al. Biliary cancer: utility of next-generation sequencing for clinical management. Cancer 2016;122:3838–47.

Published OnlineFirst May 30, 2018; DOI: 10.1158/1078-0432.CCR-18-0078

Clinical Cancer Research

Comprehensive Molecular Profiling of Intrahepatic and Extrahepatic Cholangiocarcinomas: Potential Targets for Intervention

Maeve A. Lowery, Ryan Ptashkin, Emmet Jordan, et al.

Clin Cancer Res 2018;24:4154-4161. Published OnlineFirst May 30, 2018.