


Application of Immune Checkpoint Inhibitors in the Treatment of Cholangiocarcinoma

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Abstract

Cholangiocarcinoma is a general term for intrahepatic and extrahepatic malignant tumors deriving in the biliary system. According to the location, it is divided into intrahepatic cholangiocarcinoma, hilar cholangiocarcinoma, and distal cholangiocarcinoma. Progressive cholangiocarcinoma yields poor outcomes with radiotherapy; therefore, there is an urgent need for new therapeutic breakthroughs. Immune checkpoint inhibitor (ICI) therapy brings the treatment for cancer into a new field, with the use of drugs targeting PD-1/PD-L1 and CTLA-4 considerably extending the survival of patients with melanoma, lung cancer, and other solid tumors. The FDA has approved the application of pembrolizumab for solid tumors with high microsatellite instability and defective mismatch repair, including cholangiocarcinoma. Moreover, the combination of ICIs with chemotherapy and radiation therapy showed good promise. The aim of the present study was to review the application of ICIs in the treatment of cholangiocarcinoma and to summarize the reported individualized immunotherapy-based protocols and ongoing clinical trials for clinical reference.

Keywords

cholangiocarcinoma, immune checkpoint inhibitors, PD-1

Abbreviations

MSI-H, high microsatellite instability; ICIs, immune checkpoint inhibitors; GS, gemcitabine + cisplatin; MHC, major histocompatibility complex; dMMR, defective mismatch repair; TMB-H, high tumor mutation burden; OS, overall survival; DFS, disease-free survival; HLA, human leukocyte antigen; BTC, biliary tract carcinoma; CR, complete remission; PR, partial remission; TMB, tumor mutation burden; MSS, microsatellite stability; pMMR, normal mismatch repair; SOX, Oxaliplatin + Tegafur; ORR, objective response rate; PFS, progression free survival; TACE, transhepatic arterial chemotherapy and embolization; ECC, extrahepatic cholangiocarcinoma.

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Introduction

Cholangiocarcinoma is a malignant tumor originating from the intrahepatic biliary system. It can be divided into intrahepatic cholangiocarcinoma, hilar cholangiocarcinoma, and distal cholangiocarcinoma according to the location.^{1,2} In general, the incidence of cholangiocarcinoma is low worldwide (6/100,000), accounting for ~3% of all gastrointestinal tumors. Due to the high degree of malignancy and rapid progress, however, the median survival time of patients is less than 2 years.^{1,3} Because of the different anatomical locations and predisposing factors of intrahepatic and extrahepatic cholangiocarcinoma, there are substantial differences in the oncology characteristics. Moreover, the epidemiological data show that there are huge geographic differences in the incidence

and subtypes of cholangiocarcinoma. For example, in countries and regions such as Chile, Bolivia, South Korea, and northern Thailand, the incidence is much higher than the world average, which indicates that the reason might be correlated with race, genetics, and exposure to different risk factors.⁴ The incidence of intrahepatic cholangiocarcinoma has been

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on the rise globally since the end of the last century and has reached a plateau in the last decade. In contrast, the incidence of hilar and distal cholangiocarcinoma has been slowly declining in recent years.² Most patients with cholangiocarcinoma have no obvious clinical symptoms before the occurrence of bile duct obstruction, lack highly specific serum tumor markers, and have atypical image features. Consequently, many patients are found to have metastasis at the time of diagnosis.⁵⁻⁷

In terms of treatment, surgery is the most effective treatment available. The location of the tumor, vascular, or lymphatic invasion, and distant metastasis are factors that affect whether or not the tumor can be surgically removed. Even if the patient undergoes surgical resection, however, there is still a high probability of relapse.^{8,9} Concerning chemotherapy, the gemcitabine + cisplatin (GS) regimen is currently used as the standard first-line chemotherapy for progressive cholangiocarcinoma based on the results of the phase III clinical trial ABC-02. Recent results from the phase III clinical trial FUGA-BT showed that gemcitabine + S1 was not inferior to the GS regimen.¹⁰ The results of the phase II clinical trial of gemcitabine + cisplatin + S1 in Japan showed prolonged median survival and 1-year survival in patients who undertook the gemcitabine + cisplatin + S1 regimen compared with those who undertook the GS regimen. Furthermore, it is expected that the results of the phase III MITSUBA clinical trial will be obtained in the future.^{11,12} Although there is no standard second-line treatment for cholangiocarcinoma, fluorouracil is the most common clinical choice. In terms of the targeted agent, the development of pemigatinib, an FGFR inhibitor, brings hope to patients with cholangiocarcinoma.¹³ In addition, IDH1/2, BRAF, BRCA1/2, ATM, and PIK3CA mutations and c-MET and HER2/neu overexpression are potential therapeutic targets.^{10,14}

Immune checkpoint inhibitors (ICIs) are a hot topic in the field of tumor therapy and have made great breakthroughs in the treatment of tumors of different tissue and organ origins, such as lung cancer and melanoma. In the case of hepatic cancer, the FDA approved nivolumab for patients with advanced hepatocellular carcinoma resistant to sorafenib in 2017 based on the results of the CheckMate-040 study. Subsequently, in November 2018, the FDA approved pembrolizumab for the treatment of advanced hepatocellular carcinoma. Currently, clinical studies of ICIs associated with intrahepatic and extrahepatic cholangiocarcinoma are ongoing. Several case reports and clinical trials suggest that ICIs are effective in the treatment of genotype-specific cholangiocarcinoma.

Tumor Immunity and Related Gene Expression in Cholangiocarcinoma

The processes in which the body recognizes and kills tumor cells are as follows. The tumor cells release tumor-related antigens. Antigen-presenting cells, such as macrophages and

dendritic cells, capture, and recognize the tumor-related antigens. The antigen-presenting cells present the antigens to T cells through the major histocompatibility complex (MHC) molecules. T cells are activated and migrate to the location of the tumor by circulation. The effector T cells infiltrate the tumor tissues and recognize and kill the tumor cells. The killed tumor cells release tumor antigens and continue to activate the immune system with positive feedback. PD-1 is a programmed cell death protein expressed on the surface of the activated T cells and is normally used for self-protection of the body. PD-1 initiates the programmed death of the activated T cells by binding to its ligand, PD-L1, preventing overactivation of T cells, which results in autoimmune disease. However, by expressing PD-L1, the tumor cells and tumor-associated macrophages allow the programmed death of the effector T cells in the tumor, thus realizing the immune escape. CTLA-4 is a CD28 homolog expressed in the activated T cells to inhibit T cell activation by competing for binding of the CD28 ligand, CD80/86. Blocking the CTLA-4 signaling pathway considerably enhances the immune effect and reduces the suppressive state of T cells.

Currently, ICIs are divided into the following categories: (1) PD-1 inhibitors such as camrelizumab, nivolumab, pembrolizumab, and toripalimab; (2) PD-L1 inhibitors such as avelumab, atezolizumab, and durvalumab; and (3) CTLA-4 inhibitors, including ipilimumab and tremelimumab. In 2017, the FDA approved the application of pembrolizumab for the treatment of any solid tumors with specific genetic characteristics (high microsatellite instability [MSI-H] and defective mismatch repair [dMMR]), including cholangiocarcinoma. It is currently believed that patients with solid tumor positive for PD-L1, MSI-H, dMMR, and high tumor mutation burden (TMB-H) may respond well to immunotherapy.^{15,16}

PD-1 and PD-L1 are targets of ICIs. Dirk et al. reported that the positivity of PD-L1 in extrahepatic cholangiocarcinoma in the Western population was ~11.6%.¹⁷ However, due to differences in race, environment and other factors, the positivity of PD-L1 in the Asian population is quite different to that in the Western population.¹⁸ Takashi et al. reported that the positivity of PD-L1 in Japanese patients with cholangiocarcinoma was 36% to 45% and that the expression of PD-L1 in tumor tissues was significantly higher than that in adjacent normal tissues.^{19,20} Some researchers have also reported that the PD-L1 positivity in patients with cholangiocarcinoma in China is 32.3% to 42.86%.^{21,22} In addition to tumor cells, tumor-associated macrophages showed positivity of PD-L1 expression as high as 30.4% to 74.2%,^{17,22} suggesting that these patients may have good responsiveness to the PD-L1 inhibitors.

Considering the immune escape mechanism, high expression of PD-L1 may be correlated with rapid tumor progression and poor prognosis.²³ A meta-analysis of 11 studies on cholangiocarcinoma and PD-1 showed that expression of PD-L1 in cholangiocarcinoma tumor cells was closely correlated with the TNM staging. High expression of PD-L1 indicated a low

trend in overall survival (OS), while in terms of disease-free survival (DFS), high PD-L1 expression indicated a shorter DFS duration.²⁴ Similarly, it was reported by Ma et al. that PD-L1 positivity was associated with high TNM staging, lymphatic metastasis, and poor prognosis.²¹

However, there are still many contradictory experimental conclusions. Dirk et al. reported that PD-L1 had no effect on the prognosis of patients.¹⁷ In addition, there are some opposite results. Yu et al. reported that the infiltration density of CD3⁺ T cells and CD8⁺ TIL cells in the PD-L1 positive patients was higher and that the PD-L1-positive tumors had less venous invasion and longer progression free survival (PFS) and OS.²² The discrepancy in these results may be due to the insufficient sample size, the differences in the application of PD-1/PD-L1 antibodies in the immunohistochemistry, and the differences in the criteria for determining positive.

The immune response of the tumor microenvironment is diverse and complex, and it is difficult to use PD-L1 expression as a single prognostic factor. Moreover, MHC expression, MSI, dMMR, and lymphocyte infiltration are guiding indicators for the prognosis of the patient and targeted PD-1 therapy.²⁵ The results of a recent genomic study that included 500 cases of cholangiocarcinoma suggest that upregulation of the individual immune checkpoint genes, with the exception of PD-1/PD-L1, is associated with a favorable prognosis.²⁶ MHC, called the human leukocyte antigen (HLA) in humans, is an important molecule in the process of antigen presentation. HLA class I determines the recognition of tumor cells by the killer T cells, and 50% of extrahepatic cholangiocarcinomas lack the expression of HLA class I.²⁷ In tumors with high HLA class I expression, the proportion of CD8⁺ killer T cells was significantly higher than that in the low expression group. By immunohistochemistry, the percentage of HLA class I deletion in the intrahepatic cholangiocarcinoma specimens is as high as 60% and the positivity of PD-L1 is 30%. HLA class I deletion together with significantly shorter survival in PD-L1-positive patients^{22,28} suggests that ICIs may work better in patients with HLA class I deletion and high expression of PD-L1. In addition, the tumor MSI can be used as a guiding marker for PD-1/PD-L1 targeted therapy in cholangiocarcinoma. However, some studies have highlighted that the percentage of high MSI (MSI-H) in cholangiocarcinoma is extremely low at 1.3% to 10.0%.²⁴ Recently, some researches pointed out the important role of DNA damage repair genes in biliary tract cancer (BTC). These studies demonstrated the possibility of Poly(ADP-ribose)polymerase inhibitors to treat BTC patients with BRCA-mutation, which showed the great promise to improve survival results.^{29,30}

Individualized Therapeutic Regimens for ICIs in the Treatment of Cholangiocarcinoma

The results of RCTs of the ICIs for cholangiocarcinoma have not been reported. However, there have been some case reports of advanced unresectable or recurrent metastatic

cholangiocarcinoma with complete remission (CR) or partial remission (PR) achieved by immunotherapy, and these individualized regimens provide important references for the treatment of patients with advanced cholangiocarcinoma.

Long-term occupational exposure to 1,2-dichloropropane and methylene chloride predisposes individuals to cholangiocarcinoma. The Japanese Institute of Cancer Research defines it as “occupational cholangiocarcinoma”.³¹ Whole-exome sequencing showed that patients with occupational cholangiocarcinoma had an average of 44.8 mutations per Mb, about 30 times more than the other types of cholangiocarcinoma. In addition, in occupational cholangiocarcinoma, most tumor cells express PD-L1.³² Tanaka et al. reported a case of PD-L1-positive occupational cholangiocarcinoma that progressed after S-1 + gemcitabine + cisplatin chemotherapy and achieved CR with the regimen of nivolumab + gemcitabine + cisplatin³¹ (Table 1). Similarly, a study reported that a patient with advanced metastatic intrahepatic cholangiocarcinoma with 80% of PD-L1 and 19.3 mutations per Mb of TMB, but with microsatellite stability (MSS) and normal mismatch repair (pMMR), achieved CR after chemotherapy with the regimen of pembrolizumab + SOX. Samstein reported data in several cancer types suggesting that TMB correlates with clinical response to ICIs. The top 20% TMB in each cancer type associated with better prognosis.³³ Recently, a molecular profiling of BTC revealed that 23.4% (352/1502) of BTC have TMB profiled and ~4% (14/352) of them were TMB-H with a cutoff value of 17 somatic missense mutations/MB.³⁴ Keynote-028 also revealed that TMB is associated with higher response rate in Pembrolizumab treatment cohort including BTC (5/23).³⁵

The base insertion/deletion mutations (indels) tend to produce large amounts of high HLA class I affinity tumor antigens. Sui et al. reported that 2 patients with recurrent stage IIIb intrahepatic cholangiocarcinoma, with the sequence results of low expression of MSS, pMMR and PD-L1, together with TMB-low but high base indels, achieved CR for up to 13 and 16 months with the therapy of pembrolizumab plus Tegafur, respectively³⁶ (Table 1).

Furthermore, Liu et al. reported 3 cases of achieving PR or CR with PD-1 targeted therapy + stereotactic radiotherapy in patients with low TMB, low MSI, pMMR, and PD-L1 negativity.¹⁵ It was speculated that the mechanism might be that after killing the tumor cells through stereotactic radiotherapy, a large number of tumor antigens were released, which to some extent would activate the antitumor immunity, and with PD-1 targeted therapy, good results are achieved. This provides new ideas for the combination therapy of PD-1 drugs.

It was suggested in these case reports that immunotherapy for cholangiocarcinoma might not be limited to patients with PD-L1 positivity, MSI-H, dMMR, and TMB-H.

Clinical Trial of ICIs in Cholangiocarcinoma

ICIs Monotherapy

Currently, numerous clinical trials of immunotherapy for cholangiocarcinoma are underway (Table 2), including the phase I

Table 1. Individualized Therapeutic Regimens for ICIs in the Treatment of Cholangiocarcinoma.

Types of Tumors	Therapeutic Effect	Relief Time	Therapeutic Protocol	Genetic Characteristics
iCCA	CR	—	Pembrolizumab (150 mg q3w) + [SOX] (oxaliplatin 130 mg/m ² , d1, tegafur 60 mg BID, d1-14, q3w) 4 cycle	MSS, pMMR, PD-L1 80%, TMB 19.3 mut/Mb
iCCA	CR	16 months	Pembrolizumab (150 mg, q3w, 15 cycle) + Tegafur(40 mg BID, q3w, 3 cycle)	MSS, pMMR, PD-L1 low, TMB 2.95 mut/Mb, 48%
iCCA	CR	13 months	Pembrolizumab (150 mg, q3w, 6 cycle) + Tegafur(40-60 mg BID,q3w 5 cycle)	MSS, pMMR, PD-L1 low, TMB 7.09 mut/Mb, Insertion-deletion mutation : 66.87%
iCCA	PR	7 months	Nivolumab (200 mg, q2w, 15 cycle) + SBRT (Cyberknife 55Gy/5F)	MSS, pMMR, PD-L1 <1%, TMB 1.2 mut/Mb
iCCA	PR	5 months	Pembrolizumab (5 cycle) + SBRT (Cyberknife 52Gy/4F)	MSS, pMMR,PD-L1 <1%, TMB of 3.8 mut/Mb
iCCA	CR	11 months	Pembrolizumab (200 mg, q3w, 16 cycle) + SBRT (Cyberknife 52Gy/4F) + Furfurcil (6 cycle) + recombinant human endostatin (4 cycle)	MSS, pMMR,PD-L1 <1%, TMB : 0.98 mut/Mb
iCCA	CR	12 months	Nivolumab (3 mg/m ² , q2w, 16 cycle + q3w, 6 cycle) + GEM + CIS	TMB 44.8 mut/Mb
iCCA	CR	—	Nivolumab (3 mg/kg, q2w,20 months) + lenvatinib (10 mg/day,20 months)	dMMR, MSI-H, PD-L1 <1%, TMB 18.46 mut/Mb

Abbreviations: SBRT, stereotactic radiotherapy; MSS, microsatellite stabilization; dMMR, defective mismatch repair; pMMR, mismatch repair normal; TMB, tumor mutation load; CR, complete remission; PR, partial remission.

Table 2. Clinical Trial of ICIs in Cholangiocarcinoma.

Code	Pharmacy	Target	Type	Regimen
NCT04068194	Avelumab	PD-L1	I/II	Hypofractionated RT + avelumab VS hypofractionated RT + nedisertib + avelumab
NCT03201458	Atezolizumab	PD-L1	II	Atezolizumab VS atezolizumab + cobimetinib
NCT03267940	Atezolizumab	PD-L1	Ib	PEGPH20 + Gemcitabine + Cisplatin VS PEGPH20 + Atezolizumab + CIS + GEM VS CIS + GEM
NCT04157985	Atezolizumab Ipilimumab	PD-L1 CTLA-4	III	Continuous PD-1/PD-L1 inhibitor VS discontinuous PD-1/PD-L1 to 1 inhibitor
NCT03991832	Durvalumab	PD-L1	II	Olaparib VS Durvalumab
NCT03257761	Durvalumab	PD-L1	Ib	Durvalumab + Guadecitabine
NCT03473574	Durvalumab Tremelimumab	PD-L1 CTLA-4	II	Durvalumab + Tremelimumab + Gem VS Durvalumab + Tremelimumab + Gemcitabine + Cisplatin VS Gemcitabine + Cisplatin
NCT03473574	Durvalumab Tremelimumab	PD-L1 CTLA-4	II	Durvalumab + Tremelimumab VS Durvalumab + Tremelimumab + TACE VS Durvalumab + Tremelimumab + RFA VS Durvalumab + Tremelimumab + Cryo
NCT03898895	Camrelizumab	PD-1	II	Radiotherapy + Camrelizumab VS Gemcitabine + Cisplatin
NCT03250273	Nivolumab	PD-1	II	Nivolumab VS Entinostat
NCT03684811	Nivolumab	PD-1	I/II	Nivolumab VS FT-2102
NCT03872947	Nivolumab	PD-1	I	TRK-950 + Nivolumab VS TRK-950 + Pembrolizumab
NCT02834013	Nivolumab Ipilimumab	PD-1 CTLA-4	II	Nivolumab + ipilimumab VS nivolumab
NCT02982720	Pembrolizumab	PD-1	II	Pembrolizumab + Sylatron
NCT03895970	Pembrolizumab	PD-1	II	Lenvatinib + Pembrolizumab
NCT03111732	Pembrolizumab	PD-1	II	Pembrolizumab + Oxaliplatin + Capecitabine
NCT03849469	Pembrolizumab	PD-1	I	XmAb@22841 Monotherapy VS XmAb@22841 + Pembrolizumab
NCT03937895	Pembrolizumab	PD-1	I/II	Allogeneic NK Cell (“SMT-NK”) + Pembrolizumab
NCT04003636 (KEYNOTE-966)	Pembrolizumab	PD-1	III	Pembrolizumab + Gemcitabine + Cisplatin VS Placebo + Gemcitabine + Cisplatin
NCT02628067 (KEYNOTE-158)	Pembrolizumab	PD-1	II	Pembrolizumab
NCT03982680	Toripalimab	PD-1	II	Toripalimab + Gem/5-FU
NCT03867370	Toripalimab	PD-1	I/II	Toripalimab

studies of the safety and efficacy of PD-1/PD-L1 monoclonal antibody drugs, NCT03991832, NCT03250273, and NCT02628067 (KEYNOTE-158), together with the combination immunotherapy of PD-1/PD-L1 monoclonal antibody with CTLA-4 monoclonal antibody. As data shown by NCT02628067 (KEYNOTE-158), among 104 enrolled advanced BTC patients treated with Pembrolizumab, the ORR was 5.6% and 12 months OS rate was 32.7%, despite of the PD-L1 expression level, revealing a durable antitumor activity and tolerable adverse events.³⁷ Another clinical trial enrolled BTC patients who progressed on at 1 line but no more than 3 lines of systemic therapy. After nivolumab treatment, 22% and 37.8% achieved PR and SD, respectively. The median OS reaches 14.22 months in evaluable patients.³⁸ However, Ueno et al.³⁹ reported a conflicting median OS of 5.2 months with nivolumab monotherapy. Phase I clinical trial (NCT01938612) reported and median duration of response of 9.7 months and median OS of 8.1 months for Durvalumab monotherapy.⁴⁰ Further studies on Durvalumab and Tremelimumab are listed in Table 2. Promising benefits are observed in ICIs monotherapy despite of the PD-1/PD-L1 and MMR/MSI status.

ICIs Combination Therapies

Pembrolizumab in combination with GM-CSF, a macrophage modulator, showed a PR of 21% and a minor regression 2 MSS patients for 11 and 16 months.⁴¹ Chemotherapy is believed to upregulate the effect of ICIs. Nivolumab in combination with gemcitabine plus cisplatin provided an ORR of 36.7% and median OS of 15.4 months.³⁹ In Lenvatinib plus Pembrolizumab or Nivolumab cohort, PR and DCR was 21.4% and 92.9%, respectively. Median PFS was 5.9 months.⁴²

Nivolumab, a PD-1 inhibitor, can outperform the single drug immunotherapy when in combination with the CTLA-4 inhibitor ipilimumab. Data from the phase III study of nivolumab + ipilimumab versus sunitinib in patients with intermediate- and high-risk advanced kidney cancer in IMDC prognostic evaluation reported in the ESMO meeting in 2017 revealed that the combination of nivolumab + ipilimumab outperformed the current standard therapy with sunitinib in terms of PFS, ORR, and OS. Moreover, according to 2 clinical studies, CheckMate 069 and CheckMate 067, it was suggested that the combination of nivolumab + ipilimumab might prolong the survival in progressive melanoma. The NCT02834013 trial aimed to investigate the therapeutic effect of nivolumab + ipilimumab in cholangiocytic carcinoma. In addition, the NCT04157985 trial aimed to determine the therapeutic effect of the PD-L1 monoclonal antibody atezolizumab in combination with ipilimumab. The CTLA-4 monoclonal antibody tremelimumab may inhibit the progression of hepatitis C-associated advanced hepatic cancer for more than 12 months. Two studies, NCT03473574 and NCT03473574, were designed to explore the effectiveness of the PD-1 monoclonal antibody durvalumab in combination with the CTLA-4 monoclonal antibody tremelimumab in the treatment of advanced unresectable biliary

malignancies in combination with first-line chemotherapy or TACE.

Moreover, the phase III clinical trial KEYNOTE-966 is studying the PD-1/PD-L1 monoclonal antibody in combination with the first-line chemotherapy gemcitabine + cisplatin versus placebo + gemcitabine + cisplatin. This randomized, controlled, double-blind trial started on September 24, 2019 and included 788 patients with biliary tumors, with pembrolizumab + gemcitabine + cisplatin in the experimental group and placebo + gemcitabine + cisplatin in the control group. The primary endpoint was PFS and the secondary endpoint was OS. The aim of the study was to explore the potential of pembrolizumab in combination with gemcitabine + cisplatin as the first-line treatment for biliary malignancies (Table 2).

As note that 11.4% of all BTCs have at least 1 of the 3 predictive markers (PD-L1, MSI-H, and TMB-H) associated with ICIs response. While considering the subtypes, ICC has a much higher prevalence than ECC (13.0% vs 6.9%).³⁴ Several case reports with inspiring prognosis have been discussed previously in this paper, and most of them are ICC patients (Table 1). All these indicate that ICC may have a better clinical response with ICIs treatment. Further study is needed to verify the differences between these 2 subtypes.

Summary and Perspective

Immunotherapy has brought revolutionary changes in the field of cancer treatment, bringing cancer treatment into the era of individualized treatment based on the genetic characteristics of tumors rather than the origin of tumor tissue. In melanoma, lung cancer, kidney cancer, and hepatic cancer, immunotherapy has produced breakthroughs; however, there are still many problems to be solved in cholangiocarcinoma. Whether the differences in the prevalence and genetic characteristics of different populations will affect the results of clinical trials had not yet been ascertained. For example, an integrated genomic and transcriptomic analysis of hepatic tumors (hepatocellular carcinoma and cholangiocellular carcinoma) in Asians and Caucasians showed greater T-cell infiltration in tumors in Asians, suggesting that tumor immunotherapy might have better prospects in the Asian population. Whether the difference in the genetic characteristics of different tumor locations, such as intrahepatic, hilar, and distal cholangiocarcinoma, will affect the tumor's response to immunotherapy remains unclear. Further research is required to determine whether there are completely different genetic characteristics in cholangiocarcinoma caused by different underlying diseases, such as hepatitis-related diseases, bile duct stones, and congenital cystic dilatation of the bile duct. These are factors that affect the immunotherapy and may need to be further subdivided for more precise clinical trials to be conducted in the future.

Currently, most clinical trials indicate that targeted drugs for cholangiocarcinomas, such as EGFR inhibitors, VEGF inhibitors, VEGFR inhibitors, multikinase inhibitors, and MEK inhibitors, have little effect. However, the progress of FGFR kinase inhibitors has been very effective. About 11% of patients

with intrahepatic cholangiocarcinoma have FGFR gene fusions, such as FGFR2–BICC1 (REF. 79), FGFR2–KIAA1598 (REF. 80), FGFR2–TACC3 (REF. 80), FGFR2–AHCYL1 (REF. 78), FGFR2–MGEA5 (REF. 68), FGFR2–KCTD1, and FGFR2–TXLNA29. The fusion genes can promote the activation of FGFR kinase, thereby changing the cell morphology and increasing cell proliferation.⁴³ However, this change has not yet been found in other hepatic tumors. The FDA has announced accelerated approval of the FGFR2 inhibitor pemigatinib for the treatment of advanced cholangiocarcinoma carrying the FGFR2 fusion gene. The BRAF mutations account for about 5% of intrahepatic cholangiocarcinoma. Studies have reported that the combination of dabrafenib and trametinib is effective in patients with BRAF V600E mutation.^{44,45} The combination of these new targeted drugs and immunotherapy is promising.

Despite that present available predictive markers are relatively low in BTCs compared with other solid tumors, ICIs monotherapy has shown durable results, and combination therapy either with chemotherapy or local therapy also provided inspiring outcomes. Further efforts should be made on developing new predictive markers. Adjuvant therapy based on ICIs and chemotherapy may shade light on BTC postsurgery treatment. Attempts of ICIs based neoadjuvant therapy (eg, Lenvatinib plus PD-1 inhibitor) on BTC especially on ICC have also showed a high response rate and resection rate. We believe that the application of ICIs will expand from advance BTC chemotherapy to preoperation neoadjuvant therapy and bring sweeping change on BTC treatment.


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