# Prognosis and Clinicopathologic Features of Patients With Advanced Stage Isocitrate Dehydrogenase (IDH) Mutant and IDH Wild-Type Intrahepatic Cholangiocarcinoma

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

Key Words. Cholangiocarcinoma • Isocitrate dehydrogenase • Prognosis • Metastatic • Phenotype

## ABSTRACT

**Background.** Conflicting data exist regarding the prognostic impact of the isocitrate dehydrogenase (*IDH*) mutation in intrahepatic cholangiocarcinoma (ICC), and limited data exist in patients with advanced-stage disease. Similarly, the clinical phenotype of patients with advanced *IDH* mutant (IDH*m*) ICC has not been characterized. In this study, we report the correlation of *IDH* mutation status with prognosis and clinicopathologic features in patients with advanced ICC.

**Methods.** Patients with histologically confirmed advanced ICC who underwent tumor mutational profiling as a routine part of their care between 2009 and 2014 were evaluated. Clinical and pathological data were collected by retrospective chart review for patients with IDH*m* versus IDH wild-type (IDH*wt*) ICC. Pretreatment tumor volume was calculated on computed tomography or magnetic resonance imaging.

**Results.** Of the 104 patients with ICC who were evaluated, 30 (28.8%) had an *IDH* mutation (25.0% *IDH1*, 3.8% *IDH2*). The median overall survival did not differ significantly between IDH*m* and IDH*wt* patients (15.0 vs. 20.1 months, respectively; $p = .17$). The pretreatment serum carbohydrate antigen 19-9 (CA19-9) level in IDH*m* and IDH*wt* patients was 34.5 and 118.0 U/mL, respectively ($p = .04$). Age at diagnosis, sex, histologic grade, and pattern of metastasis did not differ significantly by *IDH* mutation status.

**Conclusion.** The *IDH* mutation was not associated with prognosis in patients with advanced ICC. The clinical phenotypes of advanced IDH*m* and IDH*wt* ICC were similar, but patients with IDH*m* ICC had a lower median serum CA19-9 level at presentation. *The Oncologist* 2015;20:1019–1027

**Implications for Practice:** Previous studies assessing the prognostic impact of the isocitrate dehydrogenase (*IDH*) gene mutation in intrahepatic cholangiocarcinoma (ICC) mainly focused on patients with early-stage disease who have undergone resection. These studies offer conflicting results. The target population for clinical trials of IDH inhibitors is patients with unresectable or metastatic disease, and the current study is the first to focus on the prognosis and clinical phenotype of this population and reports on the largest cohort of patients with advanced *IDH* mutant ICC to date. The finding that the *IDH* mutation lacks prognostic significance in advanced ICC is preliminary and needs to be confirmed prospectively in a larger study.

## INTRODUCTION

Intrahepatic cholangiocarcinoma (ICC) is a rare and often fatal malignancy of the intrahepatic bile ducts, and its incidence is steadily rising [1]. Although surgery is the only curative treatment for ICC, most patients present with advanced disease and encounter a median overall survival of less than 1 year [2–4]. Combination chemotherapy with gemcitabine and cisplatin is the mainstay of treatment for patients with advanced biliary tract cancers, including ICC [4]. In an attempt

to identify novel, actionable molecular targets in ICC, we and others have identified somatic mutations in the gene encoding isocitrate dehydrogenase (*IDH*) in 10%–28% of patients with ICC [5–11]. These active site mutations in the cytoplasmic IDH1 and mitochondrial IDH2 enzymes acquire neomorphic activity that converts α-ketoglutarate (α-KG) to the oncometabolite 2-hydroxyglutarate (2HG). 2HG accumulates in high concentrations in tissues that express mutant IDH [8] and may be

**Table 1.** Baseline characteristics for patients with advanced IDH mutant versus IDH wild-type intrahepatic cholangiocarcinoma

| Characteristic | IDH mutant (n = 30) | IDH wild type (n = 74) | p value |
|---|---|---|---|
| Age at diagnosis of advanced disease, median (range), yr | 59 (24–77) | 61 (23–83) | .26 |
| Male sex, n (%) | 12 (40) | 35 (47) | .50 |
| Baseline CA19-9,[a] median (range), U/mL | 34.5 (1–533) | 118.0 (1–94,432) | .04 |
| Baseline CEA,[a] median (range), U/mL | 2.95 (0.3–77.8) | 2.3 (0.2–887.5) | .99 |
| Tumor volume,[a] median (range), cm$^3$ | 184.0 (1.87–1,074.0) | 118.7 (0.8–1,487.5) | .40 |
| Ratio of CA19-9 to tumor volume,[a] median (range) | 0.51 (0.0045–4.25) | 1.37 (0.0034–846.2) | .04 |
| Baseline total bilirubin level,[a] median, mg/dL | 0.5 (0.3–6.3) | 0.6 (0.1–22.1) | .75 |
| Site of metastasis at any time, n (%) | | | |
|     Liver | 23 (76.7) | 57 (77.0) | .78 |
|     Lymph node | 14 (46.7) | 48 (64.9) | .06 |
|     Lung | 9 (30.0) | 31 (41.9) | .24 |
|     Peritoneum | 7 (23.3) | 24 (32.4) | .32 |
|     Bone | 7 (23.3) | 10 (13.5) | .26 |
|     Other | 0 (0) | 9 (12.2) | .06 |
| Histology, n (%) | | | .28 |
|     Well differentiated | 2 (6.7) | 7 (9.46) | |
|     Well to moderately differentiated | 0 (0) | 1 (1.35) | |
|     Moderately differentiated | 7 (23.3) | 24 (32.4) | |
|     Moderately to poorly differentiated | 2 (6.7) | 9 (12.2) | |
|     Poorly differentiated | 11 (36.7) | 13 (17.6) | |
| Presentation, n (%) | | | .76 |
|     Primary unresectable or metastatic | 21 (70.0) | 54 (73.0) | |
|     Recurrent metastatic | 9 (30.0) | 20 (27.0) | |

[a]At the time of diagnosis of advanced intrahepatic cholangiocarcinoma.
Abbreviations: IDH, isocitrate dehydrogenase.

involved in tumorigenesis by inhibiting a class of $\alpha$-KG-dependent dioxygenases involved in epigenetic regulation, extracellular matrix maturation, and cell signaling [12, 13].

Since the discovery of *IDH* mutations in ICC, phase I trials of IDH1 and IDH2 inhibitors (NCT02073994 and NCT02273739; http://www.clinicaltrials.gov) have been initiated that include patients with advanced *IDH*-mutant ICC. Tumor mutational profiling will likely become the standard of care in the management of patients with advanced ICC, and knowledge of the clinical phenotype of *IDH*-mutant ICC can trigger oncologists to identify patients early who have a high likelihood of harboring the mutation, such as is done in *EGFR*- and *ALK*-positive non-small cell lung cancer. Additionally, prognostic information about the *IDH* mutation can help guide physicians in their decision-making as it does in glioma, where the *IDH1* mutation is associated with a better prognosis [14–17].

Whereas previous studies have evaluated prognosis and pathology in the IDH mutant (IDH*m*) versus IDH wild-type (IDH*wt*) ICC populations, the data are conflicting. *IDH* mutations in ICC have been associated with either improved, worse, or no impact on overall survival [9–11]. They have been associated with poorly differentiated and clear-cell histology [5] and have had no association with histologic grade [11]. Furthermore, previous studies have addressed these questions in resected populations [5, 9, 10] or heterogeneous populations of different tumor stages [8, 11], but none to our knowledge have focused

on patients with unresectable or metastatic ICC. Therefore, the purpose of this study was to evaluate the correlation of *IDH* mutation status with overall survival and clinicopathologic features in patients with advanced ICC.

## PATIENTS AND METHODS

### Patient Population
Patients with histologically confirmed advanced intrahepatic cholangiocarcinoma who underwent tumor mutational profiling at the Massachusetts General Hospital Cancer Center as a routine part of their care between 2009 and 2014 were examined in this study; one patient had testing done at Foundation One (Boston, MA). Advanced disease was defined as unresectable or metastatic disease. The diagnosis of ICC was confirmed by the independent review of histology by a pathologist (V.N.) and independent review of clinical records by two medical oncologists (L.G. and A.X.Z.). All patients gave consent for mutational profiling on a protocol approved by the institutional review board.

### Mutational Analysis
Tumor mutational analysis was performed on DNA extracted from formalin-fixed, paraffin-embedded tissue. A tumor genotyping assay based on the SNaPshot multiplex platform system (Applied Biosystems, Carlsbad, CA, http://www.appliedbiosystems.com), was used to simultaneously query
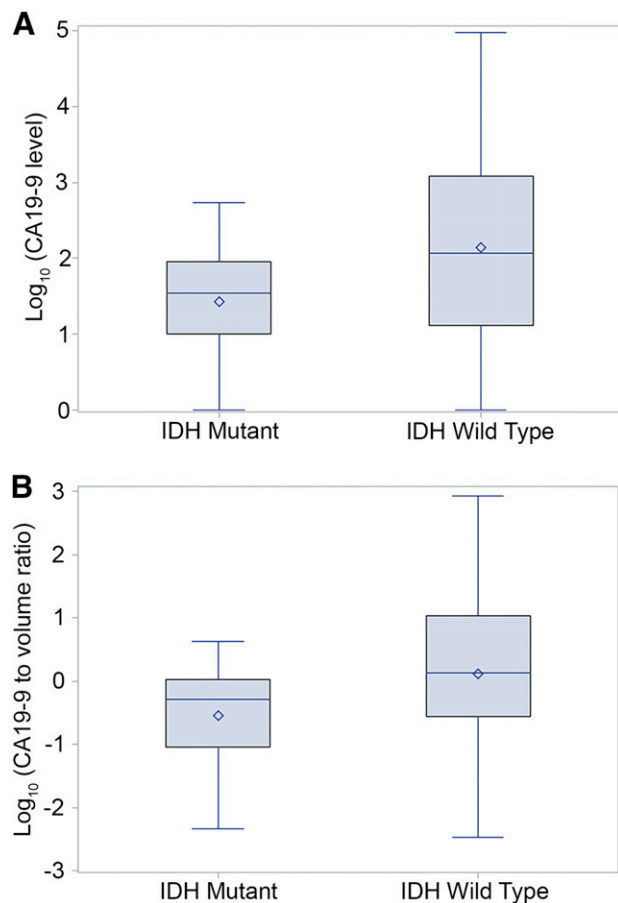
**Figure 1.** Comparison of CA19-9 in pateints with IDH*m* versus IDH*wt* advanced intrahepatic cholangiocarcinoma (ICC). **(A):** Comparison of the $\log_{10}$ pretreatment CA19-9 level in patients with IDH*m* versus IDH*wt* advanced ICC. The median $\log_{10}$ pretreatment CA19-9 level for patients with IDH*m* versus IDH*wt* disease was 1.42 and 1.96, respectively ($p = .04$). **(B):** Comparison of the $\log_{10}$ pretreatment CA19-9 to tumor volume ratio in patients with IDH*m* versus IDH*wt* advanced ICC. The median $\log_{10}$ pretreatment CA19-9 to tumor volume ratio for patients with IDH*m* versus IDH*wt* disease was −0.30 and 0.14, respectively.

Abbreviations: CA19-9, carbohydrate antigen 19-9; IDH*m*, isocitrate dehydrogenase mutant; IDH*wt*, isocitrate dehydrogenase wild type.

more than 150 previously described hotspot mutations across 16 cancer genes (supplemental online Table 1), as previously reported [18]. The SNaPshot genotyping assay is a fast, high-throughput, multiplex mutational profiling method that has the advantage over conventional dideoxynucleotide (Sanger) sequencing in that mutations can be detected when mutant DNA composes as little as 5% of the total DNA. These hotspot mutations tested on this platform included *IDH1* R132X, *IDH2* R140X, and *IDH2* R172X, and the primers used have been reported [8]. An earlier version of this assay, which did not test for *IDH2* mutations, was used on 54 samples, 12 of which were found to have *IDH1* mutations. Mutational profiling was performed at the Clinical Laboratory Improvement Amendments-certified Translational Research Laboratory at the Massachusetts General Hospital Cancer Center. Methods used for next-generation sequencing by Foundation Medicine Inc. (Cambridge, MA, http://www.foundationmedicine.com) have been reported [19].

## Data Collection

A pathologist (V.N.) confirmed the histology for all enrolled patients with available samples, using the World Health Organization classification system for tumor grade. A retrospective chart review was conducted to assess the following variables: date of diagnosis, age at diagnosis, sex, pretreatment carbohydrate antigen 19-9 (CA19-9) and carcinoembyonic antigen (CEA) levels, pretreatment total bilirubin level, histologic grade, pattern of metastasis, date of recurrence, and date of last follow-up or death. Date of diagnosis was defined as the date of biopsy confirming cholangiocarcinoma. In the case of patients with recurrent disease after primary resection or radiation, the date of first radiological recurrence was used when a biopsy was not performed. Sites of metastasis at any time during the course of treatment were determined based on the final available imaging prior to death or loss of follow-up.

For calculation of the pretreatment tumor volume at the time of diagnosis of advanced disease, a radiologist (R.A.S.) with subspecialty training in abdominal imaging used the patient's initial staging CT scan of the chest, abdomen, and pelvis; when a CT scan was not available, an abdominal MRI was used. Calculations were done using a standard clinical three-dimensional (3D) image analysis software package (iNtuition; TeraRecon, Foster City, CA, http://www.terarecon.com). To use the software, axial slices ranging in thickness from 1.25 to 5 mm were transferred to a 3D workstation, and the radiologist manually identified the intrahepatic and extrahepatic malignant lesions. Then the software package's segmentation analysis tools automatically circumscribed the lesions, and the radiologist manually adjusted the regions of interest in three orthogonal planes to accurately reflect the size of lesion. Finally, the software calculated the tumor volumes of the regions of interest. Lymph nodes ≥1.5 cm along the short axis in the porta hepatis and ≥1.0 cm along the short axis in all other locations were considered to be involved with tumor; this was done to standardize a definition, given that lymph nodes were usually not sampled to confirm or exclude tumor involvement.

## Statistical Analysis

Categorical variables such as sex, histologic grade, and sites of metastasis were described as totals and frequencies; continuous variables such as age, pretreatment tumor markers, and pretreatment tumor volume were described as medians and ranges. Univariate comparisons of each variable by *IDH* mutation status were assessed using the chi-square test, Fisher exact test, *t* test, or Wilcoxon rank-sum test, as appropriate. Recurrence-free survival (RFS) was defined as the time from the date of surgery to the date of recurrence confirmed by biopsy, or confirmed radiologically when a biopsy was not performed. Overall survival (OS) was defined as the time from initial diagnosis by biopsy to the date of death. Patients who were not known to have died were censored at the date of last follow-up. The Kaplan-Meier method was used to estimate RFS and OS and these were compared using the log-rank test. The Greenwood method was used to calculate the 95% confidence intervals for median RFS and OS. A *p* value of less than .05 was considered significant. Statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC, http://www.sas.com).

## RESULTS

### Characteristics of the Overall Study Population

The overall study population included 104 patients with unresectable or metastatic ICC (Table 1). Seventy-four patients (71.2%) presented with advanced disease at initial diagnosis, and 30 patients (28.8%) developed recurrent metastatic disease after surgery with curative intent ($n = 28$) or liver-directed radiation ($n = 2$) as their primary therapy. The two patients who received primary radiation had early-stage, resectable disease at presentation, but they were poor surgical candidates because of medical comorbidities. The median age at diagnosis of advanced disease in the overall population was 61 years (range: 23–83 years), and 45.2% of patients were male. Analysis by sex revealed that the median age at diagnosis of advanced disease was similar for men and women: 60 years and 63 years, respectively.

Pretreatment tumor marker and tumor volume analysis at the time of diagnosis of advanced disease were performed. The median CA19-9 and CEA values were 64 U/mL (range: 1.0–94,432 U/mL) and 2.5 ng/mL (range: 0.2–8,875 ng/mL), respectively. The median tumor volume was 132.9 cm$^3$ (range: 0.8–1,487.5 cm$^3$), and the median ratio of CA19-9 level to tumor volume was 0.59 (range: 0.0034–846.2).

The most common site of metastasis at the time of the last radiological scan was the liver (76.9%), followed by lymph nodes (59.6%), lung (38.5%), peritoneum (29.8%), and bone (16.3%). The other sites of metastases included the adrenal gland (5.7%), kidney (1.0%), brain (1.0%), and adnexa (1.0%). Majority of metastatic sites were suspected radiologically because of imaging characteristics and/or interval growth, but not confirmed by biopsy specimen. Many patients had more than one site of metastasis. The most common histologic grade was moderately differentiated (29.8%).

On mutational analysis of 104 patients with advanced ICC, *IDH* mutations were identified in 28.8% ($n = 30$). Other mutations identified included *KRAS* (8.7%), *PIK3CA* (4.8%), *BRAF* (2.9%), *TP53* (2.9%), *NRAS* (1.9%), *ERBB2* (1.0%), *MAP2K1* (1.0%), and *CDH1* (1.0%). One of the patients with a *TP53* mutation had a concurrent mutation in *MET*. No mutation was identified in 47.1% of patients ($n = 49$).

### Clinicopathologic Characteristics of IDH Mutant versus IDH Wild-Type ICC

The *IDH* mutation frequency among patients with advanced ICC in this study was 28.8%. Table 1 provides a comparison of the baseline and tumor characteristics of patients with IDH*m* versus IDH*wt* ICC. The most notable difference was that patients with IDH*m* ICC had a lower median pretreatment CA19-9 value compared with patients with IDH*wt* ICC (34.5 vs. 118.0 U/mL, respectively; $p = .04$) (Fig. 1A). The percentage of patients with an undetectable pretreatment CA-19-9 level at the time of diagnosis of advanced disease was similar in the IDH*m* and IDH*wt* groups (3.8% and 4.8%, respectively). No difference was noted in the median baseline total bilirubin level between the IDH*m* and IDH*wt* groups (0.5 vs. 0.6 mg/dL, respectively; $p = .75$), suggesting that differences in biliary obstruction rates did not account
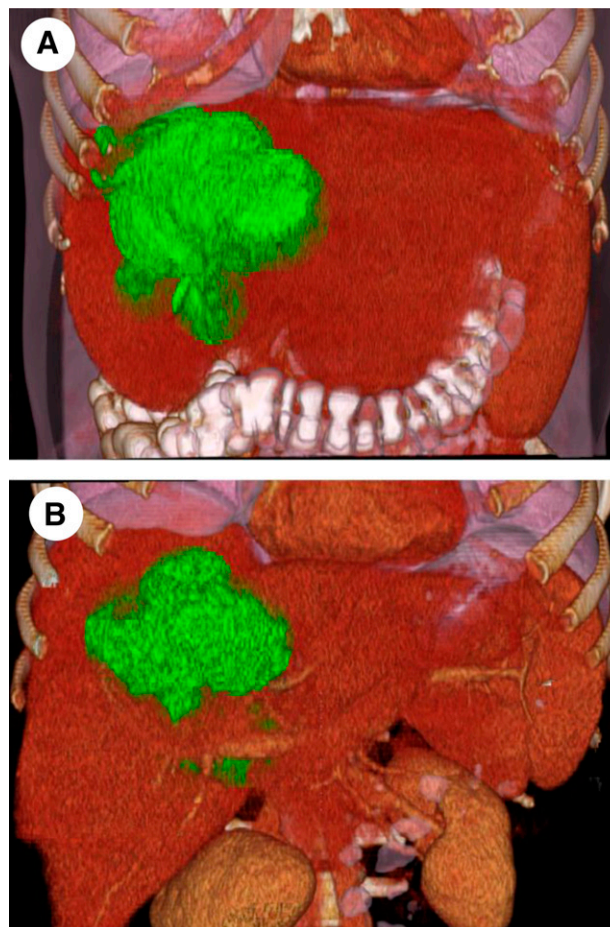


**Figure 2.** Tumor volume (TV) assessment based on abdominal computed tomography scans. Frontal projections of three-dimensional reconstructions demonstrate liver tumor volumes (green) overlaid on anatomic structures in two patients. Reconstructions and semiautomatic tumor volume calculations were generated by a commercial software package (iNtuition). **(A):** Computed tomography scan of a 47-year-old white man with primary, unresectable isocitrate dehydrogenase (IDH) wild-type intrahepatic cholangiocarcinoma (ICC) with a baseline carbohydrate antigen 19-9 (CA19-9) level of 5,688 U/mL and a baseline TV of 1,487 cm$^3$. **(B):** Computed tomography scan a 59-year-old white woman with primary, unresectable IDH-mutant ICC with a baseline CA19-9 level of 24 U/mL and TV of 1,007 cm$^3$.

for the difference in CA19-9 levels. To account for the potential impact of tumor volume (TV) on CA19-9 level, the pretreatment TV and the ratio of pretreatment CA19-9 to TV were calculated for each patient. The median pretreatment TV was not significantly different between patients with IDH*m* and IDH*wt* ICC (184.0 vs. 118.7 cm$^3$, respectively; $p = .40$), but the median ratio of the pretreatment CA19-9 level to tumor volume remained significantly different (0.51 vs. 1.37, respectively; $p = .04$) (Figs. 1B, 2), suggesting that the difference in CA19-9 levels was not due to difference in tumor burden at presentation.

The most common site of metastasis in both groups was the liver, and a trend toward a lower rate of metastasis to lymph nodes was also noted in the IDH*m* group compared with the IDH*wt* group (46.7% vs. 64.9%, respectively; $p = .06$). The most common histologic grade in the IDH*m* group was poorly differentiated (36.7%) and the most common in the IDH*wt*

**Table 2.** Clinicopathologic characteristics of patients with IDH mutant intrahepatic cholangiocarcinoma (n = 30)

| Identifier | Mutation | Age[a], yr | Sex | Histology | Baseline CA19-9 level,[a] U/mL | Sites of metastasis |
|---|---|---|---|---|---|---|
| 001 | IDH1 R132C | 56.0 | F | Moderately differentiated | 533.0 | LN, LV, LG |
| 007 | IDH1 R132C | 61.7 | M | Moderately differentiated | 393.0 | LN, LV |
| 009 | IDH1 R132C | 64.0 | F | Well differentiated | 91.0 | LN |
| 014 | IDH1 R132C | 64.4 | F | N/A | 95.0 | LV, LG, B |
| 017 | IDH1 R132C | 53.9 | F | Moderately differentiated | Undetectable | LV |
| 020 | IDH1 R132C | 58.1 | F | Well to poorly differentiated | 34.0 | LV |
| 025 | IDH1 R132C and KRAS G12D | 67.4 | F | N/A | 6.0 | LV, LG, B |
| 029 | IDH1 R132C | 64.1 | M | N/A | 19.0 | LN, LV, LG, P |
| 031 | IDH1 R132C | 67.3 | F | Poorly differentiated | 45.6 | LN |
| 033 | IDH1 R132C | 56.6 | M | N/A | 74.0 | LV |
| 037 | IDH1 R132C and TP53 R273H | 54.0 | F | Moderately differentiated | 18.6 | B |
| 041 | IDH1 R132C | 60.1 | M | Moderately differentiated | 10.0 | LN, LV, LG, P |
| 072 | IDH1 R132C | 46.4 | M | Moderately to poorly differentiated | 13.6 | LN, LG |
| 079 | IDH1 R132C | 46.0 | M | Poorly differentiated | Undetectable | LV |
| 092 | IDH1 R132C | 76.9 | F | Poorly differentiated | 7.0 | LV |
| 101 | IDH1 R132C | 48.3 | F | N/A | Undetectable | LV |
| 004 | IDH1 R132L | 77.3 | F | Moderately differentiated | 109.0 | LN, LV |
| 008 | IDH1 R132L | 66.9 | M | N/A | N/A | LV, B |
| 053 | IDH1 R132L | 67.7 | F | N/A | 98.0 | LN, LV |
| 055 | IDH1 R132L | 55.8 | F | Moderately differentiated | 137.0 | LN, LV, LG, B, P |
| 057 | IDH1 R132L | 67.2 | M | Poorly differentiated | 64.0 | LN |
| 061 | IDH1 R132L | 50.4 | M | N/A | 78.0 | LN, LV, B, P |
| 070 | IDH1 R132L | 43.1 | F | Poorly | N/A | LN, LV, B |
| 081 | IDH1 R132L | 63.5 | F | Moderately to poorly differentiated | 39.0 | LN |
| 093 | IDH1 R132L | 60.1 | F | N/A | 35.0 | LN, LV, P |
| 100 | IDH1 R132L | 23.9 | M | Poorly differentiated | 19.0 | LV, P |
| 042 | IDH2 R172W | 61.7 | M | Moderately differentiated | N/A | LV |
| 058 | IDH2 R172W | 44.1 | M | N/A | Undetectable | LV, LG |
| 076 | IDH2 R172T | 58.4 | F | N/A | 24.0 | LN |
| 097 | IDH2 mutation[b] | 52.2 | F | Moderately to poorly differentiated | N/A | LV, LG |

[a]At the time of diagnosis of advanced disease.
[b]From Foundation Medicine Inc.
Abbreviations: B, bone; CA19-9, carbohydrate antigen 19-9; IDH, isocitrate dehydrogenase; LG, lung; LN, lymph node; LV, liver; N/A, not available; N, no; P, peritoneum; Y, yes.

group was moderately differentiated (32.4%), but the difference in histologic grade was not statistically significant (p = .28). Definitive histologic grade was ascertained in 76 patients (74.1%), and the remaining patients did not have tissue available for review. Additional comparison of pathologic features, such as nodal, vascular, and perineural involvement on surgical specimens, was deferred as only 8 of the 28 resected specimens were IDH mutant, thereby precluding meaningful analysis. There was no significant difference in the age of diagnosis or sex distribution of patients with IDHm versus IDHwt ICC.

**Clinicopathologic Characteristics of IDH Mutants**
The mutation frequencies of IDH1 and IDH2 were 25.0% and 3.8%, respectively. The specific mutation and the clinicopathologic characteristics of the 30 patients with IDH mutant ICC are reported in Table 2. Among the 26 IDH1

mutants, 16 (61.5%) had an R132C mutation and 10 (38.5%) had an R132L mutation. Two patients with an IDH1 R132C mutation had a second mutation; one had mutation in KRAS G12D and the other in TP53 R273H. Among the 4 IDH2 mutants, 2 (50.0%) had an R172W mutation, 1 (25.0%) had an R172T mutation, and 1 patient had testing at Foundation Medicine Inc., and the report was not available for review. The cohort of IDH2 mutants was too small to make meaningful comparisons of survival and clinicopathological characteristics among IDH1- and IDH2-mutant patients.

**Survival Analysis**
The median follow-up duration was 16.9 months. Of the 104 patients, 100 (96.2%) had at least 3 months of follow-up, and 89 (85.6%) had at least 6 months of follow-up. The median OS
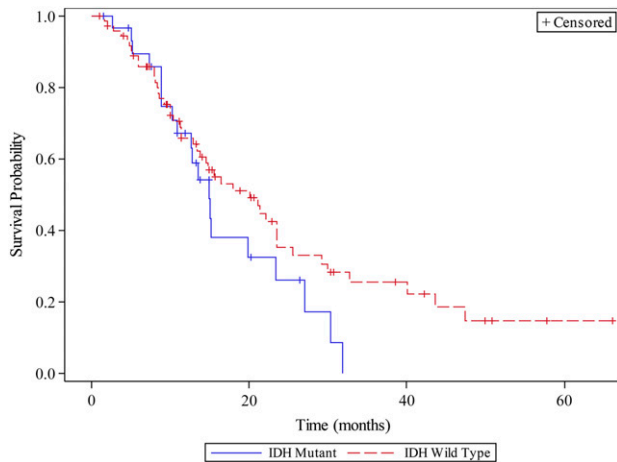
**Figure 3.** Overall survival of patients with isocitrate dehydrogenase (IDH) mutant (IDH*m*) versus IDH wild-type (IDH*wt*) unresectable or metastatic intrahepatic cholangiocarcinoma since date of diagnosis of advanced disease. The median overall survival of patients with IDH*m* versus IDH*wt* advanced-stage disease was 15.0 months versus 20.1 months, respectively ($p = .17$).

from diagnosis of advanced disease in the overall population of 104 patients was 16.4 months (95% confidence interval [CI]: 13.6–23.4 months). When analyzed by mutation status, the median OS of IDH*m* and IDH*wt* populations was 15.0 months and 20.1 months, respectively ($p = .17$) (Fig. 3). In the 74 patients with primary unresectable or metastatic disease, no significant difference in OS was seen between the IDH*m* and IDH*wt* populations.

The median OS from the date of initial diagnosis of ICC was also calculated; this time period was longer because 30 patients (28.8%) were initially diagnosed with early-stage disease and then experienced recurrence. The median OS from initial diagnosis in the overall population was 23.6 months (95% CI: 16.9–30.4 months), and no significant OS difference was seen between the IDH*m* and IDH*wt* patients (20.3 vs. 24.6 months, respectively; $p = .22$). The median RFS of the 30 patients in the overall population with recurrent metastatic ICC was 9.6 months (95% CI: 6.2–14.0 months). The median RFS of the *IDH* mutant ($n = 9$) and *IDH* wild-type ($n = 21$) patients was 9.0 months and 12.5 months, respectively ($p = .48$).

**Site of Genotyping Analysis**

We looked at the frequency of *IDH* mutation based on whether genotyping analysis was conducted on the primary liver tumor or a site of metastasis. Albeit a small sample, the rationale for this descriptive analysis was to gain preliminary insight into the potential difference of *IDH* mutation frequency in primary versus metastatic lesions. Among the 30 patients with recurrent metastatic disease, molecular genotyping was performed on the primary liver tumor in 23 cases (76.7%) and on a recurrent metastatic site in 7 cases (23.3%). The *IDH* mutation frequency among the 23 primary tumor specimens was 21.7% (5 *IDH1* and 0 *IDH2* mutations) and the frequency among the 7 recurrent metastasis specimens was 57.1% (3 *IDH1* and 1 *IDH2* mutations). Similar analyses were not conducted in patients with primary metastatic disease

because it was difficult to determine definitively in retrospect whether the liver biopsies were performed on the dominant liver mass or a metastatic liver lesion.

We also looked at *IDH* mutation frequency by organ site of mutational analysis. Of the 104 patients, genotyping was done on a biopsy or resection specimen of the liver in 93 cases (89.4%), extrahepatic site in 10 cases (9.6%), and unknown site in 1 case (1.0%). The extrahepatic sites included lymph nodes ($n = 4; 3.8\%$), peritoneum ($n = 4; 3.8\%$), sternum ($n = 1; 1.0\%$), and hernia sac ($n = 1; 1.0\%$). The *IDH* mutation frequency among the 93 liver specimens was 28.0% (23 *IDH1* and 3 *IDH2* mutations), and the *IDH* mutation frequency among the 9 extrahepatic specimens was 44.4% (3 *IDH1* mutations and 1 *IDH2* mutation).

## DISCUSSION

With IDH inhibitors in clinical development and entering phase I trials, characterizing the survival and clinical behavior of patients with unresectable or metastatic *IDH* mutant ICC is critical to assessing the impact of these drugs. The current study is the first to focus on prognosis and phenotype of patients with advanced IDH*m* ICC and evaluates the largest cohort of these patients published to date. In the pre-IDH inhibitor era, this study offers historical control data that can be used to benchmark future trial results. No significant difference was found in the median overall survival of patients with IDH*m* versus IDH*wt* advanced ICC ($p = .17$), which may, in part, have been due to insufficient power. Similarly, no significant difference was found in majority of the phenotypic variables compared, except that patients with IDH*m* advanced ICC were found to have a lower median serum CA19-9 level at presentation ($p = .04$).

Debate continues regarding the prognostic significance of the *IDH* mutation in ICC, as evidenced by three previous studies with conflicting results (Table 3). In one study, Wang et al. [10] showed a longer RFS and OS in the IDH*m* population compared with the IDH*wt* population in a cohort of 326 resected ICC patients ($p = .021$). Conversely, Jiao et al. [11] showed a shorter median OS in the IDH*m* population among 32 patients with IDH*m* or IDH*wt* ICC of different stages ($p = .0034$). However, 50% of the IDH*m* patients in this study had stage IV disease, whereas only 15% of the IDH*wt* patients had stage IV disease, and this may have skewed the prognostic data. In a third study, Zhu et al. [9] evaluated a cohort of 200 resected ICC patients and found no difference in RFS or OS based on *IDH* mutation status ($p > .05$). Our study represents another effort to assess the prognostic significance of the *IDH* mutation, specifically in patients with advanced stage ICC, but this area requires further investigation.

Despite assessing a variety of variables, no distinct clinical phenotype of IDH*m* advanced ICC emerged from this study. The clinical significance and the mechanism of a lower pretreatment serum CA19-9 level in patients with *IDH* mutant CCA is unknown. CA19-9 is a sialylated Lewis[a] blood-group antigen present on mucins that may be secreted into the plasma by cancer cells. It is an established diagnostic biomarker in cholangiocarcinoma, but the sensitivity (53% to 78%) and specificity (80% to 98.5%) in this disease vary widely [20–24]. In this study, the median pretreatment bilirubin level and tumor volume did not differ significantly

**Table 3.** Studies assessing the prognostic significance of IDH mutation in intrahepatic cholangiocarcinoma

| Study | Population | N | Patients with IDH mutations, n (%) | Patients with IDH1 vs. IDH2 mutations, n (%) | Survival data (IDH mutant vs. IDH wild type, p value) | Overall prognosis of IDHm patients compared with IDHwt patients |
|---|---|---|---|---|---|---|
| Wang et al. [10] | Resected only | 326 | 34 (10.4) | 23 (7.1) vs. 11 (3.4) | Median OS ($p = .028$ favoring IDHm)<br><br>Time to tumor recurrence ($p = .021$, favoring IDHm) | Better |
| Jiao et al. [11] | All stages | 32 | 6 (18.8) | 4 (12.5) vs. 2 (6.3) | 3–yr OS (33% vs. 81%; $p = .0034$) | Worse |
| Zhu et al. [9] | Resected only | 200 | 40 (20.0) | 31 (15.5) vs. 9 (4.5) | Median OS (31.3 vs. 31.4 months; $p > .05$) | No difference |
| Current study | Unresectable or metastatic only | 104 | 30 (28.8) | 26 (25) vs. 4 (3.8) | Median OS (15.0 vs. 20.1 months; $p = .17$) | No difference |

IDH, isocitrate dehydrogenase; IDHm, isocitrate dehydrogenase mutant; IDHwt, isocitrate dehydrogenase wild-type; OS, overall survival.

between the mutant and wild-type groups, so CA19-9 was not a surrogate for different rates of biliary obstruction or tumor burden at baseline. In terms of considering cellular mechanisms for the difference, it is worth noting that the biosynthesis of CA19-9 depends on the activity of the enzymes fucosyltransferase-2 (FUT2) and fucosyltransferase-3 (FUT3) [25–27]. Individuals who lack FUT3 activity are unable to synthesize Lewis antigens and are, therefore, unable to express the CA19-9 epitope [28–30]. Patients with primary sclerosing cholangitis without FUT3 activity have been shown to have a lower rate of CA19-9 production [31]. Whether IDHm ICC tumors have lower FUT2 and/or FUT3 activity or lower expression of other proteins responsible for CA19-9 secretion compared with IDHwt tumors remains unclear.

Our study confirmed findings seen in previous studies comparing patients with IDHm and IDHwt intrahepatic cholangiocarcinoma [8–11]. The IDH1 and IDH2 mutations were mutually exclusive, suggesting that these events are redundant, and that mutations in both genes concurrently does not confer a further advantage. The most common IDH mutations were IDH1 R132C and R132L, and no mutations were found in IDH1 R132H or IDH2 R140Q, the mutations commonly seen in glioma [17, 32] and acute myeloid leukemia (AML) [33–35], respectively. Recent studies have indicated that the mutant enzyme encoded by these mutations differ in their activity catalyzing 2HG production [36, 37], suggesting that pathways targeted by IDH mutations may differ by tumor type. Finally, a higher rate of poorly differentiated tumors was seen among IDHm compared with IDHwt patients, as has been previously reported [5], but this finding was not statistically significant in our study.

Two findings confirmed in this study regarding IDHm ICC may have future therapeutic implications. The first is the coexistence of other oncogenic mutations with mutations in IDH. In our study, two patients had concurrent mutations with IDH1 R132C: one patient had a mutation in KRAS G12D and the other in TP53 R273H. Mutations in these genes have been reported to coexist with IDH mutations in patients with ICC, as have mutations in PIK3CA, MAP2K1, BRAF, MET, and EGFR [6, 8–10]. Although the exact sequence of events is unknown in ICC, the opportunity for a double hit may arise if IDH mutations occur early in disease pathogenesis, as

observed in glioblastoma [38] and AML [38, 39]. Mutant IDH has been shown to block HNF-4α-mediated hepatocyte differentiation, leading to ICC with progenitor-like features. This environment may be ripe for transformation by additional oncogenic hits, and coexisting mutations may cooperate to activate progenitors and drive progression to metastatic ICC [40]. This phenomenon may underlie transient treatment responses and/or resistance to monotherapy with IDH inhibitors, and may suggest the need for combination strategies of systemic therapies.

The second finding that may have therapeutic implications is the clustering of somatic mutations around IDH1 Arg 132 and IDH2 Arg 172, the codons that mediate the conformational switch in isocitrate dehydrogenase. In the current study, all the patients with a known IDH mutation had a mutation at one of these two codons, as has been seen in other reports in ICC. Our platform specifically identifies hotspot mutations in the IDH gene at these codons and Arg 140, but other studies using whole-exome sequencing have identified these as the primary sites of IDH mutations [10, 11]; one study did identify a single mutation in IDH1 Ile99Met in a patient with ICC [10]. Spatial clustering is a common signature of mutations that provide an adaptive advantage to cancer cells and, consequently, undergo positive selection during clonal evolution of tumors. It can also be a sign of a driver mutation, as passenger mutations often distribute more randomly. Mutational clustering potentially allows for increased drug specificity, thereby allowing for improved safety and efficacy. Clustering can also be exploited for diagnostic purposes by allowing for efficient targeted screening efforts.

The current study has several limitations. Although it is the largest study to correlate IDH mutation status with clinicopathologic characteristics and survival in patients with advanced stage ICC, the sample size is still relatively small and the study is retrospective, limiting access to complete and accurate data. A larger prospective study is needed to validate these findings. Selection bias may impact the overall study population data, as the patients selected for genotyping at our institution are most often those who may be candidates for clinical trials; however, it is unlikely to effect the IDHm versus IDHwt comparison data, as the mutation status of the patients was not known a priori. Nearly half the samples were tested on

a genotyping platform that did not include *IDH2* testing, so the study may underestimate the frequency of *IDH2* mutations. Last, this is a single-institution study, so although it allowed for standardization of diagnostic technique and confirmation of pathology and tumor volumes, a multi-institutional study may be beneficial for discovering more generalizable results.

Several questions remain for further study in patients with IDH*m* ICC. The prognostic value of individual hotspot mutations and their predictive value in determining sensitivity to the current class of IDH1 and IDH2 inhibitors remain to be seen. Mutations coexisting with *IDH* mutations may play a role in drug resistance to monotherapy with IDH inhibitors, and combination strategies with other targeted agents or cytotoxic chemotherapy may require consideration in the future. Additionally, the performance of CA19-9 as a diagnostic and response biomarker in comparison with circulating oncometabolite 2-hydroxyglutarate [7] remains to be seen. Overall, the discovery of targetable point mutations in *IDH1* and *IDH2* represents a promising step forward in our understanding of ICC, and further investigation into the clinical and biological characteristics of patients with *IDH* mutant ICC will hopefully bring us closer to identifying effective therapies for them.

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See http://www.TheOncologist.com for supplemental material available online.

## For Further Reading:

Jeffrey S. Ross, Kai Wang, Laurie Gay et al. New Routes to Targeted Therapy of Intrahepatic Cholangiocarcinomas Revealed by Next-Generation Sequencing. *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.