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Original Research

Therapeutic relevance of targeted sequencing in management of patients with advanced biliary tract cancer: DNA damage repair gene mutations as a predictive biomarker

Heejung Chae [a,1], Deokhoon Kim [b,e,1], Changhoon Yoo [a,*,2], Kyu-pyo Kim [a,**,2], Jae Ho Jeong [a], Heung-Moon Chang [a], Sang Soo Lee [c], Do Hyun Park [c], Tae Jun Song [c], Shin Hwang [d], Ki-Hun Kim [d], Gi-Won Song [d], Chul Soo Ahn [d], Jae Hoon Lee [d], Dae Wook Hwang [d], Song Cheol Kim [d], Se Jin Jang [b], Seung-Mo Hong [b], Tae Won Kim [a], Baek-Yeol Ryoo [a]

[a] *Department of Oncology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea*
[b] *Department of Pathology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea*
[c] *Department of Gastroenterology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea*
[d] *Department of Surgery, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea*
[e] *Asan Institute for Life Sciences, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea*

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Abstract ***Purpose:*** In biliary tract cancer (BTC), standard chemotherapy has limited benefit and no molecular targeted agents have been approved. This study investigated the genetic profile of BTC to identify potential new therapeutic targets and predictive biomarkers.
Methods: Targeted exome sequencing was performed for 124 patients with BTC [gallbladder cancer (GBC), 25; intrahepatic cholangiocarcinoma (ICC), 55; extrahepatic cholangiocarcinoma (ECC), 44]. Survival analysis was performed in 112 patients who received palliative chemotherapy for locally unresectable or metastatic disease.
Results: Genetic alterations were observed in 104 patients (83.8%); the most commonly mutated genes were *TP53* (44.4%), *KRAS* (29.0%), *ARID1A* (12.1%) and *IDH1* (9.7%).

* *Corresponding author*:
** *Corresponding author*: Fax: +82 2 3010 6961.
E-mail addresses: yooc@amc.seoul.kr (C. Yoo), kkp1122@amc.seoul.kr (K.-p. Kim).
[1] Heejung Chae and Deokhoon Kim contributed equally as co-first authors. [2] Changhoon Yoo and Kyu-pyo Kim contributed equally as co-corresponding authors.

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IDH1/2 mutations appeared more frequently in ICC (23.6%, P = 0.0002) than in GBC (4.0%) or ECC (2.3%), while *ERBB2/3* mutations were found only in GBC (20.0%) and ECC (11.4%). Patients harbouring *TP53* mutations had shorter overall survival (OS; median 15.2 vs. 37.8 months, $P = 0.018$), while *IDH1* mutations showed a tendency for longer progression-free survival (PFS; 10.6 vs. 6.1 months, $P = 0.124$). Potentially actionable genetic alterations were found in 54.8%, and 7.1% received appropriate molecular targeted therapy in the clinical trial setting. Germline or somatic mutations in DNA damage repair (DDR) genes were found in 63.5% of patients and were significantly associated with longer PFS (6.9 vs. 5.7 months, $P = 0.013$) and OS (21.0 vs. 13.3 months, $P = 0.009$) in patients who received first-line platinum-containing chemotherapies (n = 88).

Conclusions: A subgroup of patients with BTC may benefit from targeted therapy by the aid of genetic information. In particular, DDR alterations may be a predictive biomarker for response to platinum-containing chemotherapy in patients with BTC.

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1. Introduction

Biliary tract cancer (BTC), which includes gallbladder cancer (GBC), intrahepatic cholangiocarcinoma (ICC) and extrahepatic cholangiocarcinoma (ECC), is an aggressive disease with dismal prognosis [1]. Gemcitabine plus cisplatin (GemCis) is a standard systemic therapy for advanced BTC [2], but the prognosis remains poor, with a median overall survival (OS) of <1 year. No molecular targeted agents or immune checkpoint inhibitors have been approved for BTC.

Large molecular profiling studies using whole-exome sequencing or targeted sequencing have characterised the genetic landscape of BTC [3,4], suggesting that BTC may be molecularly classified, and precision medicine using targeted or immunotherapy may enhance the survival of patients with BTC. However, more data are needed to reveal the predictive or prognostic implications of genetic alterations in the management of advanced BTC.

In this study, we performed a targeted sequencing analysis to assess the molecular profile of Korean patients with BTC and to identify novel therapeutic targets and predictive biomarkers in patients with advanced BTC. In addition, the role of DNA damage repair (DDR) pathway gene alterations as a predictive biomarker for the response to platinum-based chemotherapy was also investigated, as platinum-based agents such as cisplatin and oxaliplatin are the mainstay of the first-line chemotherapy for patients with unresectable or metastatic BTC.

2. Methods

2.1. Patients

Between August 2016 and February 2018, 137 patients pathologically confirmed with BTC were enrolled in this study. After the exclusion of 13 samples that failed to meet the minimum requirement for tumour cell content, 124 patients were included in this analysis. Sequencing was performed using specimens acquired from surgery (n = 78, 62.9%) or biopsy (n = 46, 37.1%). Written informed consent was obtained from all patients. Clinical data regarding baseline patient characteristics, treatment history and survival outcomes were obtained by reviewing the patients' medical records.

This study was approved by the Institutional Review Board of the Asan Medical Center and performed in accordance with the ethical standards of the institutional research committee and the latest Declaration of Helsinki.

2.2. Targeted exome sequencing and bioinformatics analysis

Genomic DNA was extracted from previously collected formalin-fixed, paraffin-embedded (FFPE) tissue specimens (Appendix Method A.1). Multiple sequencing assays were used in this study, including an in-house panel of the Asan Medical Center, Seoul, Korea (OncoPanel AMC, versions 2 and 3) (n = 66), the Foundation Medicine T7 assay for 404 cancer-related genes (Foundation Medicine, Cambridge, MA, USA) (n = 36) and the Oncomine focus panel, version 1, for 52 cancer-related genes (Q Squared Solutions, Valencia, CA, USA) (n = 22). The OncoPanel AMC version 2 (OP AMC v2) and version 3 (OP AMC v3) were run using the MiSeq platform (Illumina, San Diego, CA, USA) and captured 182 and 383 cancer-related genes, respectively (OP AMC v2, 170 genes for entire exons and 36 genes for partial introns; OP AMC v3, 199 genes for entire exons, 8 genes for partial introns and 184 genes for hotspots). Further details on our in-house panel have been described elsewhere [5,6]. The Foundation Medicine T7 assay and the Oncomine focus panel were run according to the service providers' protocols. Gene

lists for each targeted next-generation sequencing (NGS) panel are tabulated in Table A.1.

The sequence mapping steps for OP AMC v2 and v3 were performed in accordance with a described method [5,6]. Somatic variant calling for single nucleotide variants and short indels was conducted using VarDict [7]. Germline variants of candidates for somatic variants (found in ≥1% of samples) were filtered out with common germline variants database [dbSNP (build 141), gnomAD, common germline variants from 1100 healthy Korean] [8]. Copy number analyses were performed using CNVkit [9].

2.3. DNA damage repair gene mutations

Germline or somatic mutations in *ATM*, *ATR*, *BAP1*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CHEK2*, *FAM175A*, *GEN1*, *MLH1*, *MSH2*, *MSH6*, *MRE11A*, *NBN*, *PALB2*, *PMS2*, *RAD50*, *RAD51*, *RAD51C*, *RAD51D* and *XRCC2* were classified as DDR gene mutations in this study [10]. DDR genes covered in each panel are annotated in Table A.1. OP AMC v3 or Foundation Medicine T7 assay covered all 22 DDR genes. OP AMC v2 covered 10 DDR genes out of 22, but frequently mutated DDR genes such as *BRCA 1/2, ATM, ATR, CHEK, MLH1, MSH2/6* and *BAP1* were included. Oncomine focus panel, version 1, did not cover DDR gene mutations.

2.4. Statistical analyses

Categorical variables were compared using the chi-square or Fisher exact test, as appropriate. Progression-free survival (PFS) and OS were defined as the time from the start of first-line palliative chemotherapy until tumour progression or death from any cause and until death from any cause, respectively. Survival curves were estimated using the Kaplan-Meier method and compared by the log-rank test. A P-value <0.05 was considered statistically significant, and SPSS 20.0 (IBM, Armonk, NY, USA) was used for all statistical analyses.

3. Results

3.1. Patient characteristics

Clinicopathologic characteristics of the overall patients are summarised in Table 1. The median age was 62 years (range, 25–83), and 59.7% (n = 74) were male. By primary site, ICC (n = 55, 44.4%) was most common, followed by ECC (n = 44, 35.5%) and GBC (n = 25, 20.2%). At the time of initial diagnosis, 55 patients (46.0%), 8 patients (6.5%) and 59 patients (47.6%) had resectable, locally advanced (unresectable) and metastatic disease, respectively. At any point during the course of disease, 112 patients eventually received palliative chemotherapy for their recurring (n = 46, 41.1%), initially metastatic (n = 59, 52.7%) or locally advanced disease (n = 7, 6.3%); the other 7 patients just received best supportive care for their recurred or metastatic disease, and the rest 5 had not experienced a recurrence of cancer after surgical resection. Median time from tissue acquisition (biopsy or surgery) to initiation of palliative chemotherapy (i.e., chemotherapy for locally advanced or metastatic disease) was 1.7 months [95% confidence interval (CI), 0.1–3.2] in overall patients. For patients who previously had curative-intent resection, median time from surgery to palliative chemotherapy (i.e., chemotherapy for recurrent disease) was 9.4 months (95% CI, 6.5–12.2).

Table 1
Baseline characteristics and treatment summary of all patients.

Characteristics	Total (n = 124)
Age (years)	
Median (range)	62 (25–83)
Sex	
Male	74 (59.7%)
Female	50 (40.3%)
Primary site	
GBC	25 (20.2%)
ICC	55 (44.4%)
ECC	44 (35.5%)
Extent	
Initially metastatic	59 (47.6%)
Locally advanced	8 (6.5%)
Resectable	57 (46.0%)
Etiology	
HBV infection	9 (7.3%)
HCV infection	1 (0.8%)
IHD stone	4 (3.2%)
CS infection	1 (0.8%)
Choledochal cyst/AUPBD	2 (1.6%)
Not identified	107 (86.3%)
Stage at initial diagnosis	
I	5 (4.0%)
II	35 (28.2%)
III	15 (12.1%)
IV	68 (54.8%)
Not evaluable	1 (0.8%)
Surgery	72 (58.1%)
Curative-intent resection	57 (79.2%)
Palliative resection[a]	15 (20.8%)
Palliative first-line chemotherapy	112 (90.3%)
GemCis	90 (80.4%)
Fluoropyrimidine-oxaliplatin combination	20 (17.9%)
M7824	1 (0.9%)
Fluoropyrimidine monotherapy	1 (0.9%)

GBC, gallbladder cancer; ICC, intrahepatic cholangiocarcinoma; ECC, extrahepatic cholangiocarcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; IHD, intrahepatic duct; CS, *Clonorchis sinensis*; AUPBD, anomalous union of the pancreaticobiliary duct; GemCis, gemcitabine plus cisplatin.

[a] Palliative surgery indicates R2 resection or surgery in metastatic disease.

3.2. Landscape of genomic alterations

Genetic mutations and copy number alterations were observed in 105 (84.7%) and 55 (44.4%) patients,

respectively, while 13 patients (10.5%) did not have any genetic alterations (Fig. A.1, Table A.2). The most commonly mutated gene was *TP53* (n = 53, 42.7%), followed by *KRAS* (n = 35, 28.2%), *ARID1A* (n = 17, 13.7%), *IDH1* (n = 13, 10.5%), *ATM* (n = 12, 9.7%), *FAT1* (n = 10, 8.1%) and *BRCA2* (n = 9, 7.3%). In terms of copy number alterations, *CDKN2A*/B deletions (n = 14, 11.3%), *ERBB2* amplifications (n = 11, 8.9%), *CCNE1* amplifications (n = 10, 8.1%) and *MYC* amplifications (n = 6, 4.8%) were frequently detected. As shown in Fig. 1, the incidence of *TP53* mutations was highest in GBC (n = 15, 60.0%, $P = 0.052$), while *IDH1/2* mutations were more common in ICC (n = 13, 23.6%, $P = 0.0002$). On the other hand, *ERBB2* and *ERBB3* mutations were found only in GBC (n = 5, 20.0%) and ECC (n = 5, 11.4%). The frequency of all other genetic mutations and copy number alterations according to the primary tumour site is shown in Tables A.3 and A.4, respectively.

Information on genetic rearrangements was available for the 36 patients (29.0%) who were analysed by the Foundation Medicine T7 assay. Fusion genes were observed in eight patients (22.2%; Fig. A.2) including three (8.3%) with *FGFR* rearrangements (*FGFR3-FAM53A*, *FGFR2-CCDC6* and *FGFR2-WAC*).

3.3. Tumour mutational burden

Tumour mutational burden (TMB) could be estimated in 66 cases where sequencing was performed using the in-house panel (OP AMC v2 or OP AMC v3). The median TMB was 4.9 mutations/Mb (range, 0–23.0 mutations/Mb). Comparing the patients with a TMB of ≤10 mutations/Mb (n = 59, 89.4%) with those with >10

Fig. 1. **Genetic landscape of biliary tract cancer**. The oncoprint displays the most frequent and most clinically meaningful genetic mutations according to the primary site of malignancy, the aetiology, the disease extent at the time of diagnosis and the surgical history. The tumour mutational burden (mutations/Mb) and the sequencing method are indicated in the top rows. TMB, tumour mutational burden (mutations/Mb); FM, Foundation Medicine T7 assay; OM, Oncomine focus panel, version 1; OP2, OncoPanel AMC version 2; OP3, OncoPanel AMC version 3; AUPBD, anomalous union of the pancreaticobiliary duct; CC, choledochal cyst; CS, *Clonorchis sinensis* infection; HBV, hepatitis B virus; HCV, hepatitis C virus; IHDS, intrahepatic duct stone; IM, initially metastatic; LA, locally advanced; R, resectable; ECC, extrahepatic cholangiocarcinoma; GBC, gallbladder cancer; ICC, intrahepatic cholangiocarcinoma.

mutations/Mb (n = 7, 10.6%), there was no significant difference in PFS (median, 6.8 vs. 8.4 months, respectively; $P = 0.903$) or OS (25.7 vs. 16.8 months, respectively; $P = 0.895$).

3.4. Potentially actionable genetic alterations and treatment with targeted agents

Based on the OncoKB classification [11], 54.8% of patients (n = 68) harboured at least one potentially actionable alteration (Table 2). Seventeen (13.7%) had alterations in level 2B targets, including *ERBB2* amplifications (n = 11), *IDH1* mutations (n = 5), *MET* amplifications (n = 1) and *CDK4* amplifications (n = 1). *FGFR2* fusions, which are considered a level 3A alteration because of emerging clinical data on FGFR inhibitors in BTC, were detected in two patients with ICC.

Among 112 patients who received palliative systemic chemotherapy, eight patients (7.1%) entered clinical trials for targeted agents based on their genetic alterations. Three patients with ICC with *IDH1* mutations were treated with IDH1 inhibitors. Three patients with *FGFR* gene alterations (a patient with ECC with a *FGFR2* mutation, a patient with ICC with a *FGFR2-CCDC6* fusion and a patient with ICC with a *FGFR2-WAC* fusion) were treated with FGFR inhibitors. A patient with GBC with a *KRAS* G12V mutation and a patient with ICC with an *NRAS* A182G mutation were treated with a RAF1 inhibitor.

3.5. Correlative analysis with survival outcomes

For the 112 patients who received palliative chemotherapy for unresectable, metastatic or recurrent disease, correlative analyses between genetic alterations and survival outcomes were performed (Fig. 2). With a median follow-up duration of 16.6 months, the PFS and OS of the entire patient population were 6.3 (95% CI, 5.5–7.1) and 16.2 months (95% CI, 10.5–21.9), respectively. *TP53* mutations were marginally associated with poorer PFS [presence vs. absence; median, 5.7 months (95% CI, 4.2–7.1) vs. 6.9 months (95% CI, 5.8–8.0); $P = 0.088$] but significantly related with poorer OS [median, 15.2 months (95% CI, 10.5–19.9) vs. 37.8 months (95% CI, 7.3–68.3); $P = 0.018$]. Although these differences did not reach statistical significance, *KRAS* mutations tended to be associated with unfavourable PFS [presence vs. absence; median, 5.2 months (95% CI, 2.8–7.5) vs. 6.8 months (95% CI, 5.9–7.8); $P = 0.114$], while *IDH1* mutations tended to be associated with longer PFS [presence vs. absence; median, 10.6 months (95% CI, 3.8–17.4) vs. 6.1 months (95% CI, 5.3–6.8); $P = 0.124$].

Table 2
Actionable gene alterations detected in overall patients.

Actionable target		Patients (total N = 124)
Yes		68 (54.8%)
Level of evidence[a]	Genetic alterations	
2B	*ERBB* amplification	11 (8.9%)
	IDH1 mutation	5 (4.0%)
	MET amplification	1 (0.8%)
	CDK4 amplification	1 (0.8%)
3A	*FGFR2* fusion	2 (1.6%)
3B	*PIK3CA* amplification	6 (4.8%)
	ERBB2 mutation	5 (4.0%)
	NRAS mutation	4 (3.2%)
	MDM amplification	4 (3.2%)
	FGFR1 amplification	1 (0.8%)
4	*KRAS* mutation	34 (27.4%)
	BRAF mutation	2 (1.6%)
No		56 (45.2%)

BTC, biliary tract cancer; FDA, Food and Drug Administration.
Each gene alteration was classified according to OncoKB classification (https://oncokb.org/levels and https://oncokb.org/actionableGenes) [11].
[a] Since there are no FDA-approved targeted agents for BTC to date, no patient was able to show level 1 or 2A genetic alterations.

3.6. Impact of DDR gene mutations on platinum-based therapy

For 88 patients who received first-line platinum-based chemotherapy for unresectable, metastatic or recurrent disease, the impact of DDR gene mutations on survival outcomes was analysed. DDR gene mutations were found in 55 (63.5%) patients. The most commonly mutated DDR gene was *BRCA2* (n = 16, 18.2%), followed by *ATM* (n = 12, 13.6%), *ATR* (n = 7, 8.0%), *BRIP1* (n = 6, 6.8%) and *MLH1* (n = 6, 6.8%). The median PFS and OS with first-line platinum-based therapy for these 88 patients were 6.5 (95% CI, 5.7–7.3) and 16.1 (95% CI, 14.1–18.1) months, respectively. DDR gene mutations were significantly associated with prolonged PFS [presence vs. absence; median, 6.9 months (95% CI, 4.7–9.1) vs. 5.7 months (95% CI, 3.8–7.6); P = 0.013] and OS [median, 21.0 months (95% CI, 13.9–28.1) vs. 13.3 months (95% CI, 6.7–20.0); $P = 0.009$] (Fig. 3). The impact of DDR gene mutations remained significant in multivariate analyses for PFS that included other prognostic factors [hazard ratio, 0.51 (95% CI, 0.31–0.84); $P = 0.009$], but not in multivariate analyses for OS (Table A.5). In 65 patients whose response information was available, the overall response rate (ORR) was higher in patients with DDR alterations (47.3% vs. 27.3%), although this was not statistically significant ($P = 0.193$) (Table A.6).

4. Discussion

We present the results of targeted sequencing for 124 Korean patients with BTC and their therapeutic relevance. *TP53* mutations (42.7%), *KRAS* mutations (28.2%) and *CDNK2A/B* deletions (11.3%) were the most commonly observed genetic alterations, consistent

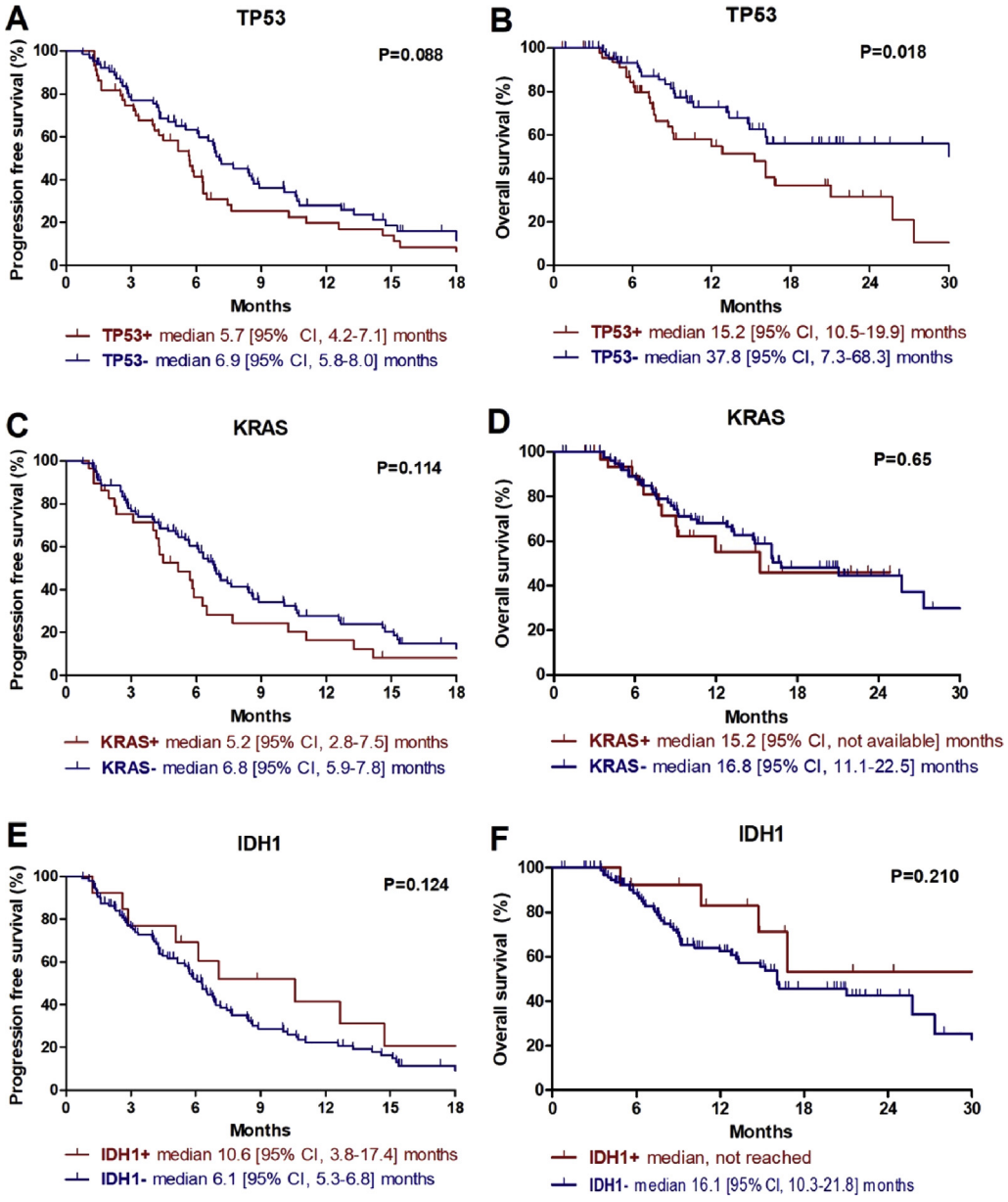
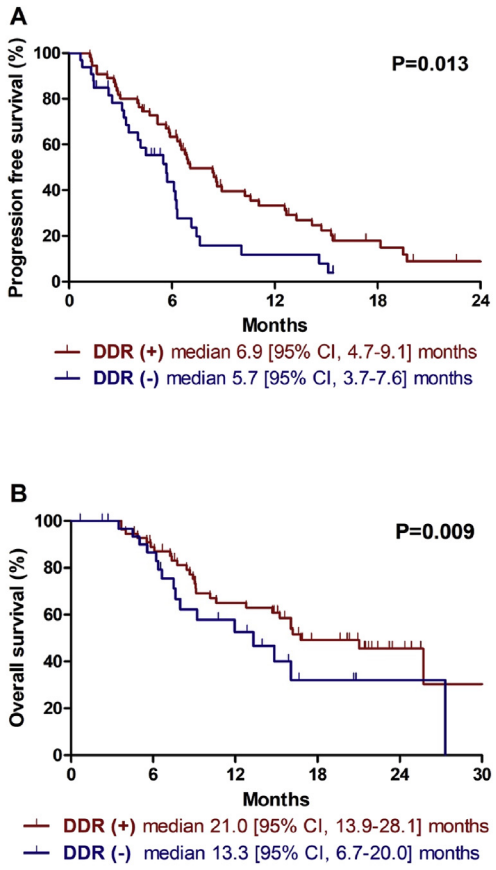


Fig. 2. Survival outcomes according to genetic mutations. CI, confidence interval.

with prior studies [3,4,12–15]. *TP53* mutations were more frequent in GBC, and *ERBB2* mutations were solely found in ECC and ICC in line with previous studies [15–17]. *IDH1/2* mutations, one of the promising targets in BTC, were found in 10.5% of patients and were more prevalent in ICC (20.0%). *FGFR* alterations, another well-described actionable target against BTC, were found in 11% of patients. Although this number is at the low end of the range that has been previously reported for ICC (11–47%) [16], this finding should be cautiously interpreted because *FGFR* gene fusions could be assessed in a subset of our patients (29.0%). Some genetic alterations were associated with survival outcomes. *TP53* mutations were significantly associated with worse survival outcomes in this study, consistent with previous reports [3,4,12,13,18]. Also, patients with *KRAS* and *IDH1* mutations tended to have worse and better PFS, respectively. Although previous studies have suggested the prognostic impact of other genetic alterations, such as *BAP1* mutations, FGF pathway alterations, *ARID1A* mutations and *CDKN2A/B* deletions [12,18], these were not detected in our study cohort. These discrepancies among studies may be due to heterogeneity in the baseline characteristics of the patient populations included.

As BTC is regarded as one of the good candidates for molecular triage program, and various targeted agents, including FGFR and IDH inhibitors, are being actively investigated [19], NGS analysis may provide precision treatment matched to individual genomic profiles. In



DDR (+), presence of DDR mutations; DDR (-), no DDR mutations.

Fig. 3. Progression-free survival (A) and overall survival (B) with platinum-based chemotherapy according to mutational status of DNA damage repair (DDR) genes. CI, confidence interval.

this study, 54.8% of patients carried at least one actionable genetic alteration according to the OncoKB classification [11], and eight patients (7.1%) received matched molecular targeted therapy with IDH1, FGFR or RAF1 inhibitors. In several phase II trials of patients with *FGFR* alterations [20–22], the ORRs to pan-FGFR inhibitors (erdafitinib, infigratinib or INCB054828) ranged from 24% to 46% and the disease control rates ranged from 76% to 85%. Patients with *FGFR* fusions were much more likely to benefit from FGFR targeted therapy than those with *FGFR* mutations [21,22]. Meanwhile, a recent study evaluating an IDH1 inhibitor (AG-120) reported that 4 of 72 (6%) patients harbouring *IDH1* mutations had a confirmed partial response and 56% achieved stable disease [23]. The efficacy and safety of AG-120 will be demonstrated in an ongoing phase III trial (ClarIDHy study) [24], and other IDH1 and/or IDH2 inhibitors are being actively developed and tested [25]. *ERBB2* amplification (8.9%) is also a promising actionable target, considering the success of trastuzumab in *HER2*-positive breast and gastric cancers. There have been anecdotal reports of the potential activity of anti-HER2 therapies in HER2-positive BTC [26], and several prospective clinical trials are ongoing (NCT03613168 and NCT03185988).

Notably, our data suggest that mutations in DDR genes may have a role as predictive biomarkers for the response to platinum-based chemotherapy in patients with BTC. It has been previously suggested that patients with defective DDR mechanisms are more likely to respond well to platinum-based chemotherapy [27–30], but data in BTC were lacking. In the present study, DDR gene mutations were found in 62.5% of patients, similar with a prior study [31], and were significantly associated with longer PFS (median, 6.9 vs. 5.7 months; $P = 0.013$) and OS (median, 21.0 vs. 13.3 months; $P = 0.009$) in patients with BTC treated with first-line platinum-based chemotherapy for unresectable or metastatic disease. In addition, the ORR tended to be higher in patients who had genetic alterations in DDR mechanisms. As platinum-based chemotherapies, such as cisplatin and oxaliplatin, are widely used in advanced patients with BTC, DDR mutations identified using targeted sequencing may be helpful to guide personalised treatment. Our findings also suggest that agents targeting DDR mechanisms, such as PARP, ATM/ATR and Chk1/2 inhibitors, require future investigation in BTC [32].

High TMB is regarded as a promising biomarker for the response to immune checkpoint inhibitors in various types of cancers [33]. In the present study, approximately 10% of patients with BTC had a relatively high mutational burden, with >10 mutations/Mb. This is much higher than the previously reported incidence of 1.8% in Western patients with ICC [34], but a Chinese group also reported that 10.9% of patients with ICC had a TMB higher than 20 mutations/Mb [35]. Because no patient with high TMB (>10 mutations/Mb) in our cohort received immune checkpoint inhibitors, we could not evaluate the relevance of high TMB as a predictive biomarker of response to immune checkpoint inhibitors in BTC. More research is necessary to identify genetic biomarkers that can predict the response to immune checkpoint blockade therapy in patients with BTC.

One of limitations in this study is that three different sequencing assays were used for genetic analysis. Because each panel covered different range of capture regions from one another, information on fusion genes and TMB was not available in all cases. In addition, this study performed retrospectively and included a relatively small number of patients that were heterogenous in stages, etiologies and primary tumour sites.

In conclusion, the genetic profile of BTC is heterogeneous and depends on the anatomic location, and some genetic alterations may be related to survival outcomes. Our results indicate that a subgroup of patients with BTC may benefit from targeted therapies such as IDH, FGFR and HER2 inhibitors. DDR gene mutation status may have a role as a predictive biomarker in patients with BTC treated with platinum-

based regimens, making personalised treatment planning possible.

Conflict of interest statement

There are no conflicts of interest relevant to this article to report.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2019.07.022.

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