ORIGINAL RESEARCH

Prognosis of Advanced Cholangiocarcinoma in the Palliative Care Setting: A Series of 201 Cases

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ABSTRACT

Objective • Prognosis of cholangiocarcinoma is poor, and palliative treatment options are limited in China. This study aimed to analyze prognostic factors affecting survival in patients with advanced cholangiocarcinoma.

Methods • Clinical data on 201 consecutive patients with cholangiocarcinoma who received treatment at a single center from May 2014 to December 2018 were analyzed retrospectively. Survival curves were plotted using the Kaplan-Meier method. Survival analyses were performed using a log-rank test.

Results • For first-line therapy, the disease control rate was 56% (85/152) and the overall response rate was 16% (24/152). The total disease control rate was 34% (23/67) for second-line therapy. The median progression-free survival was 7 months, and the median overall survival was 17 months. Next-generation sequencing was performed for 59 patients. The most frequently mutated genes were *TP53* and *PI3KCA*. No significant association was found between gene mutations and treatment response or survival. Of 5 patients with high levels of microsatellite instability, 4 (80%) were sensitive to anti–programmed death 1 antibodies and remained in partial remission at last follow-up.

Conclusions • Macroscopic tumor characteristics, rather than gene mutations, determine the prognosis of advanced cholangiocarcinoma. High microsatellite instability may be a favorable predictor of response to immunotherapy for cholangiocarcinoma. (*Altern Ther Health Med.* 2022;28(2):24-31).

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INTRODUCTION

Cholangiocarcinoma is a tumor that originates from the biliary epithelial cells. This cancer, which accounts for 3% of malignant gastrointestinal malignant tumors, has its highest incidence rate in Asia (3.3 cases per 100 000 persons).[1] The overall 5-year survival rate of advanced cholangiocarcinoma is less than 5%.[2] While radical surgery is the most effective treatment for cholangiocarcinoma, chemotherapy is the only standard treatment in the palliative care setting. Treatment outcomes of palliative care for cholangiocarcinoma have not improved appreciably in recent years.[2] Present challenges include lack of effective treatments and an absence of known factors predictive of treatment response and survival.

Standard first-line treatment for advanced cholangiocarcinoma is combination therapy including gemcitabine plus cisplatin, which was evaluated in the Advanced Biliary Cancer (ABC)-02 trial more than a decade ago.[3] Use of this combination chemotherapy is a category 1 recommendation per guidelines of both the National Comprehensive Cancer Network (NCCN) and the European Society for Medical Oncology.[4] In the NCCN guidelines, fluorouracil-centered therapy also is a first-line treatment, although not a category 1 recommendation.[5] Consensus on second-line treatment for cholangiocarcinoma has not been reached.

Various mechanisms are involved in tumorigenesis and drug resistance, including genetic mutations, hypermethylation, microsatellite instability, and translational modifications. Previous studies have shown that certain gene mutations and microsatellite instability-high (MSI-H) status can predict treatment response in patients with cholangiocarcinoma.[6,7] However, genetic analysis rarely is used to predict response to palliative treatment of advanced cholangiocarcinoma.

Hepatitis B virus infection is a potential risk factor for cholangiocarcinoma, especially for intrahepatic cholangiocarcinoma. China has the largest population of

persons with hepatitis B infection, and numerous cases of cholangiocarcinoma are diagnosed in China each year. However, palliative treatment for cholangiocarcinoma is limited in China and reports from relevant large studies are few. Accordingly, our study aimed to analyze factors predictive of survival in Chinese patients with unresectable cholangiocarcinoma.

METHODS

Study Population

We conducted a retrospective, single-center study investigating the prevalence of cholangiocarcinoma in a Chinese population, as well as treatment modalities, treatment efficacy, and survival rates among these patients. Patient inclusion criteria were histologically confirmed diagnosis of unresectable cholangiocarcinoma (including all morphological subtypes) via surgery or biopsy from January 2014 through December 2018. Patients with incomplete clinical data were excluded. We collected patient information and clinical data including epidemiological data, age, concomitant diseases, detailed information about the tumor, treatments received, follow-up data, recurrence-free survival, date of death, and overall survival (OS). Therapeutic efficacy was evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1. The ethics committee of Eastern Hepatobiliary Surgery Hospital approved this study.

Next-Generation Sequencing

Tumor tissue samples were collected and put into formalin-fixed paraffin-embedded (FFPE) sections. After centrifugation in microcentrifuge tubes, DNA was extracted from FFPE samples by using the QIAamp DSP DNA FFPE Tissue Kit (QIAGEN, USA); DNA then underwent dewaxing, proteinase digestion, and column-based purification. Using plasma isolated from peripheral blood, circulating tumor DNA and genomic DNA were extracted with the QIAamp Circulating Nucleic Acid Kit (QIAGEN, USA). The concentration, quality, and fragment size of DNA were measured. A barcoded, next-generation DNA sequencing library was prepared by using the KAPA Library Preparation Kit for Illumina sequencing platforms (Roche Diagnostics Corporation, Indianapolis, IN, USA) and amplified using ligation-mediated polymerase chain reaction (LM-PCR). The amplified library was associated and hybridized with the SeqCap EZ Library (Roche Diagnostics Corporation, Indianapolis, IN, USA), and target DNA was captured, eluted, and recovered. After purification, the obtained library was further amplified by LM-PCR. Library concentration and size were measured by the Qubit 2.0 Fluorometer and Agilent 2100 Bioanalyzer, and the library was sequenced using the Illumina HiSeq X Ten Sequencing System (Illumina, Inc., San Diego, CA, USA).

Raw DNA sequencing data were processed. Low-quality bases and sequences were removed, and sequencing errors were corrected. Processed sequences were aligned to a reference genome to locate matching positions. Alignment quality was evaluated, and local splicing was performed to correct alignment errors. We screened DNA sequences for possible mutations (such as single base mutations, structure variations, copy-number alterations, and gene fusion) and calculated mutation frequency and/or multiple copy-number alterations. Mutations were annotated by comparing the major sequence databases and the possible effects of each mutation on gene function, as well as correlations between protein function and disease.

Microsatellite Instability Assay

For all patients whose tumor tissue samples were sequenced, the samples were also tested for MSI-H status by using a pentaplex polymerase chain reaction (PCR) assay comprised of 2 mononucleotide repeat markers (BAT-25 and BAT-26) and 3 dinucleotide repeat markers (D2S123, D5S346, and D17S250). After amplification, fragment analysis chromatograms for each microsatellite were reviewed manually to identify instability in each microsatellite. Differences between the tumor samples and normal samples from the same patient were assessed. Instability in at least 2 of 5 microsatellites was defined as MSI-H status.

Sample Size Calculation

The primary outcome of interest was progression-free survival (PFS) among patients with moderately differentiated cholangiocarcinoma versus among those with poorly differentiated cholangiocarcinoma. Based on findings from a pilot study, we calculated that a sample size of 100 patients per group was needed to detect a 2-month difference in PFS with a power of 80% and a two-sided P value of .05.

Statistical Analysis

Data on continuous variables with a normal distribution were presented as means and standard deviations; medians and interquartile ranges (IQRs) were presented for continuous variables with a screwed distribution. Data on categorical variables were described using frequencies or percentages. Survival time was defined as the time between diagnosis and death. Survival curves were plotted using the Kaplan-Meier method, and survival time was evaluated using a log-rank test. Cox regression was used for univariate and multivariate survival analyses. Multivariate analyses included variables for which $P \leq 0.1$ in univariate analysis. All statistical analyses were performed using IBM SPSS Statistics, Version 21.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Patient Characteristics

A total of 201 patients were included in the study, including 34 patients with locally advanced cholangiocarcinomas and 167 patients with distant metastases. Demographic and clinical characteristics of the patients are presented in Table 1.

Treatment

Of the 201 patients, 24 did not receive chemotherapy due to poor physical status or jaundice. Among 177 patients who received chemotherapy, first-line treatment consisted of a gemcitabine-based regimen for 109 (62%) patients and a fluorouracil-based regimen for 40 (23%) patients. Six patients received transcatheter arterial chemoembolization, and 5 patients received immunotherapy. Twenty-five patients died before completing 2 treatment cycles. Of 152 patients for whom treatment efficacy was evaluable, 67 (44%) showed no response to therapy; the disease control rate was 56% (85/152), and the overall response rate was 16% (24/152). Among patients who received gemcitabine-based regimens, the disease control rate was 72% (69/96) and the overall response rate was 23% (22/96). In patients treated with fluorouracil-based regimens, the disease control rate was 60% (24/40) and the overall response rate was 5% (2/40).

Second-line treatment was used in 84 of 201 patients (42%), including 21 treated with gemcitabine-based regimens and 12 treated with fluorouracil-based regimens. Ten patients received a FOLFIRI regimen (fluorouracil, leucovorin, and irinotecan). Other second-line drugs used in this patient population included paclitaxel, anti–programmed death 1 (PD-1) antibodies, and tyrosine kinase inhibitors. Efficacy of second-line treatment was evaluable in 67 patients, with disease control rates of 34% (23/67) overall, 43% (9/21) for gemcitabine-based therapies, and 42% (5/12) for fluorouracil-based regimens. FOLFIRI achieved stable disease in 4 of 10 (40%) patients. No patients had partial response to second-line treatment.

Third-line treatments, including tyrosine kinase inhibitors and anti–PD-1 antibodies, were used in 12 patients.

Table 1. Characteristics of 201 Patients With Unresectable Cholangiocarcinoma

Patient characteristic	Data value
Age, y	
Median (IQR)	58 (30-79)
Sex, n (%)	
Female	85 (42.3)
Male	116 (57.7)
ECOG performance status, n (%)	
0	120 (59.7)
1	66 (32.8)
2	15 (7.5)
Tumor location, n (%)	
Intrahepatic	110 (54.7)
Perihilar	20 (10.0)
Extrahepatic, distal	17 (8.5)
Gallbladder	54 (26.9)
Disease stage, n (%)	
Locally advanced	34 (16.9)
Metastatic	167 (83.1)
CA 19-9 ≥ upper limit of normal, n (%)	152 (75.6)
HBV infection status, n (%)	
Cured	3 (1.5)
Chronic infection	29 (14.4)
No infection	135 (67.2)
Unknown	34 (16.9)

Abbreviations: CA 19-9, carbohydrate antigen 19-9; ECOG, Eastern Cooperative Oncology Group; HBV, hepatitis B virus; IQR, interquartile range.

Patient Survival

The median follow-up time was 12.1 months (IQR, 0-58.8 months). The median PFS and OS were 7 months and 17 months, respectively.

Univariate analyses demonstrated that tumor differentiation, tumor location, and first-line chemotherapy were significantly associated with PFS. PFS was significantly shorter among patients with low tumor differentiation (6 months) than among patients with moderate to high tumor differentiation (8 months) [hazard ratio (HR), 1.05; 95% confidence interval (CI), 1.06 to 2.13; $P = .02$] (Figure 1, Table 2). Patients with extrahepatic bile duct carcinoma had significantly longer PFS compared with patients with intrahepatic cholangiocarcinoma (HR, 0.39; 95% CI, 0.22 to 0.72; $P = .002$) (Figure 2, Table 2). Compared with patients treated with gemcitabine plus cisplatin, patients treated with gemcitabine plus oxaliplatin had significantly improved PFS (HR, 0.66; 95% CI, 0.44 to 0.99; $P = .04$) and those treated with gemcitabine plus tegafur, gimeracil, and oteracil potassium capsules had significantly worse PFS (HR, 2.63; 95% CI, 1.14 to 6.04; $P = .02$) (Table 2). Results of multivariate Cox regression analysis demonstrated that PFS was independently associated with these aspects of tumor location, differentiation, and first-line chemotherapy, excluding the gemcitabine plus oxaliplatin regimen, after adjusting for other relevant factors (Table 2).

Both univariate and multivariate Cox regression analyses demonstrated that only tumor differentiation was significantly associated with OS, which was significantly longer among patients with moderate to high tumor differentiation (20 months) than among those with low tumor differentiation (11 months) (HR, 1.92; 95% CI, 1.28 to 2.89; $P = .002$). Both magnitude and strength of this statistical association were unchanged by controlling for other relevant factors in the multivariate model (Figure 1, Table 3). In Cox regression analyses, no other potential explanatory variables pertaining to tumor location, metastases, or chemotherapy were significantly associated with OS (Figure 2, Table 3).

Next-Generation Sequencing

Sequencing of DNA was performed on tumor tissue samples from 59 patients. Of these 59 patients, 26 patients (44%) carried tumor protein p53 (*TP53*) gene mutations, 10 (17%) carried phosphatidylinositol 3-kinase (*PI3KCA*) gene mutations, 9 (15%) carried RAS gene mutations, 7 (12%) carried receptor tyrosine-protein kinase erbB-2 (*ERBB2*) gene mutations, 7 (12%) carried *MET* gene mutations, and 6

Figure 1. Progression-Free and Overall Survival by Level of Tumor Differentiation. (A) Progression-free survival among patients with tumors with low differentiation was 6 months, versus 8 months among those with tumors with moderate to high differentiation (P = .02). (B) Overall survival among patients with tumors with low differentiation was 11 months, versus 20 months among those with tumors with moderate to high differentiation (P = .002).

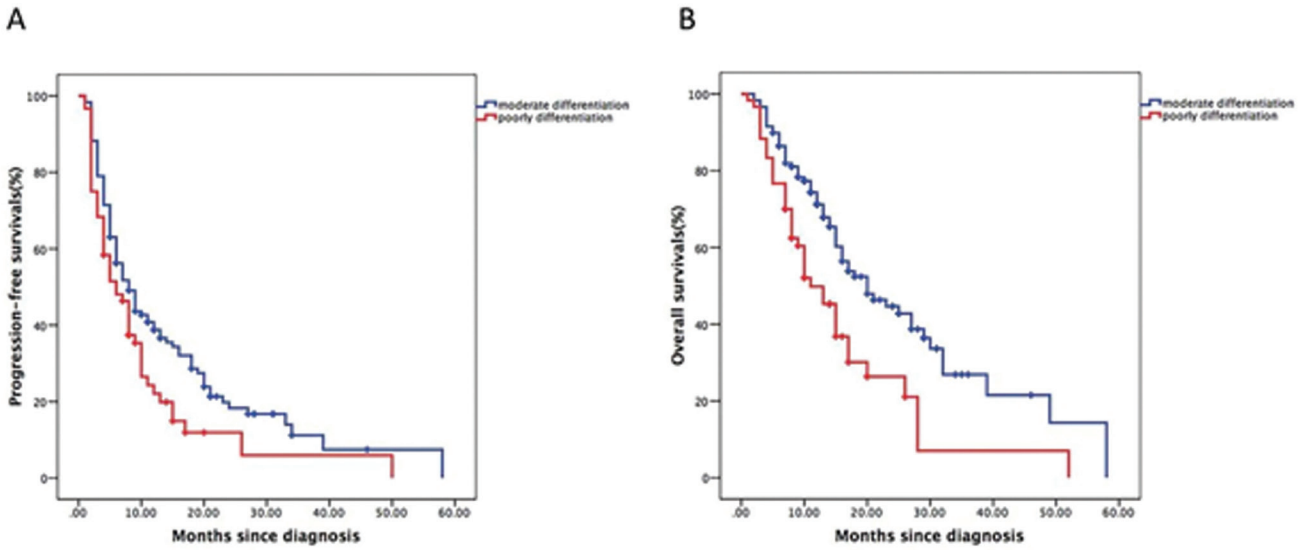
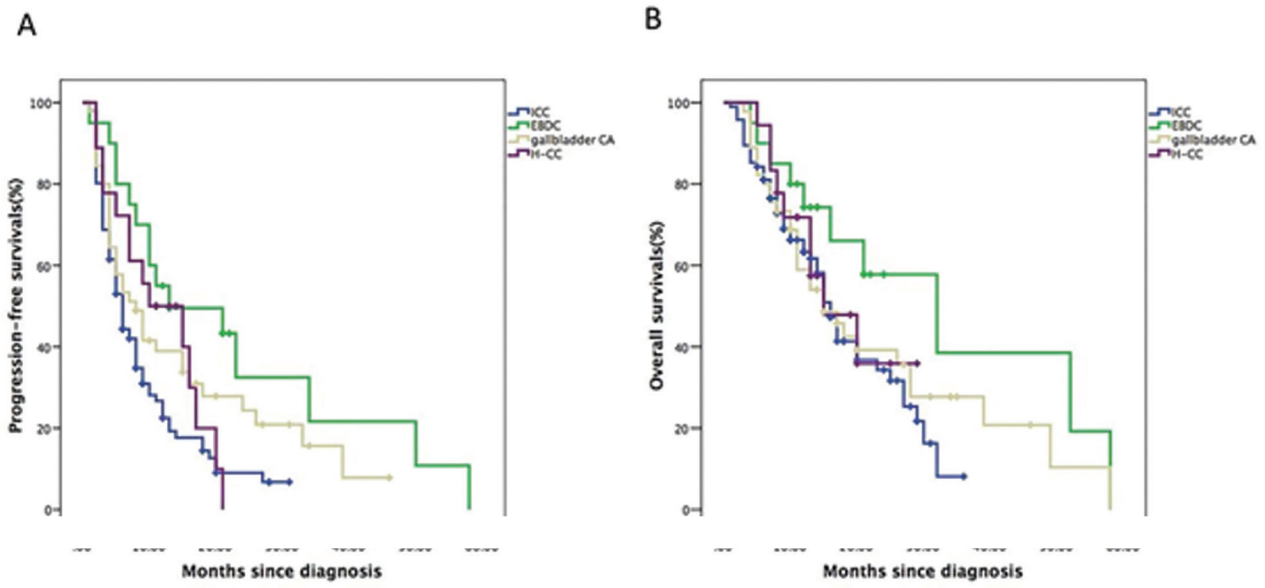


Table 2. Univariate and Multivariate Analyses of Clinical and Treatment Characteristics as Predictors of Progression-Free Survival

Characteristic		Univariate analyses		Multivariate analyses	
		HR	95% CI (P value)	HR	95% CI (P value)
Location	Intrahepatic cholangiocarcinoma	Reference		Reference	
	Extrahepatic bile duct carcinoma	0.39	0.22 to 0.72 (.002)	0.35	0.19 to 0.65 (.001)
	Hilar cholangiocarcinoma	0.67	0.44 to 1.00 (.05)	0.63	0.42 to 0.96 (.03)
	Gallbladder carcinoma	0.69	0.39 to 1.22 (.20)	0.67	0.38 to 1.19 (.17)
Differentiation	Moderate to high	Reference		Reference	
	Low	1.05	1.06 to 2.13 (.02)	1.52	1.05 to 2.19 (.03)
Lymph node metastasis	No	Reference			
	Yes	1.29	0.90 to 1.83 (.16)		
Distant metastasis	No	Reference			
	Yes	1.38	0.85 to 2.24 (.19)		
Chemotherapy	GP	Reference		Reference	
	Gemox	0.66	0.44 to 0.99 (.04)	0.67	0.44 to 1.03 (.07)
	Gs	2.63	1.14 to 6.04 (.02)	3.55	1.51 to 8.31 (.004)
	Sox	0.77	0.28 to 2.10 (.61)	0.58	0.21 to 1.60 (.30)

Abbreviations: CI, confidence interval; HR, hazard ratio; Gemox, gemcitabine + oxaliplatin; GP, gemcitabine + cisplatin; Gs, gemcitabine + tegafur, gimeracil, and oteracil potassium capsules; PFS, progression-free survival; Sox, tegafur, gimeracil, and oteracil potassium capsules + oxaliplatin.

Figure 2. Progression-Free and Overall Survival by Tumor Location. (A) Tumor location was a significant predictor of progression-free survival (P = .005). (B) Tumor location was not significantly associated with overall survival (P = .3).



Abbreviations: CA, cancer; EBDC, extrahepatic bile duct cancer; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma.

Table 3. Univariate and Multivariate Analyses of Clinical and Treatment Characteristics as Predictors of Overall Survival

		Univariate analyses		Multivariate analyses	
		HR	**95% CI (*P* value)**	**HR**	**95% CI (*P* value)**
Location	Intrahepatic cholangiocarcinoma	Reference			
	Extrahepatic bile duct carcinoma	0.51	0.25 to 1.04 (.06)		
	Hilar cholangiocarcinoma	0.85	0.54 to 1.34 (.48)		
	Gallbladder carcinoma	0.82	0.40 to 1.66 (.57)		
Differentiation	Moderate to high	Reference		Reference	
	Low	1.924	1.28 to 2.89 (.002)	1.92	1.28 to 2.89 (.002)
Lymph node metastasis	No	Reference			
	Yes	1.41	0.92 to 2.11 (.12)		
Distant metastasis	No	Reference			
	Yes	1.09	0.63 to 1.88 (.77)		
Chemotherapy	GP	Reference			
	Gemox	0.85	0.54 to 1.35 (.49)		
	Gs	1.60	0.64 to 4.00 (.31)		
	Sox	1.04	0.38 to 2.88 (.94)		

Abbreviations: CI, confidence interval; Gemox, gemcitabine + oxaliplatin; GP, gemcitabine + cisplatin; Gs, gemcitabine + tegafur, gimeracil, and oteracil potassium capsules; HR, hazard ratio; Sox, tegafur, gimeracil, and oteracil potassium capsules + oxaliplatin.

Table 4. Characteristics of Patients Treated With Anti–PD-1 Antibodies

Patient number	Tumor location	Sex	Age, y	Immuno-histochemical analysis	PCR-based analysis	TMB (mut/Mb)	Therapy	Efficacy	PFS	OS
1	Perihilar	Female	60	PD-L1*-positive (2%)	MSI-H	27.95	First-line: PD-1 antibody	PR	Not reached	Not reached
2	Perihilar	Female	61	PD-L1-negative	MSI-H	10	First-line: gemcitabine + oxaliplatin; Second-line: PD-1 antibody	First-line: PR; Second-line: PR	First-line: 9 mo; Second-line: not reached	Not reached
3	Intrahepatic	Male	64	PD-L1-negative	MSI-H	50.16	First-line: PD-1antibody	PR	Not reached	Not reached
4	Intrahepatic	Female	73	PD-L1-negative	MSI-H	32.1	First-line: PD-1 antibody	CR	Not reached	Not reached
5	Gallbladder carcinoma	Male	58	PD-L1-positive (20%)	MSI-H	Not available	First-line: gemcitabine and oxaliplatin; Second-line: PD-1 antibody	First-line: PD; Second-line: PD	First-line: 1.8 mo; Second-line: 1.7 mo	6.8 mo

Abbreviations: CR, complete response; Mb, megabase of DNA; MSI-H, microsatellite instability-high; mut, mutations; OS, overall survival; PCR, polymerase chain reaction; PD, progressive disease; PD-1, programmed death-1; PD-L1, programmed death ligand 1; PFS, progression-free survival; PR, partial response; TMB, tumor mutational burden.

(10%) carried isocitrate dehydrogenase 1 (*IDH1*) gene mutations. No significant associations were identified between these gene mutations and treatment response or survival in this patient population.

Therapeutic Efficacy of Anti–PD-1 Antibodies

Of the 59 patients with DNA sequencing results, 5 (8%) had MSI-H status and received anti–PD-1 antibodies as first-line treatment. Among the 5 patients treated with anti–PD-1 antibodies, 4 patients were responsive to the immunotherapy and remain so at the time of writing this manuscript writing; 1 patient achieved complete response, and 3 additional patients achieved partial response. However, 1 patient with gallbladder carcinoma did not respond to anti–PD-1 antibodies and died during the fourth cycle of immunotherapy. Patient characteristics and treatment outcomes for these patients are presented in Table 4.

DISCUSSION

In this study, approximately 87% of patients received a gemcitabine-based regimen as their standard first-line treatment for cholangiocarcinoma; second- and third-line treatments included fluorouracil-centered therapy and other therapies. Most patients who did not receive a first-line gemcitabine-based regimen had hepatocellular carcinoma and, therefore, were treated with radiotherapy and fluorouracil. At our center, the overall disease control rate for gemcitabine-based regimens as first-line therapy is 56%, which is lower than outcomes reported in the literature.[6,8] Several possible factors may explain this discrepancy. First, in real-world clinical practice, patients are not selected per inclusion criteria used in clinical trials. Second, some treatment-naive patients with possible sensitivity to gemcitabine chose fluorouracil-based therapies, resulting in a response rate of 60% in this chemotherapy subgroup. Predictive factors for sensitivity to gemcitabine-based regimens are not yet known. Our study found that patient sex, tumor differentiation, gene mutation, and disease stage were not associated with treatment outcomes.

Presently, 1 randomized, phase 2 clinical trial has published results on second-line therapy for cholangiocarcinoma[9]; published reports on further treatments are lacking. Our study demonstrated that either gemcitabine- or fluorouracil-based regimens can be used as second-line treatment when the other was used as the first-line treatment, although disease control rates dropped to approximately 42%-43% for these posterior-line therapy subgroups. Irinotecan combined with fluorouracil was used as a second-line regimen for 12% (10 of 84) of patients who received second-line treatment in our study, resulting in a disease control rate of 40% (4/10) that exceeded a previously reported disease control rate of 16.7% for this regimen.[10]

The median PFS after first-line therapy in our study was similar to that reported in previous trials. However, the median OS in our study was 17 months, which approaches the median OS reported elsewhere for patients treated with 3-drug combinations.[11] Multiple novel drugs, including tyrosine kinase inhibitors and anti–PD-1 antibodies, were used as posterior-line therapies in our study, which may explain the longer survival.

Data on risk and prognostic factors for advanced cholangiocarcinoma primarily are based on retrospective studies conducted in recent decades. Factors such as R0 resection (complete resection with no tumor remaining within 1 mm of resection margins), carbohydrate antigen 19-9 levels, tumor location and differentiation, and lymph node status have been found to be associated with survival outcomes,[12-14] which is consistent with our findings. Of the 4

types of cholangiocarcinoma represented among our study population, PFS was shortest for intrahepatic cholangiocarcinoma and longest for extrahepatic bile duct cancer, suggesting that tumor location may be a predictor of survival. Further analysis showed that all distal tumors in our study were locally advanced, whereas intrahepatic cholangiocarcinoma often metastasized to multiple organs. Our study also indicated the low tumor differentiation was a significant risk factor for poorer survival.

The genetic landscape of cholangiocarcinoma is highly diverse.[15-17] Our study found that the genes *TP53, PI3KCA,* RAS*, ERBB2,* and *IDH* were highly altered in this patient population. Another Chinese study showed that *TP53* mutation was associated with poor prognosis of cholangiocarcinoma.[13] In our study, however, neither *TP53* mutation nor other gene mutations were identified as predictors of poor prognosis, which may be due to the small number of affected patients.

It has been suggested that MSI-H status is a favorable predictor of response to anti–PD-1 antibody treatment for solid tumors.[18] Data on the prevalence of MSI-H status among patients with cholangiocarcinoma are rare. Limited retrospective studies have reported prevalences of MSI-H in the range of 1% to 5% and longer OS among patients with cholangiocarcinoma and MSI-H.[19-21] Prevalence of MSI-H status was relatively high among patients in our study (8%). Expression of programmed death ligand 1 is associated with MSI-H status[22] and is a promising predictive factor for efficacy of immunotherapy.[23,24] Additionally, a small number of case reports have indicated high tumor mutational burden is associated with increased sensitivity to anti–PD-1 antibodies in treatment of cholangiocarcinoma.[25-28]

Our study has several limitations. First, the retrospective design may introduce patient heterogeneity with respect to various therapies. Second, follow-up data were incomplete, resulting in censoring of some survival data.

CONCLUSIONS

Prognosis of cholangiocarcinoma in the palliative care setting remains poor. Multiple lines of therapy may improve patient survival. MSI-H status is a favorable predictor of response to immunotherapy when offered as treatment for cholangiocarcinoma. This large, single-center study conducted in China provided useful, real-world data on treatment modalities and their efficacy in patients with advanced cholangiocarcinoma.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to report relevant to this article.

AUTHOR CONTRIBUTIONS

TZ and CT made equal contribution to the manuscript. TZ, CT, GY, and XC were involved in data acquisition, analysis, and interpretation. ZY designed and supervised the study. TZ performed the statistical analysis. TZ and CT drafted the manuscript. TZ, CT, GY, XC, and ZY critically revised the manuscript. All authors contributed to analyzing data and drafting and revising the manuscript, gave final approval of the final version to be published, and have agreed to be accountable for all aspects of the work.

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