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Genetic Determinants of Outcome in Intrahepatic Cholangiocarcinoma

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Abstract

BACKGROUND AND AIM: Genetic alterations in intrahepatic cholangiocarcinoma (iCCA) are increasingly well characterized, but their impact on outcome and prognosis remains unknown.

APPROACH AND RESULTS: This bi-institutional study of patients with confirmed iCCA (n = 412) used targeted next-generation sequencing of primary tumors to define associations among genetic alterations, clinicopathological variables, and outcome. The most common oncogenic alterations were isocitrate dehydrogenase 1 (*IDH1*; 20%), AT-rich interactive domain–containing protein 1A (20%), tumor protein P53 (*TP53*; 17%), cyclin-dependent kinase inhibitor 2A (*CDKN2A*; 15%), breast cancer 1–associated protein 1 (15%), *FGFR2* (15%), polybromo 1 (12%), and *KRAS* (10%). *IDH1*/*2* mutations (*mut*) were mutually exclusive with *FGFR2* fusions, but neither was associated with outcome. For all patients, *TP53* ($P < 0.0001$), *KRAS* ($P = 0.0001$), and *CDKN2A* ($P < 0.0001$) alterations predicted worse overall survival (OS). These high-risk alterations were enriched in advanced disease but adversely impacted survival across all stages, even when controlling for known correlates of outcome (multifocal disease, lymph node involvement, bile duct type, periductal infiltration). In resected patients (n = 209), *TP53mut* (HR, 1.82; 95% CI, 1.08–3.06; $P = 0.03$) and *CDKN2A* deletions (*del*; HR, 3.40; 95% CI, 1.95–5.94; $P < 0.001$) independently predicted shorter OS, as did high-risk clinical variables (multifocal liver disease [$P < 0.001$]; regional lymph node metastases [$P < 0.001$]), whereas *KRASmut* (HR, 1.69; 95% CI, 0.97–2.93; $P = 0.06$) trended toward statistical significance. The presence of both or neither high-risk clinical or genetic factors represented outcome extremes (median OS, 18.3 vs. 74.2 months; $P < 0.001$), with high-risk genetic alterations alone (median OS, 38.6 months; 95% CI, 28.8–73.5) or high-risk clinical variables alone (median OS, 37.0 months; 95% CI, 27.6-not available) associated with intermediate outcome. *TP53mut*, *KRASmut*, and *CDKN2Adel* similarly predicted worse outcome in patients with unresectable iCCA. *CDKN2Adel* tumors with high-risk clinical features were notable for limited survival and no benefit of resection over chemotherapy.

CONCLUSIONS: *TP53*, *KRAS*, and *CDKN2A* alterations were independent prognostic factors in iCCA when controlling for clinical and pathologic variables, disease stage, and treatment. Because genetic profiling can be integrated into pretreatment therapeutic decision-making, combining clinical variables with targeted tumor sequencing may identify patient subgroups with poor outcome irrespective of treatment strategy.

Intrahepatic cholangiocarcinoma (iCCA) is an aggressive malignancy with rising incidence and mortality,[1,2] marked genetic heterogeneity,[3] and limited treatment options.[4] Complete resection of localized iCCA remains the only potentially curative treatment, but recurrence rates are high.[4] Furthermore, resection is not an option for most patients who present with advanced unresectable disease, for whom systemic chemotherapy remains the primary treatment strategy but has limited benefit. Recent clinical trials have suggested promising results for treatments targeting tumors with isocitrate dehydrogenase 1/2 (*IDH1*/*2*) and *FGFR2* alterations[5,6]; however, the impact of these therapies in a minority of patients with these alterations is unclear.

Lymph node metastasis and multifocal liver disease are powerful predictors of poor outcome after resection in iCCA[7,8] and are often used for prognostication and treatment allocation. However, some patients with unfavorable clinical criteria experience prolonged survival, whereas others with favorable clinical factors (e.g., solitary lesions, lymph node–negative) recur quickly and die shortly after surgery. Similarly, reliable outcome predictors for patients with advanced disease are lacking.

Recent studies have made inroads in characterizing mutational patterns and identifying genetic alterations with potential prognostic and therapeutic significance.[9,10] In most studies, however, such alterations have been noted in a minority of patients. Given the rarity and marked genomic heterogeneity of iCCA,[3] it has been difficult to characterize the complex interplay among alterations in individual somatic alterations and related pathways and to determine those alterations with prognostic value.[5,6,11] Thus, the primary aim of the present study was to characterize the mutational landscape of primary iCCA in a large cohort of patients, covering the broad spectrum of disease extent, with the goal of defining broadly applicable prognostic genomic alterations juxtaposed with known clinicopathologic predictors of outcome.

Patients and Methods

STUDY DESIGN AND PATIENTS

This study included patients with histologically confirmed primary iCCA treated at Memorial Sloan Kettering Cancer Center (MSKCC; USA) or Erasmus Medical Center (the Netherlands). The cohort included patients who underwent curative-intent resection at MSKCC (1993–2018) or Erasmus (2005–2015) (postoperative mortalities excluded) and patients treated nonoperatively at MSKCC (2008–2019). Systemic chemotherapy regimens were determined by the treating oncologist; some patients were treated with hepatic arterial infusion chemotherapy (HAIC), as described.[12,13] HAIC consisted of continuous infusion of floxuridine into the liver circulation through a surgically implanted hepatic pump in combination with concurrent systemic chemotherapy (primarily gemcitabine+platinum). Data were collected from prospectively maintained databases and supplemented with medical record review. Patients with previous clinical tumor genetic profiling or with available banked tumor tissue/pathological slides for retrospective genomic analysis were included. A pathologist blinded to tumor genotype reviewed slides to confirm the diagnosis and assess liver parenchymal disease (i.e., cirrhosis, steatosis), coded as present or absent. In the unresected cohort, cirrhosis was based on clinical grounds as cirrhotic liver morphology could not be assessed pathologically due to small tissue samples (e.g., liver biopsy). The diagnosis of chronic viral hepatitis was based on positive serum markers for chronic hepatitis B and C. Of note, data from 109 (31 resected, 78 unresected) patients have been reported.[11] The study was approved by the institutional review boards at MSKCC and Erasmus. All patients provided written informed consent for targeted-sequencing and in accordance with the ethical guidelines of the Declaration of Helsinki.

Resected tumors were staged according to American Joint Committee on Cancer's (AJCC's) eighth edition classification.[14] Early in the study time frame, hepatoduodenal ligament lymphadenectomy was performed selectively if clinically warranted but was

subsequently routine. Patients with clinically node-negative disease had comparable survival to patients with histologically node-negative disease ($P = 0.20$; Supporting Fig. S1); both were categorized as "lymph node–negative." Patients with lymph node metastasis and/or multifocal disease were considered "clinical high risk" for recurrence and death; those without these features were deemed "clinical low risk." In unresected patients, staging was based on imaging and available pathology. Suspicious lymph node metastasis on imaging was categorized as "lymph node–positive."[14] The reasons for unresectability included locally advanced disease (multifocal liver disease, lymph node metastasis, or extensive vascular and/or biliary involvement) or distant metastatic disease (M1).

GENOMIC PROFILING

All specimens were sequenced in the clinical laboratories of the Molecular Diagnostics Service at MSKCC using the MSK-Integrated Mutation Profiling of Actionable Cancer Targets (IMPACT) assay, a clinically validated hybridization capture-based targeted next-generation sequencing array that can detect mutations, copy-number alterations (CNAs), and select rearrangements.[15] The assay was expanded during the study period, and of the 412 cases, 40 were sequenced using the initial 341-gene assay, 147 using the 410-gene assay, and the remaining 225 using the 468-gene assay.[15] Sequencing and analysis were performed as described.[15,16] Briefly, all slides were re-reviewed by experienced attending pathologists (E.V. or C.S.) to identify tumor and normal liver tissue for DNA extraction. All samples had >60% tumor content, and DNA isolated from the primary tumor and matched normal liver tissue or blood was sequenced. All classes of genomic alterations were determined and called against the patient's matched normal sample. Genomic data are available at cBioPortal (www.cbioportal.org/study/summa ry?id=ihch_msk_2021).

Genes sequenced in at least 70% of samples and recurrent oncogenic alterations occurring in ≥5% of all patients were identified. Genetic alterations were filtered for oncogenic variants using OncoKB (http://oncokb.org), [17] a precision oncology knowledge base that tracks cancer gene alterations. Only oncogenic alterations (oncogenic or likely oncogenic by OncoKB) were included in statistical analyses. We evaluated 12 canonical signaling pathways from The Cancer Genome Atlas (TCGA) PanCancer Atlas, including cell cycle, Hippo, Myc, Notch, nuclear factor erythroid 2–related factor 2 (NRF2), phosphoinositide 3-kinase (PI3K), receptor tyrosine kinase (RTK)/RAS, TGF-β, tumor protein P53 (TP53), Wnt, epigenetic, and DNA damage repair (DDR).[18,19]

Tumor mutation burden (TMB) was calculated as the total number of somatic non–silent protein–coding mutations divided by the coding region captured in each MSK-IMPACT panel (341 genes, 0.98 Mb; 410 genes, 1.06 Mb; 468 genes, 1.22 Mb), as we have validated.[20]

Variation in genomic alterations stratified by disease extent was analyzed for the entire cohort. Because complete staging information was not available for all patients who did not undergo resection or exploration, patients were divided into three groups (solitary liver tumor, multifocal liver tumor, and metastatic disease [lymph node with or without distant sites]) that correlated with increasing AJCC stage.

STATISTICAL ANALYSIS

Recurrence-free survival (RFS) after resection was calculated from surgery date until documented recurrence or death. Patients alive and recurrence-free at last follow-up were censored. Patients were followed every 3–6 months; physical examination, carbohydrate antigen 19–9 (CA19–9) level, and cross-sectional imaging were performed at each visit. Time of recurrence was defined as the time of the first imaging that reported definitive or suspicious new tumors or, for patients with biopsy-proven recurrence, the date of positive cytological or histological results.

Overall survival (OS) was calculated from the time of liver resection until death. Patients alive at last follow-up were censored. Five-year outcome estimates with two-sided 95% CIs are reported. For unresected patients, OS was calculated from date of diagnosis until death or last follow-up. For the entire cohort and for resected versus unresected comparisons, OS was calculated from the date of diagnosis.

Individual genes and pathways were evaluated for association with RFS/OS using univariable Cox proportional hazards regression. Associations between mutational status and tumor histological characteristics were assessed with Fisher's exact test. Kaplan-Meier estimates were calculated for 5-year survival and median survival. Histopathologic variables were evaluated for associations with RFS/OS using univariable Cox proportional hazards regression, and HAIC was treated as a time-dependent variable. When testing multiple genes/characteristics, P values were adjusted for multiple testing using the false discovery rate approach within outcome; Q values (adjusted P values) < 0.05 were considered significant.

Clinicopathological multivariable models were constructed by including all clinical/pathological factors significant in univariable analysis and retaining factors with $P <$ 0.05. For the unresected cohort, suspicious lymphadenopathy was included due to clinical importance. The final multivariable models were constructed by adding genomic factors significant in univariable analysis to the final clinicopathological model. All tests were two-sided, and all analyses were performed using R, version 4.0.0.

Results

CLINICAL AND PATHOLOGIC FEATURES OF THE COHORT

Overall, 412 patients with iCCA from the two institutions were included, of whom 390 were treated at MSKCC and 22 at Erasmus (Table 1). The median age was 64 years, and the male:female ratio was approximately 1:1. Sixty-four percent (264/412) of the patients had multifocal or nodal with or without distant metastatic disease, and 51% underwent resection as a primary treatment.

Patients were stratified according to disease extent (Table 1). Older patients tended to present with solitary liver tumors, and patients with more advanced disease tended to be younger. Higher-grade tumors were more likely to be metastatic at the time of presentation. No other clinicopathologic variables were associated with disease extent. Of 148 patients with solitary liver tumors, 89% (132/148) underwent resection, with the remainder

frequently deemed unresectable owing to significant major vascular invasion or extensive biliary involvement. Patients with multifocal disease infrequently underwent operative intervention as multiple tumors are a relative contraindication to surgical resection[21]; in the majority of such cases, multifocality was not appreciated preoperatively. Hepatic arterial infusion chemotherapy was administered largely to patients with unresectable, multifocal liver disease, with or without regional nodal disease.

The cohort was then stratified and analyzed by treatment. A total of 209 patients underwent resection, 94/207 (45%) of whom received additional systemic treatment (the use of any systemic chemotherapy could not be confirmed in two patients), including adjuvant HAIC with 5-fluoro-2-deoxyuridine with or without systemic chemotherapy (n = 6) and adjuvant systemic therapy only (n = 70), predominantly gemcitabine with or without platinum or capecitabine (n = 51) (no perioperative targeted therapies). Of the remaining 203 patients who did not undergo resection, 107 (53%) had unresectable locally advanced disease (i.e., bilateral multifocal disease or major vascular invasion), and 96 (47%) had metastatic disease. Suspicious lymph node involvement based on imaging was present in 31% (63/203) at locoregional sites only (hepatoduodenal ligament) and 30% (60/203) at distant nodal basins. Of patients who did not undergo resection, 197/203 (97%) received palliative chemotherapy, predominantly gemcitabine+platinum (145/197, 74%). Only 9/203 (4%) patients received a mitogen-activated protein kinase kinase inhibitor; no other first-line targeted agents were used. HAIC with concurrent systemic chemotherapy (primarily gemcitabine+oxaliplatin) was used in 54/203 (27%) unresected patients.

MUTATIONAL LANDSCAPE OF iCCA

Targeted DNA sequencing on 412 samples identified a total of 1,551 genetic alterations. The most common inactivating mutations were found in *IDH1* (20%), AT-rich interactive domain–containing protein 1A (*ARID1A*; 20%), *TP53* (17%), breast cancer 1–associated protein 1 (*BAP1*; 15%), and polybromo 1 (*PBRM1*; 12%) (Fig. 1A; Supporting Table S1). Activating mutations were found in *KRAS* (10%) and *RAS*/*RAF* kinases, including *BRAF* (5%), *NRAS* (3%), *RASA1* (3%), and neurofibromin 1 (*NF1*; 1.7%). Ninety-two oncogenic fusions were identified in 78/412 patients (19%); 53 fusions in 47/412 (11%) patients involved *FGFR2*, and 38 were known in-frame *FGFR2* fusions. Focal CNAs were noted in multiple genes, with cyclin-dependent kinase inhibitor 2A (*CDKN2A*) homozygous deletions (*del*) being the most common (13%; Fig. 1A). Rare amplifications in RTKs, such as Erb-B2 receptor tyrosine kinase 2 (*ERBB2*) (n = 7), *EGFR* (n = 6), and *MET* (n = 3), were identified. The median TMB per sample was 2.6 mutations/MB (interquartile range, 1.8–3.9); only 2 patients had TMB > 40 (consistent with microsatellite instability). Overall, most genes belonged to four key pathways, with alterations in epigenetic regulators being most frequent (246/412, 60%), followed by RTK/RAS signaling (196/412, 48%), TP53 (97/412, 24%), and cell cycle (85/412, 21%) pathways.

Next, we examined mutual exclusivity and co-occurrence of enriched pathways and mutated genes (*mut*) across the cohort. *IDH1*/*2mut* tumors were significantly mutually exclusive from *FGFR2* fusions (*fus*) ($\tau = -0.19$, $Q = 0.002$), *CDKN2Adel* ($\tau = -0.17$, $Q = 0.008$), *TP53mut* ($\tau = -0.15$, $Q = 0.02$), and telomerase reverse transcriptase (*TERT*) *mut* ($\tau =$

−0.13, $Q = 0.04$). In addition, there was significant co-occurrence of *TERT* and *TP53* aberrations ($\tau = 0.19$, $Q = 0.002$) (Supporting Fig. S2). PI3K ($\tau = 0.21$, $Q = 0.001$), TGF-β ($\tau = 0.19$, $Q = 0.002$), cell cycle ($\tau = 0.18$, $Q = 0.004$), and RTK/RAS ($\tau = 0.16$, $Q = 0.01$) pathways showed co-occurrence with *TP53mut* (Supporting Table S2). In contrast, *IDH1*/*2mut* were mutually exclusive with most pathways (RTK/RAS [$\tau = -0.24$, $Q < 0.001$], cell cycle [$\tau = -0.15$, $Q = 0.02$], TP53 [$\tau = -0.15$, $Q = 0.02$], TGF-β [$\tau = -0.13$, $Q = 0.04$]) and not associated with any other pathway defined by the TCGA PanCancer Atlas.

RELATIONSHIP BETWEEN MUTATIONAL PROFILE AND CLINICAL OUTCOMES

We leveraged the size and clinical annotation of this cohort to determine the clinical significance of recurrent genetic alterations. Patients with alterations in the TP53, RTK/RAS, cell cycle, PI3K, or TGF-β pathways had shorter survival than those without (Fig. 1B). Specifically, for all patients, *TP53mut*, *KRASmut*, *TERTmut*, and *CDKN2Adel* were significantly associated with shorter OS (Figs. 1B and 2). Notably, only deep deletions in *CDKN2A* (n = 52) were associated with significantly shorter OS ($P < 0.001$), while presumed monoallelic *CDKN2A* alterations (n = 9) appeared to have little impact (Fig. 2C). Significant survival differences were not observed in patients harboring *IDH1*/*2mut* or *FGRF2fus* compared to wild type (*wt*) (Fig. 2E), although *IDH1*/*2mut* tumors showed a trend toward an improved OS ($P = 0.08$). In addition, the frequency of *FGFR2fus* ($P = 0.34$) and *IDH1*/*2mut* ($P = 0.26$) did not differ between resected and unresected patients, and there was no significant difference in the predictive power of either mutation in the resected group. Of note, *IDH1mut* and *IDH2mut* were not associated with outcome when analyzed together or separately (data not shown). *IDH1*/*2mut* tumors were less often associated with elevated CA19–9 levels (44% vs. 67%; $P = 0.002$), whereas peers with *TP53mut* (76% vs. 58% *wt*; $Q = 0.04$) and *CDKN2Adel* showed higher CA19–9 levels (83% vs. 58% *wt*; $Q = 0.01$) (Supporting Table S3). Of note, in the 29 patients with cirrhosis, 6 (21%) had *TERT* promoter alterations versus 14 (4%) of the 374 cirrhosis-free cases ($Q = 0.008$), and in the 6 patients with primary sclerosing cholangitis (PSC), 5 (83%) had a *TP53mut* ($Q = 0.007$) (Supporting Table S4).

RELATIONSHIP BETWEEN GENETIC ALTERATIONS AND CLINICAL STAGING AND TREATMENT

We next analyzed genomic alterations stratified by treatment group—resected versus unresected—to determine if mutational profiles were associated with primary treatment allocation (Fig. 3; Supporting Table S1). Across the landscape of genetic alterations, tumors in both groups had similar genetic signatures, and neither group was dominated by specific alterations in individual genes or canonical signaling pathways. However, there was an association between high-risk genetic alterations (i.e., those associated with shorter OS in the entire cohort [*TP53mut*, *KRASmut*, *CDKN2Adel*]) and disease extent (Tables 2–4). (*TERTmut* omitted due to its strong correlation with *TP53mut*.) The incidence of both *TP53mut* and *CDKN2Adel* increased progressively from early (i.e., solitary tumors) to later stage disease (i.e., multifocal liver or nodal with or without distant metastatic disease) (Table 2). *KRASmut* was more common in advanced iCCA but had similar prevalence in solitary and multifocal tumors. Indeed, in the resected cohort, 65% of *TP53mut* (n = 24/37), 53% of *KRASmut* (n = 10/19), 64% of CDKN2A*del* (n = 14/22), and 64% (40/63) of

all genetic high-risk patients had either multifocal disease or metastatic disease (nodal or distant). In contrast, of the resected patients without any high-risk genetic alterations, only 25% (37/146) had multifocal or metastatic disease ($P < 0.001$). The genetic high-risk cohort also comprised >50% (17/33) of the resected multifocal disease group but only 21% (11/53) of the unresected multifocal disease group ($P = 0.004$). In a multivariable model, all three high-risk genetic alterations were significant predictors of poor outcome, independent of disease extent (Table 3), with *CDKN2Adel* having the strongest impact (HR, 2.5; 95% CI, 1.7–3.8).

The relative impact of individual genomic alterations was then analyzed within each disease extent category (Table 4). *TP53mut* and *KRASmut* appeared to have a greater impact on OS in patients with solitary liver tumors, while *CDKN2Adel* was a more potent predictor in patients with multifocal liver and nodal or distant metastases. When analyzed as a group, the presence of any one or more high-risk genetic alteration corresponded to shorter median OS across all disease extent categories (Table 4).

MUTATIONAL STATUS AND CLINICAL PREDICTORS OF OUTCOME IN RESECTED iCCA

Next, we integrated clinical variables with genetic predictors specifically for patients who underwent resection. Median RFS and OS for the resected cohort (n = 209) were 18.4 (95% CI, 13.4–24.2) and 46.4 (95% CI, 41.2–61.3) months, respectively; 5-year RFS and OS were 22% (95% CI, 17–29) and 42% (95% CI, 35–50), respectively. Among resected patients, the presence of any one or more high-risk genetic alterations (*TP53mut*, *KRASmut*, and/or *CDKN2Adel*) was associated with shorter RFS and OS compared to *wt* tumors (Fig. 4A). The impact of each individual alteration on median survival was *TP53mut* = 25.8 months (95% CI, 16.1–35.7) versus *TP53wt* = 59.9 months (95% CI, 46.4–74.2), *KRASmut* = 27.6 months (95% CI, 12.1–52.6) versus *KRASwt* = 52.3 months (95% CI, 43.0–68.0), and *CDKN2Adel* = 14.8 months (95% CI, 10.0–37.0) versus *CDKN2Awt* = 52.6 months (95% CI, 45.2–67.2). PI3K pathway alterations (n = 22) were also associated with a shorter OS compared to *wt* tumors: 33.0 (95% CI, 20.4-not available [NA]) versus 52.6 (95% CI, 44.9–68.0) months (Fig. 4A).

Several clinical variables were associated with a significant risk of recurrence and death, including large tumor size (>5 cm), multifocal liver disease, regional lymph node metastases, lymphovascular invasion (LVI), perineural invasion, periductal infiltration (PDI), and large bile duct (BD) type by univariable analysis (Supporting Table S5). On multivariable analysis, nodal disease, multifocal liver disease, and LVI remained independently associated with RFS and OS (Supporting Table S5), whereas large BD type remained an independent predictor for shorter time to recurrence but not to death. In the clinicopathological multivariable model, *TP53mut* and *CDKN2Adel* remained independent predictors of shorter OS, in addition to multifocal liver disease and nodal disease (Fig. 4B), whereas *KRASmut* and LVI trended toward statistical significance ($P = 0.06$). Due to overfitting, we did not formally evaluate the independent prognostic value of PDI in the multivariable analysis. However, even after inclusion of BD type and PDI, the results did not change (data not shown). *TP53mut* was associated with LVI (76% vs. 47% *wt*; $Q = 0.02$), nodal disease (43% vs. 15% *wt*; $Q < 0.001$), and large BD type (33% vs. 7% *wt*; $Q =$

0.02); *KRASmut* was associated with perineural invasion (65% vs. 27% *wt*; $Q = 0.04$) and large BD type (37% vs. 9% *wt*; $Q = 0.02$), and *CDKN2Adel* was associated with multifocal disease (55% vs. 25% *wt*; $Q = 0.04$) (Supporting Tables S6 and S7).

To assess risk stratification by genetic profile in resected patients, the low-risk (solitary, node-negative) and high-risk (multifocal and/or nodal disease) clinical groups were stratified by the presence ("genetic high risk") or absence ("genetic low risk") of at least one alteration in *TP53*, *KRAS*, or *CDKN2A* (Fig. 5A,B). Patients with both or without any high-risk clinical or genetic factors represented the extremes of outcome (median OS, 18.3 and 95% CI, 14.8–29.7 vs. OS, 74.2 and 95% CI, 61.3–95.1 months, respectively, $P < 0.001$). Within this wide range, however, the interaction between clinical and genetic variables further stratified patients into outcome groups. Specifically, RFS and OS in clinical low-risk patients were significantly reduced in the presence of at least one high-risk genetic alteration (genetic high risk) (Fig. 5A,B); similarly, outcomes in the high-risk clinical group were improved if no high-risk genetic alterations were present (genetic low-risk).

RISK STRATIFICATION IN UNRESECTABLE DISEASE

Next, we examined the utility of mutational profiling to stratify outcome in patients who did not undergo resection. In patients with unresectable disease (n = 203), median OS was 24.1 months (95% CI, 18.7–29.9) in those with locally advanced disease compared to 13.1 months (95% CI, 11.7–15.8) in those with distant metastases. On univariate analysis, *TP53mut*, *KRASmut*, and *CDKN2Adel*, as well as alterations in the RTK/RAS and TGF-β pathways, were associated with shorter OS (Fig. 4C). On multivariate analysis, only metastatic disease and *TP53mut*, *KRASmut*, and *CDKN2Adel* remained independent predictors of worse survival (Fig. 4D), recapitulating the findings observed in resected patients.

Alterations in *TP53*, *KRAS,* and *CDKN2A* stratified OS in unresected patients (locally advanced, $P = 0.001$; metastatic, $P = 0.01$; Fig. 5C). Median OS was 26.1 months (95% CI, 21.4–40.6) in patients with locally advanced, genetic low-risk tumors versus 16.8 months (95% CI, 11.1–29.8) in those with genetic high-risk tumors. In patients with metastatic disease, median OS was 15.7 months (95% CI, 12.8–23.1) for genetic low-risk tumors versus 9.7 months (95% CI, 7.0–15.5) with high-risk features, such that specific genetic alterations were universal predictors of poorer outcome, irrespective of disease extent or primary treatment modality.

In unresected patients with locally advanced iCCA treated with any chemotherapy (n = 103), survival was improved in genetic low-risk (n = 75; OS, 26.1 months, 95% CI, 23.5–40.6) compared to genetic high-risk tumors (n = 28; OS, 17.5 months, 95% CI, 11.2–29.8; $P = 0.001$). Within this group, HAIC was a strong predictor of improved survival (n = 50; HR, 0.45, 95% CI, 0.27–0.77; $P = 0.003$), independent of genomic profile. In fact, when compared with resected patients with similar disease extent (i.e., clinical high-risk, n = 77), the median OS for unresected, locally advanced patients was shorter (24.1, 95% CI, 18.7–29.9, vs. 32.0, 95% CI, 26.7–41.3, months), but the reduced HR of the HAIC treatment resulted in outcomes more comparable to resected, clinical high-risk patients. By contrast, patients in the locally advanced subgroup with genetic high-risk tumors generally had poor

outcomes across all treatments. In particular, clinical high-risk *CDKN2Adel* patients (n = 14) submitted to resection had a median OS of 12.2 months (95% CI, 10.5-NA) versus 16.8 months (95% CI, 11.3-NA) for locally advanced patients with *CDKN2Adel* tumors who did not undergo resection (n = 12) (Fig. 5D). Alterations in *TP53* or *KRAS*, on the other hand, did not negate apparent treatment outcome differences between these two groups (clinical high-risk resected: *TP53mut* 26.7 months [n = 24; 95% CI, 17.7–33.6] and *KRASmut* 27.1 months [n = 10; 95% CI, 13.3-NA] versus locally advanced unresected: *TP53mut* 11.8 months [n = 16; 95% CI, 10.1-NA and *KRASmut* 9.4 months [n = 8; 95% CI, 6.6-NA]). *CDKN2Adel* was therefore the strongest negative predictor of OS among the high-risk genetic variables across treatment groups.

Discussion

Several recent studies have highlighted the genetic heterogeneity of iCCA.[22] In the present analysis, we describe targeted genomic sequencing results from 412 iCCA tumor samples, representing one of the largest series combining genomics with detailed clinical annotation of treatment and outcomes. The data demonstrate the clinical utility of routine genomic analysis in patients with iCCA both for prognostication and for treatment recommendations.

As has been described, we observed that no single genetic alteration occurs in >25% of patients; however, we identified a high incidence of somatic alterations in the epigenetic (60%), RTK/RAS (48%), TP53 (24%), and cell cycle (21%) pathways, consistent with previous reports from other Western centers.[22,23] *FGFR2fus* were present in 11% of patients, a similar incidence as described recently (13.6%).[24] In our study, *TP53* and *KRAS* alteration prevalence was higher than that reported from other Western centers (6%−10%).[23,25,26] In the present series, we identify an independent predictive value of mutations in *TP53* and *KRAS* and deletions in *CDKN2A* for oncological outcome in patients treated for iCCA, even after adjustment for clinicopathological confounders. Specifically, the presence of any one of these high-risk genetic alterations independently predicted shorter survival, regardless of disease stage or treatment, reflecting their prognostic significance when combined with known prognostic clinical variables.[4,8,27] While the genetic and clinical high-risk features tended to coexist, there was a large proportion of patients with high-risk genetic alterations in the setting of clinically more favorable tumors.

The association of somatic mutations in *TP53* with shorter survival in resected iCCA has been reported,[22,28] suggesting an association with more aggressive disease and poor outcome.[28] In the present study, *TP53mut* was also associated with LVI, nodal disease, and large BD type, consistent with an aggressive phenotype, as shown in other liver cancers.[29] Associations between *KRASmut*, perineural invasion, large BD type, and worse outcome after iCCA resection have also been reported.[22,30,31] The prognostic implications of *TP53* and *KRAS* alterations in univariate analyses were described in a prior analysis of 321 biliary tract cancer samples, including 224 iCCAs.[32] In contrast to that study, here we demonstrate that each of these genetic high-risk mutations associates with outcomes independently for resected and unresectable disease, even when stratified by disease extent and known pathologic variables. Our study also uniquely identifies an association between alterations in *CDKN2A* and survival outcomes in patients with iCCA irrespective of disease extent or

treatment, building on our findings in advanced disease.[11] We also found that a subgroup with *CDKN2Adel* and high-risk clinical features had universally poor survival regardless of treatment, suggesting that these patients do not benefit from resection.

Divergent from previous studies that have reported predominantly large BD type iCCA (41% −59%),[33–37] the present study mainly consisted of small BD tumors. Potential reasons for this finding include a small number of patients with PSC or liver fluke infection,[35,38,39] two etiologies associated with large BD type iCCA.[36] Consistent with previous reports,[34,35,40–43] patients with iCCA submitted to resection with large BD type had worse prognosis in univariate analysis. However, histopathological subtype was not an independent predictor of postoperative survival in multivariate analysis, perhaps due to a significant association between large BD type and *KRASmut* and *TP53mut*.

There are conflicting data regarding the association between other commonly identified genetic alterations and outcomes. The most commonly reported actionable alterations in iCCA are *IDH1/2mut* and *FGFR2fus*, with significant mutual exclusivity between *IDH1/2mut* and *CDKN2Adel*, *TP53mut, TERTmut*, and *FGFR2fus*, consistent with previous work.[44] *IDH1/2* alterations may represent a distinct disease mechanism as oncogenic *IDH1/2* mutations are known to acquire neomorphic activity to produce the oncometabolite 2-hydroxyglutarate, with subsequently an inhibitory effect on α-ketoglutarate-dependent enzymes.[45,46] While *IDH1/2mut* and *FGFR2fus* remain predictive biomarkers for patients with these mutations receiving targeted therapies, particularly in advanced disease,[47,48] our data do not support a major prognostic role for all patients. For *IDH1/2mut*, this finding contrasts with a number of studies that have shown that *IDH1/2mut* may be associated with improved survival.[49,50] Other efforts to define the relationship of *IDH1/2mut* with survival have shown no impact[51,52] or even poorer survival.[23] We suspect that the heterogeneity in the literature related to inconclusive association of *IDH1/2mut* with outcome may reflect different proportions of resected and unresected patients in each study; of note, we observed an improvement in RFS in resected patients with *IDH1/2mut* and a trend toward increased OS, consistent with a prior evaluation in patients treated surgically.[49]

Multifocal disease and lymph node metastasis constituted clinical stratification, given their predictive power and availability prior to resection (versus histological features). The addition of genetic information, however, greatly improved risk stratification, identifying additional patient subgroups that may obtain either significant or marginal benefit from treatment. For instance, we demonstrate that patients with multifocal disease or lymph node involvement without high-risk genetic alterations have superior outcomes after resection. Despite relative contraindications to surgical resection in clinical high-risk patients, there appears to be a significant survival improvement in the absence of high-risk genetic features. On the other hand, patients with *CDKN2A* deletions are more likely to have multifocal disease and/or lymph node involvement, as well as universally poor outcomes irrespective of whether they underwent resection. Patients with this genetic profile combined with high-risk clinical features did not appear to benefit from resection, and they may be best treated initially with systemic therapy alone or in combination with HAIC, which resulted in survival rates better than systemic chemotherapy, consistent with our experience.[53]

Surgical resection should be reserved in these instances as there appears to be quite limited benefit.

As recent data supporting the use of targeted therapies for tumors with *IDH1* mutations[6] and *FGFR* fusions[5] now highlight the critical need for tumor mutational profiling in iCCA, we propose that deleterious mutations and alterations in *KRAS*, *TP53*, and/or *CDKN2A* are also key variables that can guide therapeutic interventions in these patients. In our large cohort, the ability to stratify by mutation, as well as treatment or disease extent, uncovers specific genetic aberrations that identify a population more likely to have a more aggressive disease course after resection. For this patient subgroup, the risk–benefit equation would seem to favor nonoperative management, given the potential morbidity associated with hepatic resection and limited benefit of intervention. Validation of these results in larger populations remains necessary, but the findings uncover a genetically defined population worthy of further investigation.

Limitations of this study include inherent selection bias associated with retrospective analysis of patients from two tertiary institutions; the findings may not fully account for geographic/demographic variation of iCCA.[54] The present cohort certainly reflects a non–liver fluke, non–hepatitis B endemic population in Western countries distinct from previous studies with mainly Asian populations. Additionally, our study used annotations in OncoKB, in which several variants of unknown significance (VUS) are often poorly characterized. However, large-scale efforts to characterize most VUS eventually demonstrate no clinical significance.[41] Finally, our effort focused primarily on genetic events in iCCA and does not offer additional insight into the epigenetic, transcriptional, and cell-extrinsic mechanisms that contribute to heterogeneous disease biology and thus outcome. Our observation that extensive clinical and genetic annotation allows prediction of outcomes in patients with iCCA highlights, however, the importance of driver mutations in tumor biological behavior.

Study patients also received various adjuvant and locoregional treatments which were not controlled for in statistical analyses. Certain pathologic characteristics were unavailable for some specimens, but this occurred in <10% of samples. Additionally, while some patients were treated prior to the introduction of contemporary chemotherapy, this group accounted for only 1% of all patients. Finally, resected tumors were staged according to AJCC guidelines, whereas staging was based on imaging and available pathology for unresected patients. We have taken great care to avoid direct statistical comparisons between the two treatment groups, and the concordance of the high-risk clinical stage based on preoperative imaging versus postsurgical histopathology was otherwise excellent (86% [180/209] in the resected cohort). Reanalysis of the risk stratification by *CDKN2Adel* status in resected and unresected patients of similar disease extent based on preoperative imaging only did not change the findings of the study (data not shown).

In conclusion, we identified high-risk mutations that stratify outcome in patients with iCCA and enhance prognostic modeling based on clinical variables alone. Pretreatment genetic profiling adds critical information to known clinicopathologic factors to inform therapeutic decision-making in patients with iCCA and to identify potential benefit from currently available treatment options.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

AJCC	American Joint Committee on Cancer
ARID1A	AT-rich interactive domain–containing protein 1A
BAP1	breast cancer 1–associated protein 1
BD	bile duct
CA19–9	carbohydrate antigen 19–9
CDKN2A	cyclin-dependent kinase inhibitor 2A
DDR	DNA damage repair
del	deletion
ERBB2	Erb-B2 receptor tyrosine kinase 2
fus	fusion
HAIC	hepatic arterial infusion chemotherapy
iCCA	intrahepatic cholangiocarcinoma
IDH1/2	isocitrate dehydrogenase 1/2
LVI	lymphovascular invasion
MSKCC	Memorial Sloan Kettering Cancer Center
mut	mutant
NA	not available
OS	overall survival

PBRM1	polybromo 1
PDI	periductal infiltration
PI3K	phosphoinositide 3-kinase
PSC	primary sclerosing cholangitis
RFS	recurrence-free survival
RTK	receptor tyrosine kinase
TERT	telomerase reverse transcriptase
TMB	tumor mutation burden
TP53	tumor protein P53
WT	wild type

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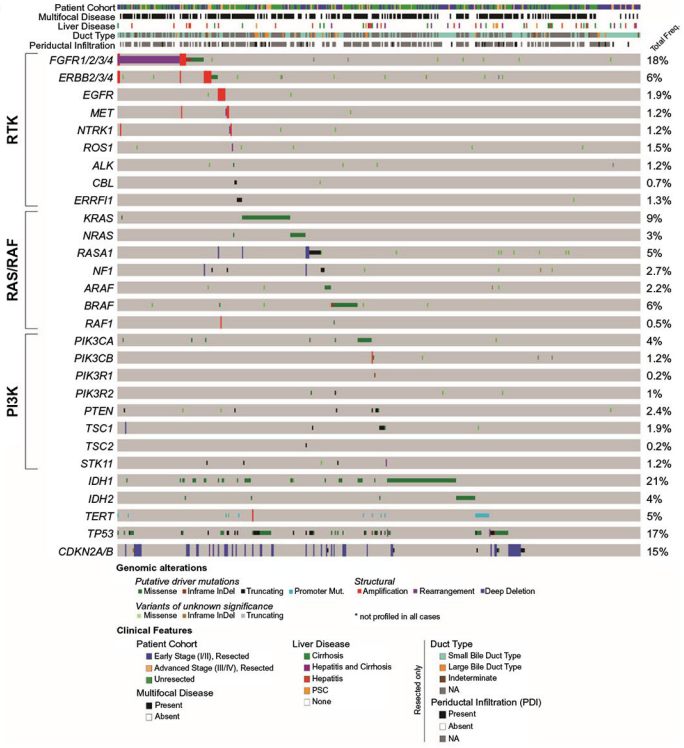
A



B

	HR	95% CI	q
BRAF	1.26	(0.76-2.09)	0.51
IDH1	0.81	(0.91-1.09)	0.27
IDH1/2	0.82	(0.62-1.07)	0.27
KRAS	1.99	(1.40-2.84)	**<0.001**
TERT	2.08	(1.25-3.45)	**0.01**
ARID1A	0.93	(0.69-1.24)	0.70
BAP1	1.10	(0.79-1.51)	0.69
CDKN2Adel	2.41	(1.74-3.33)	**<0.001**
FGFR2fus	0.89	(0.60-1.31)	0.69
PBRM1	0.78	(0.54-1.12)	0.27
TP53	2.26	(1.70-3.03)	**<0.001**
Epigenetic pathway	0.97	(0.76-1.23)	0.84
RTK/RAS pathway	1.36	(1.08-1.72)	**0.02**
TP53 pathway	2.03	(1.56-2.64)	**<0.001**
Cell cycle pathway	1.56	(1.18-2.05)	**0.01**
PI3K pathway	1.60	(1.13-2.26)	**0.02**
DDR pathway	1.04	(0.64-1.67)	0.88
TGF-β pathway	1.79	(1.11-2.89)	**0.03**

FIG. 1.

(A) OncoPrint representation of the genetic alterations occurring in the entire cohort (N = 412), including detailed breakdown of the RTK, RAS/RAF, and PI3K pathway genes, *IDH1/2*, and survival-associated genes. (B) Cox regression for overall survival by genetic features in all iCCA (N = 412, 287 events). Abbreviations: ARAF, A-Raf protooncogene; CBL, Cbl proto-oncogene; ERFFI1, ERBB receptor feedback inhibitor 1; NTRK, neurotrophic receptor tyrosine kinase; PTEN, phosphatase and tensin homolog; ROS1, ROS protooncogene 1; STK11, serine/threonine kinase 11; TSC1/2, TSC complex subunit 1/2.

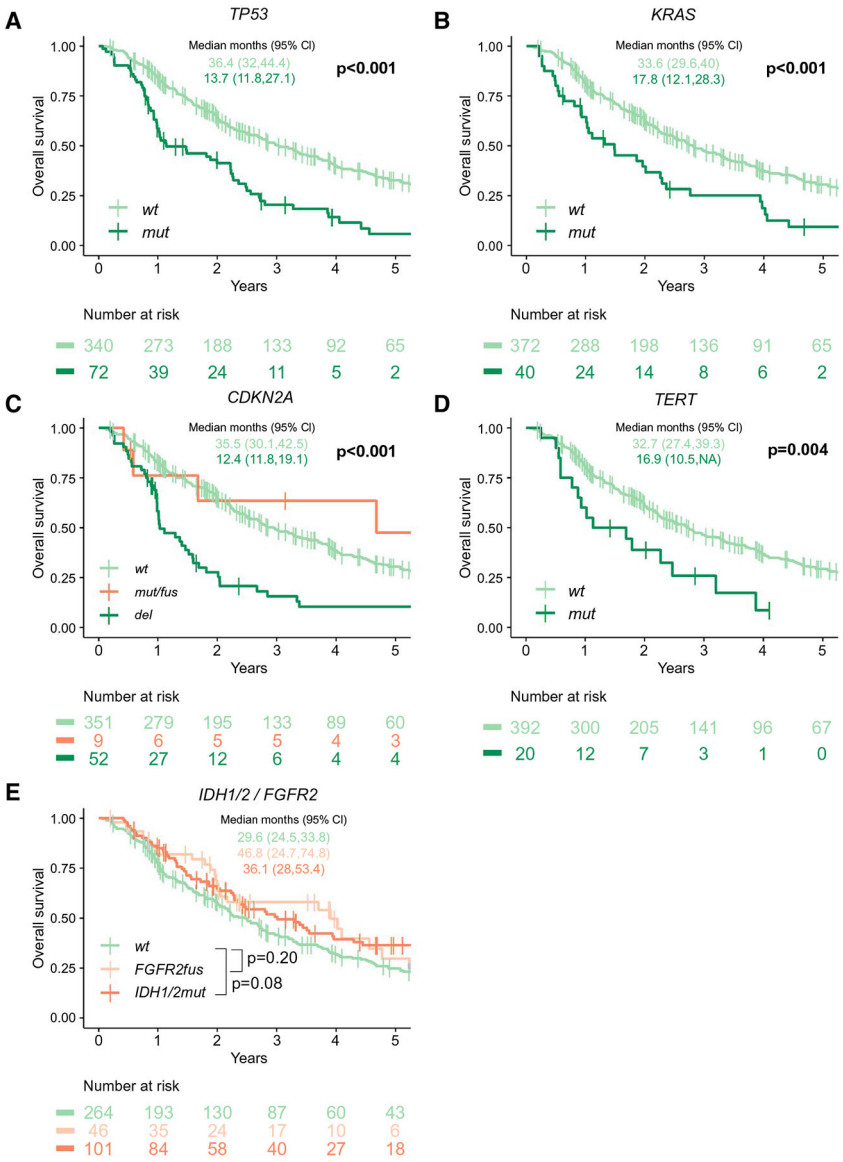


FIG. 2.

Effect of *TP53* (A), *KRAS* (B), *CDKN2A* (C), *TERT* (D), *IDH1*/*2* (E), and *FGFR2* (E) mutation status on overall survival in the whole cohort (N = 412, 287 events).

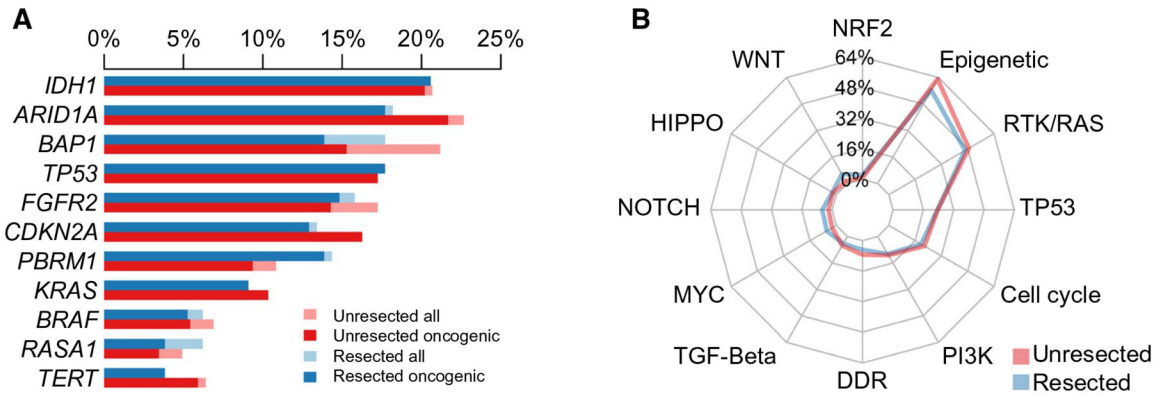


FIG. 3.

(A) Distribution of driver mutations or structural genetic alterations in iCCA occurring with a frequency of >5% in the entire cohort (N = 412), stratified by treatment group (resected and unresected). (B) Spider plot illustrating frequencies of alterations across 12 canonical signaling pathways in the entire cohort (N = 412), stratified by treatment group.

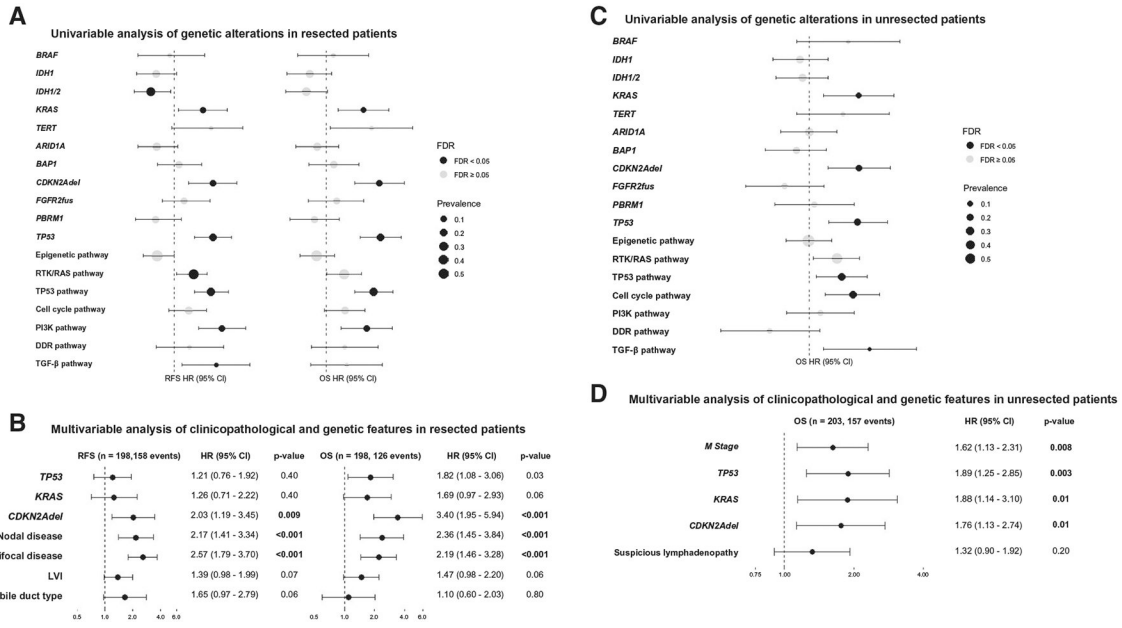


FIG. 4.

Univariable and multivariable analysis of genetic and clinicopathological features with RFS and OS in (A,B) resected and (C,D) unresected patients. (A) Univariable analysis of genetic features in resected patients (n = 209, 166 RFS events, 130 OS events). (B) Multivariable analysis of clinicopathological and genetic features in resected patients (n = 198). (C) Univariable analysis of genetic features in unresectable patients (n = 203, 157 events). (D) Multivariable analysis of clinicopathological and genetic features in unresectable patients (n = 203). *P* values were adjusted for multiple comparisons within outcome using the false discovery rate correction. Abbreviations: FDR, false discovery rate; M stage=metastatic disease.

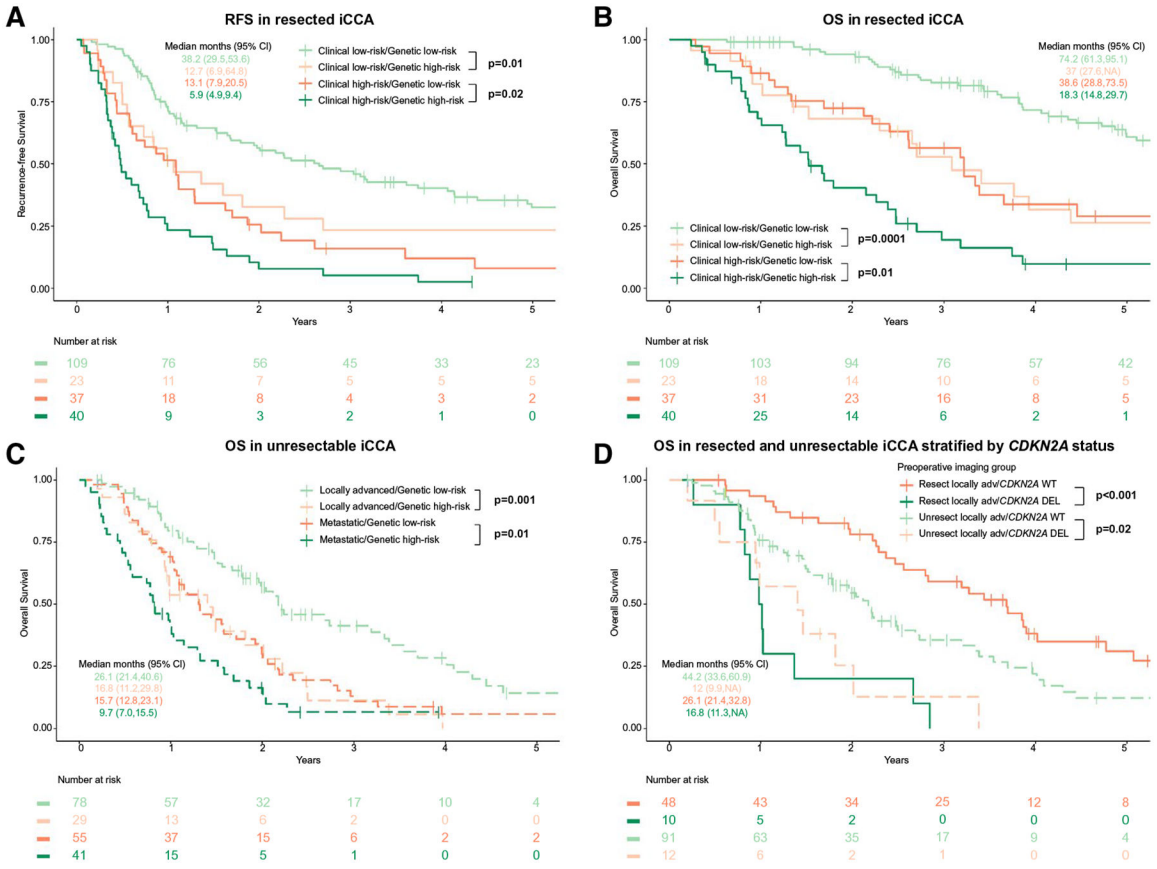


FIG. 5.

Effect of clinical and genetic high-risk group (TP53/KRAS/CDKN2A alterations) on (A) RFS (n = 209, 166 events) and (B) OS (n = 209, 130 events) after resection. (C) OS stratified by genetic risk groups and disease extent (locally advanced vs. metastatic, n = 203, 157 events) in patients with unresectable disease. (D) OS by CDKN2Adel status in resected/unresected patients of similar disease extent: resected clinical high-risk (n = 77) and unresected locally advanced (n = 103).

TABLE 1.

Demographic and Clinicopathological Characteristics

	Characteristic	Overall (N = 412)	Solitary liver tumor (n = 148)	Multifocal liver disease (n = 86)	Metastatic disease (n = 178)	*Q*
All patients	Age (years) at diagnosis, median (range)	64 (19–89)	66 (19–89)	63 (29–78)	61 (31–87)	**0.01**
	Sex					0.50
	Female	222/412 (54%)	85/148 (57%)	49/86 (57%)	88/178 (49%)	
	Male	190/412 (46%)	63/148 (43%)	37/86 (43%)	90/178 (51%)	
	BMI, median (range)	27.5 (17.6–59.8)	27.7 (19.6–59.8)	27.7 (17.6–54.7)	27.1 (17.6–52.1)	0.60
	Diabetes	84/411 (20%)	35/147 (24%)	18/86 (21%)	31/178 (17%)	0.50
	Chronic hepatitis	33/412 (8.0%)	15/148(10%)	4/86 (4.7%)	14/178 (7.9%)	0.50
	Hepatitis B	19/412 (4.6%)	8/148 (5.4%)	1/86 (1.2%)	10/178 (5.6%)	0.40
	Hepatitis C	16/412 (3.9%)	7/148 (4.7%)	3/86 (3.5%)	6/178 (3.4%)	0.80
	Cirrhosis	29/403 * (7.2%)	10/144 (6.9%)	8/85 (9.4%)	11/174 (6.3%)	0.80
	PSC	6/412 (1.5%)	2/148 (1.4%)	2/86 (2.3%)	2/178 (1.1%)	0.80
	Smoking					0.30
	Current smoker	41/409 (10%)	20/146 (14%)	9/85 (11%)	12/178 (50%)	
	Former smoker	166/409 (41%)	50/146 (34%)	39/85 (46%)	77/178 (6.7%)	
	Never smoked	202/409 (49%)	76/146 (52%)	37/85 (44%)	89/178 (43%)	
	HAIC	65/412 (16%)	10/148 (6.8%)	32/86 (37%)	23/178 (12%)	**<0.001**
	Tumor grade					**0.02**
	Well differentiated	15/392 (3.8%)	8/144 (5.6%)	4/81 (4.9%)	3/167 (1.8%)	
	Moderately differentiated	231/392 (59%)	92/144 (64%)	53/81 (65%)	86/167 (51%)	
	Poorly differentiated	146/392 (37%)	44/144 (31%)	24/81 (30%)	78/167 (47%)	
	Treatment group					**<0.001**
	Resected	209/412 (51%)	132/148 (89%)	33/86 (38%)	44/178 (25%)	
	Unresected	203/412 (49%)	16/148 (11%)	53/86 (62%)	134/178 (75%)	
	ECOG PS					**0.01**
	0	168/390 (43%)	75/139 (54%)	33/82 (40%)	60/169 (36%)	
	1	208/390 (53%)	60/139 (43%)	49/82 (60%)	99/169 (59%)	
	2–3	14/390 (3.6%)	4/139 (2.9%)	0/82 (0%)	10/169 (5.9%)	
	CA19–9 elevated (>40 U/mL)	190/307 (62%)	46/93 (49%)	38/63 (60%)	106/151 (70%)	**0.01**

Resected patients only	Characteristic	Overall (N = 209)	Solitary liver tumor (n = 132)	Multifocal liver disease (n = 33)	Metastatic disease (n = 44)	*Q*
	Systemic chemotherapy	94/207 (45%)	45/132 (34%)	13/32 (41%)	36/43 (84%)	**<0.001**
	Neoadjuvant	29/209 (14%)	6/132 (4.5%)	8/33 (24%)	15/44 (34%)	**<0.001**
	Adjuvant	76/205 (37%)	39/131 (30%)	9/32 (28%)	28/42 (67%)	**<0.001**
	Pathological tumor size (cm), median (range)	6.0 (1.1–24.0)	5.6 (1.1–18.5)	6.1 (2.0–15.0)	7.2 (2.7–24.0)	**0.04**
	LVI	108/208 (52%)	58/131 (44%)	16/33 (48%)	34/44 (77%)	**0.002**
	Perineural invasion	57/186 (31%)	33/120 (28%)	6/27 (22%)	18/39 (46%)	0.07
	Positive margin status	25/209 (12%)	14/132 (11%)	6/33 (18%)	5/44 (11%)	0.50
	Liver steatosis	67/199 (34%)	51/127 (40%)	9/32 (28%)	7/40 (18%)	**0.04**
	Periductal infiltration	23/163 (14%)	13/107 (12%)	3/23 (13%)	7/33 (21%)	0.50
	BD type					0.80
	Small	176/205 (86%)	114/132 (86%)	27/31 (87%)	35/42 (83%)	
	Large	23/205 (11%)	13/132 (9.8%)	4/31 (13%)	6/42 (14%)	
	Indeterminate	6/205 (2.9%)	5/132 (3.9%)	0/31 (0%)	1/42 (2.4%)	
	Positive lymph node status	42/209 (20%)	0/132 (0%)	0/33 (0%)	42/44 (95%)	**<0.001**

All data are n (%) unless noted. Denominators < 412 (for entire cohort) and <209 (for resected cohort) in overall column reflect missing data. Bold indicates significance.

* In a small subset of patients (n = 9), an adequate assessment of cirrhosis was not possible.

Abbreviations: BMI, body mass index; ECOG, Eastern Cooperative Oncology Group; PS, performance status.

TABLE 2.

Association Between Disease Extent and Genetic Alterations

Characteristic	Solitary liver tumor (n = 148), n (%)	Multifocal liver disease (n = 86), n (%)	Nodal ± distant metastases (n = 178), n (%)	*Q*
TP53mut	16 (11%)	15 (17%)	41 (23%)	**0.02**
KRASmut	11 (7%)	6 (7%)	23 (13%)	0.20
CDKN2Adel	8 (5%)	10 (12%)	34 (19%)	**0.003**

Bold indicates significance.

TABLE 3.

Influence of Clinicopathological and Genetic Alterations on OS (N = 412, 287 events)

Characteristic	HR	95% CI	*P*
Disease extent			
Solitary liver tumor	Ref		
Multifocal liver disease	1.16	0.71–1.88	0.60
Metastatic disease	2.13	1.39–3.24	**<0.001**
Resected	2.60	1.81–3.73	**<0.001**
ECOG PS			
0	Ref		
1	1.33	0.98–1.79	0.07
2–3	3.28	1.65–6.53	**<0.001**
Log CA19-9	1.06	1.00–1.12	0.05
CDKN2Adel	2.50	1.65–3.78	**<0.001**
TP53mut	1.87	1.31–2.68	**<0.001**
KRASmut	1.94	1.25–3.01	**0.003**

Bold indicates significance.

TABLE 4.

OS Estimates Stratified by Disease Extent and Genetic Alteration

	Solitary liver tumor			Multifocal liver disease			Nodal with or without distant metastases		
	n	**Events**	**Median OS, months (95% CI)**	**n**	**Events**	**Median OS, months (95% CI)**	**n**	**Events**	**Median OS, months (95% CI)**
All	148	85	63.6 (55.6–84.9)	86	56	30.0 (26.1–44.4)	178	146	15.8 (13.3–19.0)
TP53									
wt	132	72	69.4 (58.6–87.1)	71	44	40.6 (26.1–51.5)	137	111	17.3 (14.2–21.4)
mut	16	13	28.3 (12.3–NA)	15	12	26.6 (21.8–NA)	41	35	11.8 (9.9–26.7)
KRAS									
wt	137	74	72.5 (58.6–87.9)	80	50	30.7 (26.1–45)	155	128	17.3 (13.7–20.6)
mut	11	11	33.1 (17.8–NA)	6	6	25.5 (17.9–NA)	23	18	12.1 (5.9–24.1)
CDKN2A									
wt	140	80	63.6 (55.6–84.9)	76	47	32.8 (26.7–47.2)	144	116	17.7 (14.2–23.9)
del	8	5	74.8 (19.1–NA)	10	9	19.3 (11.8–NA)	34	30	11.9 (9.9–15.5)
Any genetic high risk									
wt	121	64	72.5 (61–88.1)	58	32	44.4 (26.8–67.7)	100	81	18.7 (15.7–25.6)
alt	27	21	47.6 (19.1–74.8)	28	24	24.4 (17.9–32)	78	65	12.1 (10.1–17.5)

P values were adjusted for multiple comparisons within outcome using the false discovery rate correction. Abbreviations: *alt*, altered; ECOG, Eastern Cooperative Oncology Group; PS, performance status.