Title

Comprehensive genomic landscape and precision therapeutic approach in biliary tract cancers.

Journal

International journal of cancer, 148(3)

ISSN

0020-7136

Authors

Okamura, Ryosuke
Kurzrock, Razelle
Mallory, Robert J
et al.

Publication Date

2021-02-01

DOI

10.1002/ijc.33230

[Research Article to *International Journal of Cancer*]

Comprehensive Genomic Landscape and Precision Therapeutic Approach in Biliary Tract Cancers

Ryosuke Okamura, MD[1, 2, *], Razelle Kurzrock, MD[1, 2, *], Robert J. Mallory[3], Paul T. Fanta, MD[2], Adam M. Burgoyne, MD, PhD[2], Bryan M. Clary, MD[3], Shumei Kato, MD[1, 2, **], and Jason K. Sicklick, MD[1, 3, **]

Affiliations list:

[1] Center for Personalized Cancer Therapy, UC San Diego Moores Cancer Center, La Jolla, CA
[2] Division of Hematology Oncology, UC San Diego Moores Cancer Center, La Jolla, CA
[3] Division of Surgical Oncology, Department of Surgery, UC San Diego Moores Cancer Center, La Jolla, CA

* Authors contributing equally to this study.
** Co-corresponding authors.

Co-corresponding authors:

Jason K. Sicklick, MD Associate Professor of Surgery Division of Surgical Oncology, UC San Diego Moores Cancer Center 3855 Health Sciences Drive La Jolla, CA 92093-0987 Mail Code 0987 Tel: 858-822-3967 Fax: 858-228-5153 E-mail: jsicklick@health.ucsd.edu Twitter: @JasonSicklick	Shumei Kato, MD Assistant Professor of Medicine Division of Hematology-Oncology, UC San Diego Moores Cancer Center 3855 Health Sciences Drive La Jolla, CA 92093-0658 Mail Code 0658 Tel: 858-822-2372 Fax: 858-822-6186 Email: smkato@health.ucsd.edu

Running title:

Precision therapy approach in biliary tract cancers

Key words: Biliary tract cancers; cholangiocarcinoma; circulating tumor DNA; liquid biopsy; molecular profiling; biomarker; personalized cancer therapy.

Abbreviations

College of American Pathologist (CAP); circulating-tumor DNA (ctDNA); clinical laboratory improvement amendments (CLIA); combined or mixed cholangio-hepatocellular carcinoma (C-HCC); complete response (CR); confidence interval (CI); Eastern Cooperative Oncology Group Performance

Status (ECOG-PS); extrahepatic cholangiocarcinoma (EHCC); gallbladder cancer (GBCA); gemcitabine plus oxaliplatin (GEMOX); hazard ratio (HR); immunohistochemistry (IHC); intrahepatic cholangiocarcinoma (IHCC); micro microsatellite instability (MSI); next-generation sequencing (NGS); overall survival (OS); partial response (PR); programmed death-ligand 1 (PD-L1); progression-free survival (PFS); progressive disease (PD); Response Evaluation Criteria in Solid Tumors (RECIST); stable disease (SD); tumor mutational burden (TMB).

ABSTRACT

Biliary tract cancers have dismal prognoses even when cytotoxic chemotherapy is administered. There is an unmet need to develop precision treatment approaches using comprehensive genomic profiling. A total of 121 patients with biliary tract cancers were analyzed for circulating-tumor DNA (ctDNA) and/or tissue-based tumor DNA (tissue-DNA) using clinical-grade next-generation sequencing: 71 patients (59%) had ctDNA; 90 (74%), tissue-DNA; and 40 (33%), both. Efficacy of targeted therapeutic approaches was assessed based upon ctDNA and tissue-DNA. At least one characterized alteration was detected in 76% of patients (54/71) for ctDNA [median, 2 (range, 0-9)] and 100% (90/90) for tissue-DNA [median, 4 (range, 1-9)]. Most common alterations occurred in *TP53* (38%), *KRAS* (28%), and *PIK3CA* (14%) for ctDNA versus *TP53* (44%), *CDKN2A*/B (33%), and *KRAS* (29%) for tissue-DNA. In 40 patients who had both ctDNA and tissue-DNA sequencing, overall concordance was higher between ctDNA and metastatic site tissue-DNA than between ctDNA and primary tumor DNA (78% versus 65% for *TP53*, 100% versus 74% for *KRAS*, and 100% versus 87% for *PIK3CA*. [But not statistical significance]). Among 80 patients who received systemic treatment, the molecularly matched therapeutic regimens based on genomic profiling showed a significantly longer progression-free survival (hazard ratio [95%confidence interval], 0.60 [0.37-0.99]. P=0.047 [multivariate]) and higher disease control rate (61% versus 35%, P=0.04) than unmatched regimens. Evaluation of ctDNA and tissue-DNA is feasible in biliary tract cancers.

NOVELTY AND IMPACT

There is an unmet need for investigating precision therapy approaches in biliary tract cancers using next-generation sequencing techniques. This study revealed that ctDNA and tissue DNA tests are complementary since they often reveal discordant alteration results likely due to tumor heterogeneity. In addition, among 80 patients who received systemic chemotherapy after these molecular profiling, matched therapies were associated with better treatment response and progression-free survival than unmatched therapies.

INTRODUCTION

Despite their low incidence, the mortality from biliary tract cancers is high. Biliary tract cancers are generally categorized as intrahepatic cholangiocarcinoma (IHCC), extrahepatic cholangiocarcinoma (EHCC), and gallbladder cancer (GBCA). Also, combined or mixed cholangio-hepatocellular carcinoma (C-HCC) which comprises histopathological features of cholangiocarcinoma and hepatocellular carcinoma is occasionally seen. Traditionally, systemic therapy approaches have been the same for all of these tumors, regardless of the tumor type as they were assumed to have similar biologies.[1, 2]

Biliary tract cancers mostly present with locally advanced disease or metastatic lesions precluding surgical resection. Moreover, they all have poor prognoses even when systemic chemotherapy is administered. In several clinical trials, the median progression-free survival (PFS) and median overall survival (OS) of multi-agent regimens in the advanced settings remain dismal despite being limited to patients with good performance status and without hyperbilirubinemia (median PFS: 5.8-8.0 months for gemcitabine plus cisplatin, 4.2-5.7 months for gemcitabine plus oxaliplatin [GEMOX], 5.8 months for GEMOX with erlotinib, and 11.8 month for gemcitabine cisplatin plus nab-paclitaxel; median OS: 11.2-11.7 months for gemcitabine plus cisplatin, 9.5-15.4 months for GEMOX, 9.5 months for GEMOX with erlotinib, and 19.2 month for gemcitabine cisplatin plus nab-paclitaxel).[3-7]Thus, the goals of chemotherapy in advanced biliary tract cancer patients are mostly palliative in nature.[1] As a result, personalized, molecular targeted approaches have emerged as a potential approach for treating malignancies with high mortality.[8, 9] In a meta-analysis of 32,149 patients with diverse cancers who underwent early phase clinical trials, targeted therapy approaches without specific biomarkers had significantly worse clinical outcomes (i.e., objective response rate, PFS, and OS) when compared to patients who received targeted therapies based upon biomarkers.[10] However, previous clinical trials that utilized targeted therapies in biliary tract cancers have not shown clinically significant improvements in overall response rates so far (e.g., 31-33% for GEMOX or gemcitabine/irinotecan with panitumumab targeting EGFR among *KRAS* wild-type biliary tract cancers; and 15% for an FGFR inhibitor among FGFR-altered cholangiocarcinoma).[11-13] Some of the limitations to the previous targeted therapy approaches may be due to spatial or temporal tumor heterogeneity that may lead to the lack of response with single targeted approaches.[14] Also, tissue

biopsies of biliary tract cancers can be challenging to safely obtain with adequate tissue quality for comprehensive molecular testing. Thus, the blood-derived circulating tumor DNA (ctDNA) technique has some advantages over tissue-DNA sequencing since it is less-invasive and potentially enables real-time monitoring of genomic evolution. Herein, we assessed the genomic landscape of ctDNA along with tissue-DNA using clinical-grade next-generation sequencing (NGS), as well as also investigated the efficacy using genomic profiling data from both approaches to administer molecularly matched targeted therapies to patients with biliary tract cancers.

METHODS

Patients

We collected the genomic and clinical data of patients pathologically diagnosed as IHCC, EHCC, GBCA, or C-HCC, who were presented to the UC San Diego Moores Cancer Center between March 2012 and March 2019. The study was conducted consistent with the IRB-approved protocol *Profile Related Evidence Determining Individualized Cancer Therapy* (UCSD-PREDICT study: NCT02478931) parameters and any investigational therapies for which the patients gave consent. All investigations were performed in accordance with the guidelines of the UC San Diego Internal Review Board and the Declaration of Helsinki.

Clinical Grade Next-Generation Sequencing

Blood-derived circulating tumor DNA: ctDNA assay for all blood samples was performed by a clinical laboratory improvement amendments (CLIA) licensed and College of American Pathologist (CAP) accredited clinical laboratory, *Guardant Health, Inc*. (Redwood City, CA) (http://www.guardanthealth.com; panels of 68-73 genes; **Supplementary Table 1**) and sequenced cancer-associated genes to identify somatic alterations with high analytic sensitivity and high specificity, as previously described.[15] In this study, only characterized genomic alterations were used for analysis (synonymous alterations or variants of unknown significance were excluded).

Tumor tissue-DNA: Tissue-DNA assay for all tumor samples was performed by a CLIA-licensed CAP-accredited laboratory, *Foundation Medicine, Inc.* (Cambridge, MA) (https://www.foundationmedicine.com; panels of 236-324 genes. **Supplementary Table 2**). Also in tissue-DNA, only characterized alterations were analyzed. The sequencing was designed to include all genes somatically altered in human solid malignancies that were validated as targets for therapy, either approved or in clinical trials, and/or that were unambiguous oncogenic drivers based on available recent knowledge.[16] Micro microsatellite instability (MSI) and tumor mutational burden (TMB) were also evaluated in tumor tissues as the biomarkers which have entered clinical practice for immunotherapies.[17-20]

Definition and Statistical Analysis

In this series, hilar cholangiocarcinoma was classified as EHCC. Genomic concordance between ctDNA and tissue-DNA tests was assessed in the three most commonly altered genes in ctDNA at the gene level and described with overall concordance rate. The Kappa values were interpreted by commonly used agreement categories: from 1 (perfect agreement) to 0 (no agreement, the same as would be expected by chance). When patients were stratified according to tissue biopsy site and time interval between blood draw and tissue biopsy, the difference in concordance rate was compared by Fisher's exact test. All statistical analysis was done using SPSS Statistics version 24 software (IBM Corporation, Armonk, NY).

***Matched Targeted Therapy Based on Molecular Profiling*:**

We assessed the efficacy of precision oncology approaches based on ctDNA and/or tissue-DNA molecular profiling. For this analysis, treatment regimens that were initiated after the dates of blood draw for ctDNA analysis and tissue biopsy were only studied (the first regimen initiated after molecular profiling for each patient). When at least one drug was administered and it targeted at least one genomic alteration in either ctDNA or tissue-DNA or both, treatment was considered "matched therapy" as previously described.[8] We also considered checkpoint inhibitors matched to mismatch-repair gene alteration (e.g., alteration in *MLH1*, *MSH2*), programmed death-ligand 1 (PD-L1) immunohistochemistry (IHC), or high/intermediate tumor mutational burden (TMB: high [≥20 mutations/mb]; and intermediate [6-19 mutations/mb]) and certain alterations (including but not limited to *PDL1* amplification) as "matched therapy". In addition, even when treated with a conventional platinum-based regimen (e.g., cisplatin plus gemcitabine), the patient was considered "matched" if the genomic profiling includes at least one *BRCA*-associated gene alteration (e.g., *BRCA2*, *BAP1*, *ATM*). Tumor response was assessed by means of computed tomography or magnetic resonance imaging at every 8-12 weeks, using modified Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 evaluation: complete response (CR), partial response (PR); stable disease (SD); and progressive disease (PD).[21] PFS was defined as the time from the initiation of the regimen to progressive disease (PD) or all cause death (counted as censored if a patient still survives without

progression on the date of data cutoff [April 2019] or if the regimen was switched to another regimen without PD on imaging [e.g., due to toxicity or patient's preference]). The sample size was mainly determined by the number of patients for whom data were available among the patients who were consented to the UCSD-PREDICT study (*ClinicalTrials.gov*, NCT02478931).

RESULTS

Patient Demographics and Genomic Landscape in Next-Generation Sequencing

A total of 121 patients with biliary tract cancers were evaluated: 40 patients (33%) had both ctDNA and tissue-DNA analyses, 31 (26%) had only ctDNA analysis, and 50 (41%) had only tissue-DNA analysis (**Supplementary Figure 1**). Fifty one percent of the 112 patients were male, and the median age at disease diagnosis was 63 years (**Table 1**). Tumor type was IHCC in 49% (N=59), EHCC in 22% (N=26), GBCA in 24% (N=29) and C-HCC in 5.8% (N=7), respectively. Median follow-up time from disease diagnosis was 27.8 months (95% confidence interval [CI], 23.4-32.2).

ctDNA NGS in Biliary Tract Cancer Patients [N=71]: The ctDNA analyses were performed in advanced disease setting (metastatic, locally advanced, or recurrent disease), except for 3 GBCA cases and 1 IHCC case whose ctDNA were analyzed postoperatively (**Table 1**). Of the 71 patients with ctDNA analysis, 76% (N=54) had at least one characterized alteration in ctDNA. The median number of characterized alterations per patient was 2 (range, 0-9), and a total of 147 characterized alterations were observed, including 112 mutations (76%), 32 gene amplifications (22%), 2 gene fusions (1.4%), and 1 indel (0.7%). These characterized alterations involved 36 unique genes and included 97 distinct alterations (**Figure 1A**). The most common genes altered in ctDNA were *TP53* (38%, N=27), followed by *KRAS* (28%, N=20), and *PIK3CA* (14%, N=10). Overall, 85% of these characterized alterations (125 of the 147 alterations) were theoretically targetable with FDA-approved agents (on- or off-label use) (**Supplementary Table 3**). In other words, 75% of the patients (N=53) had at least one characterized alteration targetable with FDA-approved agents (on-, or off-label). Only two patients harbored molecularly identical portfolios (*PIK3CA* amplification) in ctDNA.

Tissue-DNA NGS in Biliary Tract Cancer Patients [N=90]: Seventy eight percent of the tissue-DNA analyses (N=70 of 90) used primary tumor samples while the remaining 22% (N=20) utilized biopsies from metastatic sites (**Table 1**). Interestingly, all 90 patients had at least one characterized alteration in the tissue-DNA (median number of characterized alterations per patient [range], 4 [1-9]). A total of 362 characterized alterations were observed in tissue-DNA, including 190 mutations (53%), 105 gene amplifications (29%), 52 allelic loss/deletions (14%), and 15 gene

fusions/truncations/duplications (4%), which involved 106 different genes and 236 distinct alterations (genes altered in ≥3 samples were shown in **Figure 1B**). The most common genes altered in tissue-DNA were *TP53* (44%, N=40), followed by *CDKN2A/B* (33%, N=30), and *KRAS* (29%, N=26). Of the 362 characterized tissue-DNA alterations, 70% of alterations (252/362) were theoretically targetable with FDA-approved agent while 96% of the patients (N=86) had at least one tissue-DNA characterized alteration which was pharmacologically targetable with FDA-approved agents. No two patients had molecularly identical tissue-DNA portfolios.

Genomic Concordance Between ctDNA and Tissue-DNA Sequencing [N=40]

Overall, 40 patients had both ctDNA and tissue-DNA NGS. When comparing *TP53*, *KRAS*, and *PIK3CA* genes, the overall concordance rate between ctDNA and tissue-DNA was 68%, 80%, and 90%, respectively (Kappa values ranged 0.27-0.55) (**Table 2**). When comparing according to tissue biopsy site, ctDNA alteration was numerically more concordant with metastatic site DNA than primary tumor DNA in these three genes (overall concordance [Kappa], 78% versus 65% [0.57 versus 0.17] for *TP53*; 100% versus 74% [1.00 versus 0.41] for *KRAS*; and 100% versus 87% [1.00 versus 0.45] for *PIK3CA*). But there were no statistical differences observed (the *P*-values ranged 0.16-0.69) (**Table 2** and **Figure 1C**). In terms of temporal effects in the genomic concordance, no clear differences were observed for samples from ≤6 months versus >6 months apart (i.e., between blood draw for ctDNA and tissue biopsy) in these genes although the Kappa values were likely higher in the ≤6 months group (74% versus 54% [0.40 versus 0.03] for *TP53*; 82% versus 77% [0.60 versus 0.32] for *KRAS*; and 100% versus 87% [0.61 versus 0.00] for *PIK3CA.* [*P*-values ranged 0.28-0.99]).

Treatment Outcome of Personalized Matched Therapy Approaches in Advanced Biliary Tract Cancers [N=80]

Among the 121 patients with biliary tract cancers, 80 patients had systemic therapies initiated after the molecular profiling in locally advanced or metastatic disease setting (adjuvant intent chemotherapy was excluded) (**Supplementary Figure 1**). Of these 80 treated patients, 43% (N=34)

were administered at least one drug matched to their profiling results (detailed genomic information was shown in **Supplementary Table 4**). The matched targeted therapies include molecular targeting therapies for genomic alterations in ctDNA and/or tissue-DNA (N=29), immunotherapies for PD-L1 IHC status (N=3) or mismatch repair deficiency (N=1), and a combination of molecular targeting therapy with immunotherapy for TMB status (N=1). These matched patients received a median of 2 drugs (range, 1 - 3), and the regimens were administered as their first-line treatments in 67% of the patients (N=23). Eleven patients (32%) were treated with gemcitabine with platinum agents and their tissue-DNA included at least one alteration in *BRCA*-associated genes (i.e., alterations in *ATM, BAP1, BRCA2, CHEK2, FANCL, or RAD50* gene). Patients with *FGFR* fusion (ID#33) and *IDH1* alteration (ID#38) received anti- FGFR and IDH therapies, respectively. On the other hand, the remaining 46 of the 80 patients (57%) were treated with unmatched regimens, which mostly used gemcitabine-based regimens (gemcitabine with platinums [N=22]; gemcitabine with capecitabine [N=6]; gemcitabine monotherapy [N=9]) and other regimens [N=9]. Additionally, 87% (N=40) of the unmatched patients were treated with these regimens as their first-line treatments. For instance, 31% of the treated patients (N=25/80) harbored *KRAS* alterations in either of the tissue DNA or ctDNA testing or both (N=14, only in tissue; N=6, only in ctDNA; and N=5, in both), and 5 patients of them (ID#23, #37, #38, #52, and #59) received matched treatment regimens including trametinib, a MEK inhibitor (**Supplementary Table 4**). Also, 5 patients (ID#12, #33, #34, #66, and #86) received other matched regimens based on their tissue DNA and/or ctDNA testing (e.g., a FGFR inhibitor for *FGFR2* fusion [ID#33]). The remaining 15 patients mostly received gemcitabine-based unmatched regimens. The matched and unmatched patients were similar in regard to key basic characteristics such as pretreatment physical conditions (age, ECOG-PS, or total bilirubin level), tumor site, or extent of disease (**Supplementary Table 5**). RECIST evaluation was available in 76 of the 80 treated patients (95%), and the PR rate was significantly higher in the matched regimen group versus the unmatched regimen group (24% [N=8 of 33] versus 4.7% [N=2 of 43], P=0.02) while the PD rate was significantly lower in the matched regimen group (39% [N=13 of 33] versus 65% [N=28 of 43], P=0.04) (**Figure 2A**). Consistent with the response analysis, Kaplan-Meier curves showed that the matched regimen group had a significantly longer PFS time than the unmatched regimen group (median PFS time, 4.3 versus 3.0 months, P=0.04) (**Figure 2B**). Importantly, the matched regimens

remained significantly associated with better PFS even when age, sex, performance status, tumor type, extent of disease, presence of prior radical surgery, number of prior regimens, and the number of drugs administered were considered as confounding factors in the multivariate analysis (HR [95%CI], 0.60 [0.37-0.99]; *P*=0.047) (**Table 3**).

DISCUSSION

Most biliary tract cancers are unresectable at presentation and often have metastases to intrahepatic sites, lymph nodes, or the peritoneum[22]. Even in surgically resectable cases, involvement of surgical margin often occur and is associated with high rates of disease recurrence.[23] At present, gemcitabine-based combination regimens are globally accepted as the systemic chemotherapy regimen for advanced biliary tract cancer patients. However, the prognosis remains poor.[3-7] Thus, there is an unmet need for novel therapeutic approaches for these cancers. Precision oncology approaches have recently shown promising responses in diverse cancer types.[7-10, 24] To our knowledge, the detailed comprehensive genomic landscape of biliary tract cancers by using clinical-grade ctDNA as well as its concordance analysis with tissue-DNA are limited.[25] We now demonstrate that each biliary tract cancer patient has distinct pattern of ctDNA and tissue-DNA genomic alterations which are frequently druggable and that targeted matched therapies based on the molecular profiling are associated with higher response rates and longer progression-free survival.

Interestingly, 76% of the patients had at least one characterized ctDNA alteration while 100% had at least one characterized alteration found in tissue-DNA. In addition, the median number of alterations per patient (range) was 2 (0-9) in ctDNA and 4 (1-9) in tissue-DNA. The most common alterations occur in *TP53* (38%), *KRAS* (28%), and *PIK3CA* (14%) for ctDNA while in *TP53* (44%), *CDKN2A/B* (33%), and *KRAS* (29%) for tissue-DNA, respectively. The frequencies in both ctDNA and tissue-DNA NGS were consistent with previous reports.[26, 27] In the majority of the commonly altered genes in this series, such as *TP53, KRAS, ARID1A,* and *IDH*, similar percentages were seen between ctDNA and tissue-DNA. However, *CDKN2A*/B alterations were detected in only 3% of patients by ctDNA, as compared with 33% by tissue-DNA NGS. This discrepancy might be attributable to the possibility that earlier versions of the ctDNA panel did not capture allelic loss in this gene. Moreover, among 40 patients who had both ctDNA and tissue-DNA NGS analyses, no two patients had identical results from these molecular profiling. Moreover, no IHCC patient had characterized *SMAD4* or *ERBB2* alterations in either ctDNA or tissue-DNA NGS in this series. In terms of druggability, 75% of patients who had ctDNA analysis and 96% of patients whose tissue-DNA were analyzed had at least one alteration that was theoretically targetable with FDA-approved drugs (on- or off-label), respectively. A previous study reported that 55% of patients with biliary tract cancers had therapeutically relevant

characterized ctDNA alterations although genes considered as targetable were somewhat different with our study.[25] Altogether, nearly all biliary tract cancer patients had unique pattern of genomic alterations in ctDNA and tissue-DNA NGS, indicating that these two different sequencing techniques can be complementary. These findings also suggest the potential for precision clinical trials in biliary tract cancers, as well as that customized molecular targeting based on each individual genomic portfolio may be necessary to improve outcomes.[8, 28-30]

Overall concordance between ctDNA and tissue-DNA was 68-90% for *TP53*, *KRAS*, and *PIK3CA* genes. Discrepancies between the two tests may be due to the inter- and intra-tumor genetic heterogeneity.[14] Also, cases with positive in ctDNA and negative in tissue-DNA for certain genes, such as *TP53* or *KRAS,* may be explained by age-related or therapy-related clonal hematopoiesis.[31, 32] When stratified according to tissue biopsy sites, ctDNA was numerically more concordant with metastatic site tissue-DNA than primary tumor DNA, although there were no statistical differences observed (78% versus 65% in *TP53*, 100% versus 74% in *KRAS*, and 100% versus 87% in *PIK3CA*). This finding likely suggests that additional mutations develop in metastatic tumors and that ctDNA may be able to detect DNA shed into bloodstream from the tumors throughout the patient's whole body.[14, 33] Also, biomarker profiling of either metastatic site tissue-DNA or ctDNA may be more effective in selection of therapy than interrogating primary tumor sites. Moreover, there was a temporal effect on concordance with shorter time apart between blood draw and tissue biopsy (≤6 months): higher concordances in *TP53* and *KRAS* and greater Kappa values in *TP53, KRAS,* and *PIK3CA* than >6 months. However, further studies with larger sample sizes are required for validation.

Given the emerging role of precision matched therapies, we assessed the efficacy of molecularly matching drugs in patients with biliary tract cancers.[8, 9] Importantly, the matched targeted regimens had a higher response rate and longer PFS (PR rate, 24% versus 4.7%, P=0.02; median PFS time, 4.3 versus 3.0 months, P=0.04) than regimens unmatched to NGS results. It should be noted that about half of unmatched regimens were gemcitabine with platinum agents, which are commonly used as the first line for biliary tract cancers, but the median PFS time was 3 months in current study which is shorter compared to previous reports.[3, 6] This discrepancy may result from the issue that this study was not performed in prospective exploratory setting such as randomized control trials, but in more pragmatic setting including patients with history of previous aggressive therapy

(23%), poor performance status (ECOG-PS 2-3, 26%), or abnormal total bilirubin levels (> the institutional upper limit of normal [1.2 mg/dL], 23%). On the other hand, 11 patients were treated with gemcitabine plus platinum regimens in the setting of ≥1 characterized alterations in *BRCA*-associated genes that were considered as the molecularly matched group. Previous studies have suggested that similar to ovarian and breast cancers, cholangiocarcinoma harboring *BRCA*-associated gene alterations are more sensitive to platinum-based therapy.[34-36] In fact, when we investigated the patients treated with gemcitabine and platinums in this series, the matched group tended to have a longer median PFS, although there was no statistical significance (5.8 versus 3.1 months; **Supplementary Figure 2**). We also assessed the treatment outcomes according to the matching score (the number of targeted gene alterations divided by the total number of alterations observed in NGS; unmatched patients had a score of 0%) as reported previously.[8, 28, 37] However, in this series, the number of patients with higher matching score (>50%) was small (13%). Thus, the matching score failed to discriminate the treatment response and PFS with statistical differences (**Supplementary Figure 3**). In terms of the efficacy of the matched therapy approaches in patients' overall survival, the matched patients had a longer median OS time (11.9 versus 7.9 months) and 12-month-OS rate (47% versus 39%) over the unmatched patients, although these were not statistical significant (**Supplementary Figure 4**). Among treated patients, the unmatched regimens were more often administered as a first line setting than the patients with matched regimens and had a shorter interval between advanced disease diagnoses to initiation of the treatment (**Supplementary Table 5**). Therefore, the introduction of matched regimens in earlier phase of disease may require in the future. Historically, several molecularly targeted trials in biliary tract cancers have failed to show favorable outcomes.[38] Negative studies to date may be due to the lack of biomarker selection or the existence of oncogenic co-alterations. Thus, individually customized targeted therapy regimens may be required to improve clinical outcomes of patients with advanced biliary tract cancers.

The current study has several limitations. First, not all patients had both ctDNA and tissue-DNA NGS results and there can be discordance between ctDNA and tissue DNA results**.** Further studies utilizing both ctDNA and tissue-DNA NGS are warranted. Also, our classification of matched or unmatched treatment may not be accurate due to the lack of either ctDNA or tissue-DNA NGS, whereby some unmatched patients may actually have received an unrecognized molecularly

matched therapy. In addition, the small number of patients precludes further investigation of the concordance between ctDNA and tissue-DNA. Additional studies with larger sample sizes are needed. Finally, it is possible that other unmeasurable, or unknown, but important confounding factors were not considered in comparison of treatment strategies. For these issues, randomized controlled trials may be more ideal.

In conclusion, our study demonstrates that biliary tract cancer patients mostly had at least one characterized alteration in ctDNA (76% of blood samples) and tissue-DNA (100% of tissue samples). Concordance was higher between ctDNA and metastatic site tissue-DNA than between ctDNA and primary tumor DNA. Among patients who received chemotherapy following the genomic profiling, molecularly matched regimens based on biomarkers showed a statistically longer PFS and higher disease control rate over unmatched regimens. Further investigations of biomarker-driven therapies using clinical-grade NGS in blood and tissue are warranted for developing new and better treatment strategies for patient with biliary tract cancer.

DATA ACCESSIBILITY

The data that support the findings of our study are available upon request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

This study was approved by UC San Diego Institutional Review Board. Written signed informed consent was obtained from all participants.

ACKNOWLEDGMENT

We thank Lee Suzanna, Rebecca E. Jimenez, David Philips-Moses, and Sophia C. Gumas for their critical roles in the PREDICT registry and data management. This work was funded in part by Foundation Medicine Inc., as well as the Joan and Irwin Jacobs Fund philanthropic fund, the Jon Schneider Memorial Cancer Research Fund and by NIH P30 CA023100. The authors also acknowledge the support of NIH grant (R01 CA226803, JKS) and FDA (R01 FD006334, JKS).

DISCLOSURES

Dr. Kurzrock receives research funding from Genentech, Merck Serono, Pfizer, Boehringer Ingelheim, TopAlliance, Takeda, Incyte, Debiopharm, Medimmune, Sequenom, Foundation Medicine, Konica Minolta, Grifols, Omniseq, and Guardant, as well as consultant and/or speaker fees and/or advisory board for X-Biotech, Loxo, Neomed, Pfizer, Actuate Therapeutics, and Roche. She has an equity interest in IDbyDNA and CureMatch Inc and serves on the Board of CureMatch and CureMetrix.

Shumei Kato received research funds from ACT Genomics, Sysmex, Konica Minolta, and OmniSeq, as well as consultant fees for Foundation Medicine. He received speaker fees from Roche.

Jason Sicklick received research funds from Foundation Medicine Inc., Novartis Pharmaceuticals, and Amgen, as well as consultant fees from Loxo, Grand Rounds and Deciphera. He received speaker fees from Roche.

Adam Burgoyne has served as a consultant for Genentech, Deciphera, Exelixis, and Eisai.

All other authors have no relationships to disclose.

AUTHOR CONTRIBUTIONS

Study conception and design (RO, RK, SK, and JKS); data acquisition (RO, RJM, PTF, AMB,); statistical analysis (RO and SK); data interpretation (RO, RK, BMC, SK, and JKS); drafting the manuscript or revising it critically (all authors); and final approval of the manuscript (all authors).

REFERENCES

1. Sicklick JK, Fanta PT, Shimabukuro K, Kurzrock R. Genomics of gallbladder cancer: the case for biomarker-driven clinical trial design. *Cancer metastasis reviews* 2016;**35**: 263-75.

2. Sicklick JK, Fanta PT. Molecular pathogenesis of biliary tract cancer. In: Blumgart's Surgery of the Liver, Biliary Tract and Pancreas, 2-Volume Set: Elsevier, 2017:150-9. e2.

3. Valle J, Wasan H, Palmer DH, Cunningham D, Anthoney A, Maraveyas A, Madhusudan S, Iveson T, Hughes S, Pereira SP, Roughton M, Bridgewater J. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *The New England journal of medicine* 2010;**362**: 1273-81.

4. Okusaka T, Nakachi K, Fukutomi A, Mizuno N, Ohkawa S, Funakoshi A, Nagino M, Kondo S, Nagaoka S, Funai J, Koshiji M, Nambu Y, et al. Gemcitabine alone or in combination with cisplatin in patients with biliary tract cancer: a comparative multicentre study in Japan. *British journal of cancer* 2010;**103**: 469-74.

5. Andre T, Tournigand C, Rosmorduc O, Provent S, Maindrault-Goebel F, Avenin D, Selle F, Paye F, Hannoun L, Houry S, Gayet B, Lotz JP, et al. Gemcitabine combined with oxaliplatin (GEMOX) in advanced biliary tract adenocarcinoma: a GERCOR study. *Annals of oncology : official journal of the European Society for Medical Oncology* 2004;**15**: 1339-43.

6. Lee J, Park SH, Chang HM, Kim JS, Choi HJ, Lee MA, Jang JS, Jeung HC, Kang JH, Lee HW, Shin DB, Kang HJ, et al. Gemcitabine and oxaliplatin with or without erlotinib in advanced biliary-tract cancer: a multicentre, open-label, randomised, phase 3 study. *The Lancet Oncology* 2012;**13**: 181-8.

7. Shroff RT, Javle MM, Xiao L, Kaseb AO, Varadhachary GR, Wolff RA, Raghav KPS, Iwasaki M, Masci P, Ramanathan RK, Ahn DH, Bekaii-Saab TS, et al. Gemcitabine, Cisplatin, and nab-Paclitaxel for the Treatment of Advanced Biliary Tract Cancers: A Phase 2 Clinical Trial. *JAMA oncology* 2019;**5**: 824-30.

8. Sicklick JK, Kato S, Okamura R, Schwaederle M, Hahn ME, Williams CB, De P, Krie A, Piccioni DE, Miller VA, Ross JS, Benson A, et al. Molecular profiling of cancer patients enables personalized combination therapy: the I-PREDICT study. *Nature medicine* 2019.

9. Rodon J, Soria JC, Berger R, Miller WH, Rubin E, Kugel A, Tsimberidou A, Saintigny P, Ackerstein A, Brana I, Loriot Y, Afshar M, et al. Genomic and transcriptomic profiling expands precision cancer medicine: the WINTHER trial. *Nature medicine* 2019;**25**: 751-8.

10. Schwaederle M, Zhao M, Lee JJ, Eggermont AM, Schilsky RL, Mendelsohn J, Lazar V, Kurzrock R. Impact of Precision Medicine in Diverse Cancers: A Meta-Analysis of Phase II Clinical Trials. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2015;**33**: 3817-25.

11. Sohal DP, Mykulowycz K, Uehara T, Teitelbaum UR, Damjanov N, Giantonio BJ, Carberry M, Wissel P, Jacobs-Small M, O'Dwyer PJ, Sepulveda A, Sun W. A phase II trial of gemcitabine, irinotecan and panitumumab in advanced cholangiocarcinoma. *Annals of oncology : official journal of the European Society for Medical Oncology* 2013;**24**: 3061-5.

12. Javle M, Lowery M, Shroff RT, Weiss KH, Springfeld C, Borad MJ, Ramanathan RK, Goyal L, Sadeghi S, Macarulla T, El-Khoueiry A, Kelley RK, et al. Phase II Study of BGJ398 in Patients With FGFR-Altered Advanced Cholangiocarcinoma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2018;**36**: 276-82.

13. Jensen LH, Lindebjerg J, Ploen J, Hansen TF, Jakobsen A. Phase II marker-driven trial of panitumumab and chemotherapy in KRAS wild-type biliary tract cancer. *Annals of oncology : official journal of the European Society for Medical Oncology* 2012;**23**: 2341-6.

14. Dagogo-Jack I, Shaw AT. Tumour heterogeneity and resistance to cancer therapies. *Nature reviews Clinical oncology* 2018;**15**: 81-94.

15. Lanman RB, Mortimer SA, Zill OA, Sebisanovic D, Lopez R, Blau S, Collisson EA, Divers SG, Hoon DS, Kopetz ES, Lee J, Nikolinakos PG, et al. Analytical and Clinical Validation of a Digital Sequencing Panel for Quantitative, Highly Accurate Evaluation of Cell-Free Circulating Tumor DNA. *PloS one* 2015;**10**: e0140712.

16. Frampton GM, Fichtenholtz A, Otto GA, Wang K, Downing SR, He J, Schnall-Levin M, White J, Sanford EM, An P, Sun J, Juhn F, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nature biotechnology* 2013;**31**: 1023-31.

17. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, Lu S, Kemberling H, Wilt C, Luber BS, Wong F, Azad NS, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science (New York, NY)* 2017;**357**: 409-13.

18. Samstein RM, Lee CH, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, Barron DA, Zehir A, Jordan EJ, Omuro A, Kaley TJ, Kendall SM, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nature genetics* 2019;**51**: 202-6.

19. Goodman AM, Kato S, Bazhenova L, Patel SP, Frampton GM, Miller V, Stephens PJ, Daniels GA, Kurzrock R. Tumor Mutational Burden as an Independent Predictor of Response to Immunotherapy in Diverse Cancers. *Molecular cancer therapeutics* 2017;**16**: 2598-608.

20. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D. PD-1 blockade in tumors with mismatch-repair deficiency. *New England Journal of Medicine* 2015;**372**: 2509-20.

21. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *European journal of cancer (Oxford, England : 1990)* 2009;**45**: 228-47.

22. Valle JW. Advances in the treatment of metastatic or unresectable biliary tract cancer. *Annals of oncology : official journal of the European Society for Medical Oncology* 2010;**21 Suppl 7**: vii345-8.

23. Valle JW, Lamarca A, Goyal L, Barriuso J, Zhu AX. New Horizons for Precision Medicine in Biliary Tract Cancers. *Cancer discovery* 2017;**7**: 943-62.

24. Tsimberidou AM, Iskander NG, Hong DS, Wheler JJ, Falchook GS, Fu S, Piha-Paul S, Naing A, Janku F, Luthra R, Ye Y, Wen S, et al. Personalized medicine in a phase I clinical trials program: the MD Anderson Cancer Center initiative. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2012;**18**: 6373-83.

25. Mody K, Kasi PM, Yang J, Surapaneni PK, Bekaii-Saab T, Ahn DH, Mahipal A, Sonbol MB, Starr JS, Roberts A, Nagy R, Lanman R, et al. Circulating Tumor DNA Profiling of Advanced Biliary Tract Cancers. *JCO precision oncology* 2019: 1-9.

26. Javle M, Bekaii-Saab T, Jain A, Wang Y, Kelley RK, Wang K, Kang HC, Catenacci D, Ali S, Krishnan S, Ahn D, Bocobo AG, et al. Biliary cancer: Utility of next-generation sequencing for clinical management. *Cancer* 2016;**122**: 3838-47.

27. Goyal L, Kate Kelley R, Kiedrowski L, Catenacci D, Mody K, Shroff R, Parikh A, Reyes S, Maurer JR, Corcoran RB, Fanta P, Cusnir M, et al. Abstract A183: Blood-based genomic profiling of cell-free tumor DNA (ctDNA) in patients with biliary tract cancer. *Molecular cancer therapeutics* 2018;**17**: A183-A.

28. Wheler JJ, Janku F, Naing A, Li Y, Stephen B, Zinner R, Subbiah V, Fu S, Karp D, Falchook GS, Tsimberidou AM, Piha-Paul S, et al. Cancer Therapy Directed by Comprehensive Genomic Profiling: A Single Center Study. *Cancer research* 2016;**76**: 3690-701.

29. Tsimberidou AM, Hong DS, Ye Y, Cartwright C, Wheler JJ, Falchook GS, Naing A, Fu S, Piha-Paul S, Janku F, Meric-Bernstam F, Hwu P, et al. Initiative for Molecular Profiling and Advanced Cancer Therapy (IMPACT): An MD Anderson Precision Medicine Study. *JCO precision oncology* 2017;**2017**.

30. Bogenberger JM, DeLeon TT, Arora M, Ahn DH, Borad MJ. Emerging role of precision medicine in biliary tract cancers. *NPJ precision oncology* 2018;**2**: 21.

31. Genovese G, Kähler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF, Chambert K, Mick E, Neale BM, Fromer M, Purcell SM, Svantesson O, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *The New England journal of medicine* 2014;**371**: 2477-87.

32. Coombs CC, Zehir A, Devlin SM, Kishtagari A, Syed A, Jonsson P, Hyman DM, Solit DB, Robson ME, Baselga J, Arcila ME, Ladanyi M, et al. Therapy-Related Clonal Hematopoiesis in Patients with Non-hematologic Cancers Is Common and Associated with Adverse Clinical Outcomes. *Cell Stem Cell* 2017;**21**: 374-82.e4.

33. Pectasides E, Stachler MD, Derks S, Liu Y, Maron S, Islam M, Alpert L, Kwak H, Kindler H, Polite B, Sharma MR, Allen K, et al. Genomic Heterogeneity as a Barrier to Precision Medicine in Gastroesophageal Adenocarcinoma. *Cancer discovery* 2017.

34. Golan T, Raitses-Gurevich M, Kelley RK, Bocobo AG, Borgida A, Shroff RT, Holter S, Gallinger S, Ahn DH, Aderka D, Apurva J, Bekaii-Saab T, et al. Overall Survival and Clinical Characteristics of BRCA-Associated Cholangiocarcinoma: A Multicenter Retrospective Study. *The oncologist* 2017;**22**: 804-10.

35. McCabe N, Turner NC, Lord CJ, Kluzek K, Bialkowska A, Swift S, Giavara S, O'Connor MJ, Tutt AN, Zdzienicka MZ, Smith GC, Ashworth A. Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition. *Cancer research* 2006;**66**: 8109-15.

36. Xie Y, Jiang Y, Yang XB, Wang AQ, Zheng YC, Wan XS, Sang XT, Wang K, Zhang DD, Xu JJ, Li FG, Zhao HT. Response of BRCA1-mutated gallbladder cancer to olaparib: A case report. *World journal of gastroenterology* 2016;**22**: 10254-9.

37. Schwaederle M, Parker BA, Schwab RB, Daniels GA, Piccioni DE, Kesari S, Helsten TL, Bazhenova LA, Romero J, Fanta PT, Lippman SM, Kurzrock R. Precision Oncology: The UC San Diego Moores Cancer Center PREDICT Experience. *Molecular cancer therapeutics* 2016;**15**: 743-52.

38. Hezel AF, Deshpande V, Zhu AX. Genetics of biliary tract cancers and emerging targeted therapies. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2010;**28**: 3531-40.

TABLE AND FIGURE LEGENDS

Table 1. Clinical characteristics of biliary tract cancer patients (N=121).

Table 2. Concordance of common genes between ctDNA and tissue-DNA among patients with biliary tract cancers whose ctDNA and tissue-DNA were both analyzed [N=40]. Most common 3 genes in ctDNA sequencing were assessed at the gene level.

Table 3. Exploration of prognostic factors for progression-free survival among the treated patients with biliary tract cancers [N=80].

Figure 1. Genomic characterized alterations among patients with biliary tract cancers.

Panel 1A. Frequency of genomic alterations in ctDNA sequencing [N=71].

Panel 1B. Frequency of genomic alterations in tissue-DNA sequencing (genes altered in ≥3 patients were only shown) [N=90].

Panel 1C. Association between ctDNA and tissue-DNA in commonly altered genes among patients whose ctDNA and tissue-DNA were both analyzed [N=40].

Figure 2. Comparisons of treatment outcome between matched regimens and unmatched regimens among patients who received systemic therapies following molecular profiling.

Panel 2A. Best response during the treatment (76 of 80 patients [95%] were available for RECIST evaluation). Among 76 evaluable patients, PR was observed in 10 patients (13%), SD in 25 (33%), and PD in 41 (54%) as the best response during the therapies following their molecular profiling (no one had complete response from the treatments).

Panel 2B. Progression-free survival [N=80].

TABLES

Table 1. Clinical characteristics of biliary tract cancer patients (N=121).

Basic characteristics [All patients, N=121]	***N (%)***
Median age at diagnosis (range) (years)	62.6 (31.2-88.5)
Sex	
Male	62 (51.2%)
Female	59 (48.8%)
Ethnicity	
Caucasian	67 (55.4%)
Hispanic	32 (26.4%)
Asian	11 (9.1%)
Other/unknown	11 (9.1%)
Tumor type	
Intrahepatic cholangiocarcinoma (IHCC)	59 (48.8%)
Extrahepatic cholangiocarcinoma (EHCC)	26 (21.5%)
Gallbladder carcinoma (GBCA)	29 (24.0%)
Cholangio-hepatocellular carcinoma (C-HCC)	7 (5.8%)
Patients who had ctDNA analysis [N=71]	***N (%)***
Disease status at the time of blood draw for ctDNA	
Metastatic, locally advanced, or recurrent disease	67 (94.4%)
Surgically resectable (blood was biopsied postoperatively)*	4 (5.6%)
Number of patients with ≥ 1 characterized alteration	54 (76.1%)
Median number of characterized alterations per patient (range)	2 (0-9)
IHCC [N=36]	2 (0-9)
EHCC [N=19]	1 (0-6)
GBCA [N=13]	2 (0-7)
C-HCC [N=3]	0 (0-2)
Median of total %ctDNA per patient (%)	1.1 (0.0-119.7)
Patients who had tissue-DNA analysis [N=90]	***N (%)***
Disease status at the time of tissue biopsy for tissue-DNA	
Metastatic, locally advanced, or recurrent disease	73 (81.1%)
Surgically resectable	17 (18.9%)
Biopsy site	
Primary tumor	70 (77.8%)
Metastatic sites	20 (22.2%)
Number of patients with ≥ 1 characterized alteration	90 (100%)
Median number of characterized alterations per patient (range)	4 (1-9)
IHCC [N=41]	3 (1-8)
EHCC [N=20]	4 (1-7)
GBCA [N=24]	4.5 (1-9)
C-HCC [N=5]	4 (2-6)

*Blood draw was performed after radical surgery in 3 GBCA patients (ID#28, 44, and 91) and stereotactic radiosurgery in one IHCC patient (ID#111).

Abbreviations: ctDNA, circulating-tumor DNA; C-HCC, cholangio-hepatocellular carcinoma; CI, confidence interval; EHCC, extrahepatic cholangiocarcinoma; GBCA, gallbladder carcinoma; IHCC, intrahepatic cholangiocarcinoma.

Table 2. Concordance of common genes between ctDNA and tissue-DNA among patients with biliary tract cancers whose ctDNA and tissue-DNA were both analyzed [N=40]. Most common 3 genes in ctDNA sequencing were assessed at the gene level.

Patients who had both ctDNA and tissue-DNA sequencing (N=40)					
		Tissue-DNA (+)	**Tissue-DNA (-)**	***Overall concordance***	***Kappa* (SE)***
TP53	***ctDNA (+)***	7	6	67.5%	0.27 (0.16)
	ctDNA (-)	7	20		
KRAS	***ctDNA (+)***	8	3	80.0%	0.53 (0.15)
	ctDNA (-)	5	24		
PIK3CA	***ctDNA (+)***	3	4	90.0%	0.55 (0.19)
	ctDNA (-)	0	33		

Concordance depending on whether primary tumor or metastatic site was biopsied											
	Primary tumor (N=31)					***Metastatic sites (N=9)***					***P-value (primary tumor versus metastatic sites)***
	(+/+)	***(+/-, -/+)***	***(-/-)***	***Overall concordance***	***Kappa (SE)***	***(+/+)***	***(+/-, -/+)***	***(-/-)***	***Overall concordance***	***Kappa (SE)***	
TP53	N=4	N=11	N=16	64.5%	0.17 (0.18)	N=3	N=2	N=4	77.8%	0.57 (0.24)	0.69
KRAS	N=6	N=8	N=17	74.2%	0.41 (0.17)	N=2	N=0	N=7	100%	1.00 (0.00)	0.16
PIK3CA	N=2	N=4	N=25	87.1%	0.45 (0.22)	N=1	N=0	N=8	100%	1.00 (0.00)	0.56

Concordance based on time interval between blood draw and tissue biopsy											
	≤6 months (N=27)					***>6 months (N=13)***					***P-value (≤6 versus >6 months)***
	(+/+)	***(+/-, -/+)***	***(-/-)***	***Overall concordance***	***Kappa (SE)***	***(+/+)***	***(+/-, -/+)***	***(-/-)***	***Overall concordance***	***Kappa (SE)***	
TP53	N=5	N=7	N=15	74.1%	0.40 (0.19)	N=2	N=6	N=5	53.8%	0.03 (0.28)	0.28

KRAS	N=7	N=5	N=15	81.5%	0.60 (0.16)	N=1	N=3	N=9	76.9%	0.32 (0.25)	>0.99
PIK3CA	N=3	N=3	N=21	88.9%	0.61 (0.20)	N=0	N=1	N=12	92.3%	0.00 (0.00)	>0.99

* The closer the Kappa to 1, the more the concordance.

Abbreviations: ctDNA, circulating-tumor DNA; SE, standard error.

Table 3. Exploration of prognostic factors for progression-free survival among the treated patients with biliary tract cancers [N=80].

	Univariate		***Multivariate****	
Characteristics	***Median PFS (months)***	***P-value***	***HR (95%CI)***	***P-value***
Age, years**				
≥63 [N=37] vs. <63 [N=43]	3.5 vs. 3.9	0.54	--	--
Sex				
Men [N=41] vs. Women [N=39]	3.0 vs. 4.8	0.22	--	--
ECOG-PS				
2-3 [N=21] vs. 0-1 [N=59]	3.9 vs. 3.2	0.70	--	--
Total bilirubin, mg/dL***				
>3.6 [N=8] vs. ≤3.6 [N=72]	2.8 vs. 3.5	0.26	--	--
Tumor type				
IHCC [N=41] vs. not [N=39]	3.5 vs. 3.8	0.25	--	--
Extent of disease				
Metastatic [N=67] vs. locally advanced [N=13]	3.2 vs. 5.1	0.39	--	--
Extent to extrahepatic [N=63] vs. not [N=17]	3.2 vs. 5.1	0.48	--	--
Lung metastasis [N=17] vs. not [N=63]	2.8 vs. 3.8	0.12	1.35 (0.76-2.41)	0.31
Peritoneal metastasis [N=26] vs. not [N=54]	3.1 vs. 4.2	0.38	--	--
Radical surgery prior to chemotherapy				
Yes [N=30] vs. No [N=50]	3.5 vs. 3.8	0.14	0.68 (0.40-1.15)	0.15
Treatment				
Matched [N=34] vs. Unmatched [N=46]	4.3 vs. 3.0	**0.04**	0.60 (0.37-0.99)	**0.047**
Administered as 1st line [N=62] vs. ≥2nd line [N=18]	3.5 vs. 3.9	0.78	--	--
Single drug [N=19] vs. ≥2 drugs [N=61]	2.8 vs. 3.9	0.22	--	--

* Variables with ≤0.15 in the univariate analysis were included in the multivariate analysis.

** Age at diagnosis. Dichotomized by the median.

*** Total bilirubin at the time of treatment start. Dichotomized by (3 x institutional upper limit of normal [1.2 mg/dL]).

Abbreviations: CI, confidence interval; ECOG-PS, Eastern Cooperative Oncology Group Performance Status; HR, hazard ratio; IHCC, intrahepatic cholangiocarcinoma; PFS, progression-free survival.