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Review Article

Horizons on the Therapy of Biliary Tract Cancers: A State-of-the-art Review

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Abstract

Biliary tract cancers (BTCs) comprise a group of heterogeneous poor prognosis cancers with increasing incidence recent years. The combination chemotherapy with cisplatin and gemcitabine is the first-line therapy for advanced BTC. There remains no accepted standard treatment in the second-line setting. Nowadays, more and more novel treatment strategies have entered development, with some encouraging results being seen. Here, we review the current treatment status and clinical characteristics of BTC, the role of immunotherapy in BTC as well as the design of clinical trials for oncology drugs for BTC which aim to focus on the future profiles of clinical care and resolution of BTC.

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Introduction

Biliary tract cancer (BTC) is a kind of malignant tumor arising from epithelial cells of the biliary system. According to different origins, it is divided into intrahepatic cholangiocarcinoma (ICC), perchilar/hilar cholangiocarcinoma (PCC), extrahepatic cholangiocarcinoma (ECC) and gallbladder cancer (GBC).[1] The histology of BTC is mainly adenocarcinoma. Surgery is the only curable technique available for BTC. However, more than 65% of patients with BTC are unable to undergo radical surgical resection when they are discovered, with a 5-year survival rate of about 5–15% and a recurrence rate of 67% in 1 year after operation.[2,3] In the absence of surgery, BTC is not sensitive to traditional chemotherapy. Gemcitabine plus cisplatin (GC) is the first-line standard chemotherapy for advanced BTC.[3] Morizane *et al*. confirmed that gemcitabine plus S-1 (GS) is not inferior to GC in terms of overall survival rate, and recommended GS as a new choice for first-line treatment of BTC.[4] However, the survival benefits of chemotherapy with either GS or GC are still limited, and the median survival time is only about 12 months. Therefore, it is urgent to develop new clinical strategies for the treatment of BTC.

Keywords: Biliary tract cancers; Immunotherapy; Clinical trials.

Abbreviations: ACT, adoptive cell transfer therapy; BTC, biliary tract cancer; CAR-T, chimeric antigen receptor T lymphocyte; CIK, cytokine-induced killer; ECC, extrahepatic cholangiocarcinoma; ECOG, Eastern Cooperative Oncology Group; EMEA, European Medicines Agency; EORTC, European Organization for Cancer Therapy Research; ERCP, endoscopic retrograde cholangiopancreatography; FDA, Food and Drug Administration; JCOG, Japanese Clinical Oncology Cooperative Organization; GBC, gallbladder cancer; GC, gemcitabine plus cisplatin; GS, gemcitabine plus S-1; HR, hazard ratio; ICC, intrahepatic cholangiocarcinoma; LAK, lymphokine-activated killer cells; MMR, mismatch repair; MRCP, magnetic resonance cholangiography; MSI, microsatellite instability; PCC, perchilar/hilar cholangiocarcinoma; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; PTC, percutaneous transhepatic cholangiography; SWOG, Southwest Oncology Cooperative Group; TCR-T, T lymphocyte receptor chimeric T lymphocyte; TIL, tumor infiltrating lymphocytes.

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Current treatment status and clinical characteristics of BTC

GBC

GBC is the most aggressive and most common type of BTC, and the majority of cases represent adenocarcinomas. Its incidence increases with age, and the incidence in women is higher than that in men, especially for white women. GBC generally occurs locally, easily invades blood vessels, and is prone to local or extensive lymph node metastasis and distant metastasis. The clinical manifestations are similar to biliary colic or chronic cholelithiasis, so it is usually discovered at an advanced stage when it is diagnosed. Based on the data from 177 patients who underwent potentially curative resection (GBC: n=97; PCC: n=80), the median time to disease recurrence was shorter for patients with GBC compared with patients with PCC (11.5 vs. 20.3 months; p= 0.007). In total, 52 (68%) of the patients with PCC and 53 (66%) of the patients with GBC had disease recurrence at a median follow-up of 24 months. It was indicated that compared with PCC, patients with GBC have a shorter median survival time and are prone to recurrence; the survival time after recurrence is shorter as well.[5]

For patients with jaundice, if GBC is suspected, surgery must be done for the purpose of treatment. It is recommended that multidisciplinary consultations evaluate the possibility of surgery first. The assessment should include

Table 1. The summary the surgical/non-surgical treatment plans for different types of BTC

BTC type	Resectable	Unresectable
GC	Cholecystectomy + en bloc hepatic resection + lymphadenectomy ± bile duct excision for malignant involvement	GC combination therapy Fluoropyrimidine-based or other gemcitabine-based chemotherapy regimen EBRT with concurrent fluoropyrimidine Radiation therapy Clinical trial Best supportive care Pembrolizumab (only for MSI-high/MMR defect tumors)
ICC	Consider staging laparoscopy Resection Consider lymphadenectomy for accurate staging	GC combination therapy Clinical trial Fluoropyrimidine-based or other gemcitabine-based chemotherapy regimen EBRT with concurrent fluoropyrimidine Consider locoregional therapy Radiation therapy Arterially directed therapies Best supportive care Pembrolizumab (only for MSI-high/MMR defect tumors)
ECC	Surgical exploration Consider laparoscopic staging Consider preoperative biliary drainage Multidisciplinary review	GC combination therapy Clinical trial Fluoropyrimidine-based or other gemcitabine-based chemotherapy regimen EBRT with concurrent fluoropyrimidine Radiation therapy Pembrolizumab (only for MSI-high/MMR defect tumors) Best supportive care

cholangiography to determine the degree of tumor invasion to the hepatobiliary system, with non-invasive magnetic resonance cholangiography (MRCP) being preferred and the second choice being endoscopic retrograde cholangiopancreatography (ERCP) or percutaneous transhepatic cholangiography (PTC).[6]

For operable patients, biliary drainage should be considered before surgery. Cholecystectomy, hepatectomy and lymph node dissection with or without bile duct resection are performed, combined with adjuvant treatment and monitoring after surgery. It is worth noting that GBC with jaundice usually indicates a poor prognosis, so the possibility of surgery needs to be carefully evaluated.[7] For inoperable patients, biliary drainage should be performed before chemotherapy. The chemotherapy regimen can involve GS, fluorouracil or gemcitabine-based chemotherapy, combined with radiotherapy, clinical trials and supportive care.

ICC

Patients with ICC usually have no specific clinical manifestations and generally do not have symptoms of bile duct obstruction. They are often found incidentally due to a solitary mass on the liver being found upon imaging examination. Although most patients are diagnosed with advanced disease and are not suitable for surgery, complete resection is still the only curative method for patients with ICC.

For isolated intrahepatic masses, if the imaging examination findings are consistent with adenocarcinoma, it is recommended to conduct a multidisciplinary assessment immediately to determine the possibility of surgery. For operable patients, the presence of multiple liver lesions, lymph node metastasis or distant metastasis should be evaluated before surgery, since lymph node metastasis and distant metastasis beyond the hepatic hilar are contraindications to surgical resection. Partial hepatectomy is the surgical option, and while hepatectomy is usually performed, as long as the margin is negative, liver wedge resection, segmentectomy and extended resection can also be considered. It is worth noting that hilar lymph node dissection is reasonable, because it can not only provide staging information of cholangiocarcinoma but also assess the prognosis to a certain extent. However, lymph node metastasis to the hilar is usually related to a poor prognosis, and resection must be performed on highly specific patients. Patients should receive adjuvant treatment and monitor changes in their condition after surgery. For inoperable patients, GC chemotherapy, clinical trials, fluorouracil or gemcitabine-based chemotherapy, fluorouracil chemotherapy and radiotherapy, local treatment and supportive treatment could be used.

The tumor size of ICC has no significant effect on the survival rate after surgery. The influential factors include the number of tumors, vascular invasion and the status of lymph nodes. Furthermore, the number of tumors and vascular invasion only have guiding significance at N0.[8]

ECC

Patients with ECC often have symptoms of bile duct obstruction, such as jaundice, pain, and abnormal liver function, followed by abnormal lesions on imaging examination. The radical treatment for ECC is to completely remove the lesion and ensure that the margin is negative. The 5-year survival rates for hilar cholangiocarcinoma and distal cholangiocarcinoma undergoing radical resection are 20–40% and 16–52%, respectively.[9] When the above-mentioned clinical manifestations occur, it is recommended to conduct a multidisciplinary assessment immediately to determine whether there is a possibility of surgery.

The radical treatment for extrahepatic cholangiocarcinoma involves complete removal of the lesion and provision of negative resection margins. The 5-year survival rates

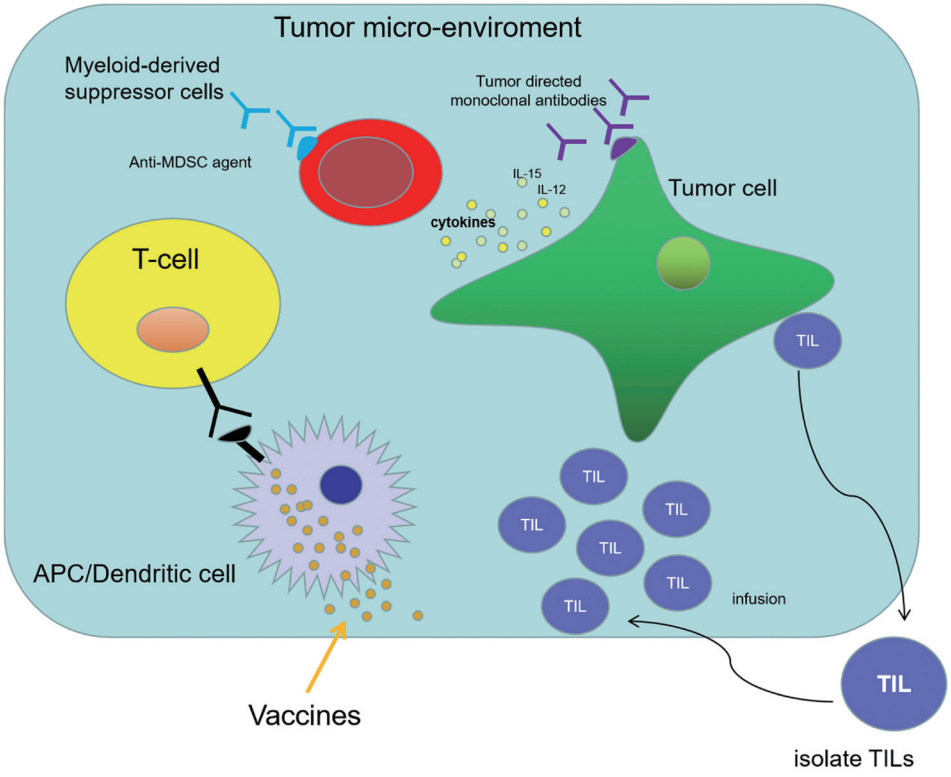

Fig. 1. Cancer immunotherapy approaches in BTC.

of hilar cholangiocarcinoma and distal cholangiocarcinoma undergoing radical resection are 20–40% and 16–52%, respectively.[9] In the presence of the above clinical manifestations, a multidisciplinary assessment is recommended immediately to determine the possibility of surgery.

For nonoperative patients, biliary drainage is recommended, referral to a transplant center if suitable for transplantation, or needle biopsy if not, followed by GC chemotherapy, clinical trials, fluorouracil or gemcitabine-based chemotherapy, fluorouracil radiotherapy, and supportive care. For operable patients, preoperative laparoscopic determination of staging and biliary drainage can be considered. For nonresectable patients found after intraoperative exploration, the treatment is the same as above. For resectable patients, surgical treatment can be performed, and postoperative adjuvant treatment and monitoring can be performed. For patients with metastases, it is recommended to use surgical bypass or endoscopy (such as ERCP) or percutaneous methods (such as PTC) for biliary drainage. Most patients often receive biliary stent implantation and biopsy at the same time. After the diagnosis of cholangiocarcinoma, the treatments are GC combined with chemotherapy, clinical trials, fluorouracil or gemcitabine-based chemotherapy and supportive care.[10] Table 1 summarizes the surgical/nonsurgical treatment plans of different types of BTCs.[11]

Will immunotherapy become a "savior" for BTC?

With the rapid development and cross-penetration of oncology, immunology, molecular biology and other related disciplines, immunotherapy has become an emerging research focusing on cancer treatment. Tumor immunotherapy began about 100 years ago, when Coley *et al*.[12] discovered that the application of streptococcus and *Staphylococcus aureus* toxins, later called Coley toxins, could control the growth of certain cancers. In the late 1980s, with the mature application of *in vitro* cell culture technology, lymphokine activated killer cells (LAKs) and tumor infiltrating lymphocytes (TILs) in clinical application, combined with chemotherapy and radiation treatment, obviously improve the curative effect of patients with cancer.

In the 21st century, medical science has continued to advance, and new cellular immunotherapy technologies have been developed rapidly. On April 29, 2010, the USA Food and Drug Administration (FDA) approved dendritic cells to treat advanced prostate cancer. This historic breakthrough enabled this treatment technology, that had undergone 15 years of lengthy clinical research, to enter into the clinical application stage.[13] Immunotherapy has become another important antitumor treatment after surgery, radiotherapy and chemotherapy, and it has been the hope of conquering malignant tumors (Fig. 1).

Potential benefit mechanisms of immunotherapy in BTC

Tumor cells survive and grow in the process of the body's antitumor immune response through an immune escape mechanism. Moreover, immunotherapy can kill tumor cells by activating and enhancing the body's antitumor immune response. At present, immunotherapy has been demonstrated to have definite effects in the treatment of various cancers, including melanoma, renal cell carcinoma and non-small cell lung cancer.[14–16] Chronic inflammation is known to promote tumor development in a number of ways and ultimately lead to immunosuppressive status in the tumor microenvironment. Inflammation also plays a key role in the occurrence and development of BTC, such as viral hepatitis,

primary sclerosing cholangitis, biliary inflammation caused by parasites or stones, etc., which are all the risk factors for BTC.[17] Therefore, it is speculated that chronic inflammation, antitumor immune response and immunosuppressive state in tumor microenvironment may have an interaction relationship in BTC, and immunotherapy could be a potential choice for the treatment of BTC (Fig. 1).[18]

In addition, a large number of studies have confirmed that infiltration of different immune cell subsets, including lymphocytes, macrophages, dendritic cells and granulocytes, can promote or inhibit tumor progression and/or metastasis in the tumor microenvironment of BTC.[19,20] Studies showed that the survival time of patients with high expression of immune-activating factors (CD4+, CD8+, Foxp3+T cells, MHC-I presenting cells, and NKG2D cells) was significantly higher than that of patients with low expression (hazard ratio [HR]: 0.52, $p<0.001$). In contrast, high expression of immunosuppressive factors (CD66b+ neutrophils, neutrophil-lymphocyte ratio, intratumoral IL-17+ cells, and PD-1+/CD8+TILs) was significantly associated with poor prognosis (HR: 1.79, $p<0.001$).[21] A study of ECC also found some similar conclusions; high expression of CD66b+ tumor-associated neutrophils ($p=0.01$), low expression of CD8+T cells ($p=0.02$), and high expression of Foxp3+ regulatory T cells ($p=0.04$) were all significantly associated with poor prognosis.[22] These studies further provide a theoretical basis for immunotherapy as a novel treatment for BTC. However, tumor-associated neutrophils and tumor-associated macrophages in the immune microenvironment have not yet become therapeutic targets in clinical trials of cholangiocarcinoma.

Immunocheckpoint inhibitors

Immunocheckpoint is an inhibitory signaling pathway that inhibits excessive inflammation in the body by modulating the autoimmune response. When a tumor appears, activation of the immune checkpoint can inhibit the activation and proliferation of T lymphocytes and induce the apoptosis of T lymphocytes, so that tumor cells can escape the immune response and increasingly reproduce. Blocking immune checkpoints can promote the activation of T lymphocytes and trigger antitumor immune response, so as to achieve the purpose of treating tumors.[23] The main targets of the present study are programmed cell death-1 (PD-1)/programmed death-ligand 1 (PD-L1) and cytotoxic T-lymphocyte-associated antigen 4. Others include lymphocyte activation gene 3 and T lymphocyte immunoglobulin myxin-3. PD-1 is an immunosuppressive transmembrane protein expressed on the surface of T lymphocytes, and PD-L1 is a PD-1 ligand induced by pro-inflammatory cytokines in tumor cells. In the tumor microenvironment, PD-L1 expressed by tumor cells can induce T lymphocyte failure through binding to PD-1, thus inhibiting the immune response of the body. Protein antibodies designed for PD-1/PD-L1 can block the recognition process of PD-1 and PD-L1 and restore the immune response of the body to achieve the therapeutic purpose. At present, PD-1/PD-L1 antibody has been used in the first-line treatment of non-small cell lung cancer, Hodgkin's lymphoma and other cancers.[24]

Potential benefit groups

Tumor mutation burden (TMB)

Studies have shown that PD-1 antibody has better immune response and antitumor effect in BTC patients with high TMB.[25,26] Lenvatinib is a multikinase inhibitor, mainly targeting vascular endothelial cell growth factor receptor, while pembrolizumab and nivolumab are both FDA-approved PD-1 inhibitors for a variety of advanced tumors. The antitumor activity of these three drugs alone has been evidenced in clinical trials. In the 2018 ASCO-GI, there was a single-center phase 2 study of lenvatinib combined with PD-1 monoclonal antibody (pembrolizumab or nivolumab) in the treatment of advanced ICC, which included a total of 14 patients who failed advanced multi-line therapy. The median progression-free survival was 5.0 months. Through the further stratified analysis of the sequencing results, a high TMB value (≥12) was found to be strongly correlated with better treatment response and longer progression-free survival time, suggesting that TMB may be used as a characteristic marker for judging prognosis.[27]

Mismatch repair (MMR) function

Microsatellite instability (MSI) refers to the phenomenon of changes in the length of tandem repeat DNA sequences caused by insertion or deletion mutations during DNA replication, often caused by MMR defects. MMR function is an important DNA repair mechanism that can accurately identify and repair base mismatches generated during DNA replication or recombination, and plays an important role in maintaining genome stability. MMR dysfunction is an abnormality in the MMR repair mechanism, which is generally highly consistent with MSI. It has been well demonstrated that MMR defects can cause immune cells to respond to cancer and that they can be used as a biomarker for PD-1 immunotherapy. However, most patients with cholangiocarcinoma do not have any mutations that can be used as therapeutic targets, which means that this is a typical highly immune-resistant cancer.

Studies have shown that patients with DNA MMR/MSI-high may represent the dominant-benefit population for BTC immunotherapy, and the incidence of MSI-high in BTC is 3%. Le *et al*.[28] reported the efficacy of pembrolizumab in solid tumors of DNA MMR in 86 patients with 12 tumor types and achieved an objective response rate of 53%. That study included four cases of cholangiocarcinoma, one case of complete remission, three cases of stable disease, and 100% disease control rate.

In May 2017, the USA FDA accelerated the approval of pembrolizumab for the treatment of MSI-high or DNA MMR refractory unresectable or metastatic solid tumors. It was the first drug that relied solely on specific genetic characteristics for treatment. The National Comprehensive Cancer Network guidelines also recommend PD-1 monoclonal antibody for BTC patients with MSI-H.

PD-1/PD-L1-positive expression

According to the immunohistochemical analysis of BTC patients, 32.3% of tumor cells and 74.2% of tumor-related macrophages can be observed to have positive expression of PD-L1, and the expression of PD-L1 is related to infiltrating lymphocytes, TILs) and human leukocyte antigen class I, and up-regulated PD-1/PD-L1 in BTC patients usually means worse overall survival.[29] In addition, the high expression of PD-L1 and the loss of human leukocyte antigen expression in BTC provide the basis for immune escape of tumor cells, which leads to worse prognosis and faster disease progression.[30] In the multicohort Ib study of KEYNOTE028 reported by the "ESMO" in 2019, pembromizumab (PD-1 monoclonal antibody) was used to treat advanced BTC with positive PD-L1 (>1%), and 42% (37/89) patients were

found to have positive PD-L1 expression (>1%). Among the 23 patients evaluated for curative effect, 4 cases were partially relieved, objective response rate was 17% (4/23), and 4 cases were stable. The results showed that the effective rate of immunotherapy for cholangiocarcinoma was similar to other solid tumors, close to the average, and had good tolerance.[31] In 2019, the ASCO reported that nivolumab alone or combined with GC was used to treat unresectable or recurrent cholangiocarcinoma. Moreover, 30 patients were enrolled in the single-drug group and combined-drug group respectively. Subgroup analysis showed that the median overall survival of patients with PD-L1 >1% in the single-drug group was better than that of patients with PD-L1 <1%. However, the relationship between the expression of PD-L1 and overall survival in the combined-drug group is still unclear.[32] In the 2020 ESMO, an open-label, one-arm, phase II clinical trial evaluated the survival benefits of chemotherapy with treprazolam, lamivudine combined with oxaliplatin and Gemox for unresectable advanced ICC patients. A total of 30 patients were included, and the results showed that PD-L1 protein expression was significantly positively correlated with objective response rate. Specifically, PD-L1+ vs. PD-L1- showed objective response rates of 100% vs. 68.8% (p=0.048) (NCT 03951597; Abstract No. 56P). It is noteworthy that the KEYNOTE-158 study reported in the 2019 ASCO, a phase 2 study, evaluated the antitumor activity and safety of pembrolizumab against advanced cholangiocarcinoma. A total of 104 patients were involved, and 6 patients were partially relieved, with objective response rate of 5.8%, median progression-free survival of 2 months and median overall survival of 9.1 months. This study found that pembrolizumab showed certain antitumor activity and controllable toxicity in patients with advanced BTC, regardless of the combined positive score of PD-L1.[33]

A clinical meta-analysis of 16,176 tumor patients, including those with BTC, showed that PD-L1 expression levels varied greatly in different tumor types; overall PD-L1 expression was associated with poor disease-free survival and overall survival was significantly positively correlated.[34] From this, we can speculate that the antitumor effects of PD-1/PD-L1 antibodies in different subtypes of BTC may also be significantly different. Therefore, in the future, more studies should be carried out with different types of BTCs to further clarify the relationship between the positive expression of PD-1/PD-L1 and the efficacy of BTC immunotherapy.

Insertion deletion variation

Studies have reported that two BTC patients with insertion deletion variation that was significantly higher than the median level (48% and 66.84% respectively, with a median level of 12.77%) were completely relieved after receiving PD-1 antibody combined with chemotherapy. Therefore, it is speculated that high-level insertion deletion variation can produce more tumor-specific antigens, and then express higher affinity with MHC class I. The high level of insertion deletion variation is a predictive factor for the good response of PD-1 treatment of BTC patients.[35]

Safety assessment

There are few reports on the adverse reactions of PD-1 antibody during BTC treatment. In the 2019 ASCO, there was a phase 2 study of nivolumab in the treatment of patients with advanced refractory BTC, in which nivolumab was used after at least first-line but no more than third-line systematic treatment. The most common treatment-related adverse events were elevated alkaline phosphatase (24.5%), and the common grade 3 to 4 adverse reactions were hyponatremia (3 cases) and elevated alkaline phosphatase (2 cases).[32]

Combination therapy-future development direction

At present, clinical trials using PD-1/PD-L1 antibody to activate the antitumor immune response to treat BTC has been carried out gradually. Combination therapy will be the main trend in the future. However, the efficacy of combination therapy remains controversial. A phase 1 study of the safety and efficacy of ramucirumab combined with pembrolizumab in patients with advanced BTC showed no significant improvement in overall survival with only 4% objective response rate, 1.6 months for median PFS, and 6.4 months for overall survival. However, the study found that PD-L1-positive patients had better overall survival than PD-L1-negative patients, which suggested that the baseline characteristics of patients may affect treatment efficacy. It is key to select the group reasonably.

In April 2020, the American Cancer Society online meeting announced a new study. That study is a multicenter, randomized phase II trial to explore the combination of PD-L1 monoclonal antibody (atrizizumab) and MEK inhibitor. In addition, the efficacy of cobimetinib is being assessed in the treatment of BTC. A total of 77 patients who had previously undergone one to two lines of treatment were included. For group A (n=37, ICC=21, ECC=7, GBC=11), aterizumab (840 mg, q2w) were injected intravenously. For group B (n=38, ICC=22, ECC=8, GBC=8) daily oral cobitinib (60 mg, taken for 21 days/7 days off) combined with intravenous aterizumab (840mg, q2w) were administered. Initial results of group B vs. group A include median progression-free survival of 3.65 months vs. 1.87 months (0.027), disease control rate of 45.2% vs. 32.4%, including one case of partial response (3.2%) in group B and 13 cases of stable disease (41.9%). As for the adverse reactions, the two groups had similar grade 3–4 treatment-related adverse events, and no treatment-related deaths. Atrizizumab combined with cobitinib reached its primary endpoint and significantly prolonged progression-free survival. The toxicity is controllable and worthy of further study in BTC.

Keynote-966 is a randomized, double-blind, placebo-controlled phase III study designed to investigate the treatment of patients with advanced cholangiocarcinoma with papolizumab combined with GC. This study includes metastatic or non-resectable local BTC patients who have not received systematic treatment. Patients are randomized 1:1 (n=788) to the pembro+GC and placebo+GC groups. The primary endpoints are progression-free survival and overall survival, and the secondary endpoints were objective response rate and duration of response. The final results will be released soon, but it is known that some positive results have been obtained thus far. Combination therapy will become the exploration trend of BTC in the future.

In January 2021, the American Cancer Society online meeting, the multicohort phase II LEAP-005 study showed the data of lenvatinib plus pembrolizumab for patients with previously treated BTC. Thirty-one BTC patients were included in this study (partial response: n=3; stable disease: n=18). objective response rate was 10% (95% confidence interval: 2–26) and DCR was 68% (95% confidence interval: 49–83). The median DOR was 5.3 months. The median PFS was 6.1 months (95% confidence interval: 2.1–6.4). The median OS was 8.6 months (95% confidence interval: not reported-5.6). Lenvastinib combined with pablizumab has shown encouraging efficacy and manageable toxicity in patients with advanced BTC who have previously received first-line treatment.[36]

Advantages of immunotherapy

The treatment effect of "immunoinflammatory" tumor is good, and the long-term survival rate is significantly improved. The treatment initiates the body's immune system to restore immune function and kill tumor cells over a long term. Meanwhile, it can also restore and improve the body's immune function, fully identify, search for and kill tumor cells, and effectively prevent tumor recurrence and metastasis. Moreover, the side effects are less than the traditional treatment. All in all, immunotherapy has a high accuracy, specificity and targeting of immune system.[37]

Existing problems

Although immune checkpoint inhibitors based on PD-1/PD-L1 antibodies have some effectiveness in the treatment of BTC, they are still faced with problems, such as low objective response rate and drug resistance. How to select the target group and control the timing of immunotherapy combination, such as sequential, intermittent, continuous, and the interval between the therapy. All these questions require further exploration. In addition, although current studies have confirmed the partial effectiveness and short-term safety of PD-1/PD-L1 antibody in the treatment of BTC, immune checkpoints are the normal physiological functions of the human body. It is still unclear whether the artificial suppression of immune checkpoints to enhance the body's immune response will cause long-term chronic tissue and organ immune loss and autoimmune diseases. At the same time, the specific mechanism of signal transduction of immunosuppressive pathway including PD-1/PD-L1 and the interaction with the tumor microenvironment are not yet fully clear. Future research directions should also focus on the above aspects.

Adoptive cell transfer (ACT) therapy

ACT refers to the isolation of immunocompetent cells from tumor patients, which are amplified *in vitro* and then returned to the patient's body, so as to achieve the purpose of stimulating the body's immune response or directly killing tumor cells. ACT therapy is currently divided into two categories, namely non-specific cell therapy (including cytokine-induced killer (CIK) therapy, TILs, etc.) and specific cell therapy (including T lymphocyte receptor chimeric T lymphocyte (TCR-T), chimeric antigen receptor T lymphocyte (CAR-T), etc.).[38]

Nonspecific cell therapy

CIK is a class of fast growing high-efficiency immune effector cells that are not restricted by MHC. The combination culture of dendritic cells that recognizes antigens and activates the immune system and CIK with highly effective anticancer activity has been used in clinical trials for tumor therapy.

It has been well demonstrated that CIK can delay tumor progression in a variety of solid tumors, including gastrointestinal malignancies. A clinical study involving 72 patients with advanced BTC who received adoptive treatment with dendritic cell-CIK showed that there were 1 complete response, 25 partial response, 34 stable disease, 12 progressive disease, and disease control rate of 83.3%. Nine cases (12.5%) of low-grade fever occurred, which were relieved after symptomatic treatment, no other adverse reactions were seen, indicating high safety. In addition, IL-6 and serum CA199 levels decreased significantly after receiving treatment. The percentages of CD8+CD38+T, CD8+DRT cells and CD3-CDL5+CD56+T and CD3+CDl6+CD56+T cells were significantly increased.[39]

TIL is a heterogeneous lymphocyte population in tumor stroma, including T lymphocytes and natural killer cells, which directly kills tumor cells by regulating the immune function of the body and releasing cytotoxins. Through immunohistochemical analysis of 375 cases of BTC patients, some studies found that TIL infiltration of different degrees could be observed in about half of the patients. The level of TIL infiltration was closely related to tumor grade and overall survival. A high level of TIL infiltration often predicted better overall survival.[40] A number of studies have shown the potential prospects of TIL adoptive therapy for BTC. A randomized controlled study showed that the 5-year progression-free survival and overall survival of the experimental group combined with TIL adoptive therapy and dendritic cell vaccine treatment were significantly higher than those of the control group that only underwent surgical resection (the experimental group progression-free survival and overall survival were respectively 18.3 and 31.9 months, while the control group showed 7.7 and 17.4 months respectively).[41]

Specific cell therapy

CAR-T and TCR-T used genetic engineering technology to genetically modify ordinary T lymphocytes in tumor patients. The modified T lymphocytes can express specific receptors and recognize specific tumor cells without MHC presentation, which can induce strong antitumor immune response without toxicity to normal cells.

Although there have been no reports of effective treatment of BTC using CAR-T and TCR-T, studies have shown that CD19 antigen-specific CAR-T technology produces sustained disease remission in clinical trials for the treatment of adult and childhood B lymphocytic leukemia and lymphoma. In addition, CAR-T and TCR-T technologies have also achieved certain results in the treatment of malignant melanoma, breast cancer, liver cancer, prostate cancer, lung cancer, and colorectal cancer.

Compared with non-specific cell therapy, CAR-T and TCR-T have the characteristics of specific killing of tumor cells and stronger immune effect, which have become hot spots in the field of ACT therapy. However, there is still a lack of breakthrough progress. Chinese researchers used CAR-T therapy targeting EGFR and CD133 for patients with metastatic cholangiocarcinoma and achieved partial remissions, lasting 8.5 months and 4.5 months respectively. However, the damage caused by CAR-T cell infusion cannot be ignored.[42]

Most of the existing studies believe that T lymphocytes injected by CAR-T and other exogenous agents failed and impaired effector function after entering the body, which may be due to adaptability of T lymphocytes and immunosuppressive state of the tumor microenvironment. How to ensure the accurate homing of T lymphocytes from peripheral blood infusion to the local solid tumor, break through the immunosuppression of tumor microenvironment, infiltrate into the tumor and ensure the continuous expansion of T lymphocytes so as to play a killing role are still difficulties currently. Furthermore, so far, studies of CAR-T cells have focused more on enhancing its function, but in almost all clinical trials there have been adverse reactions (such as cytokine release syndrome and neurotoxicity), and some may be fatal. With the transformation of CART cells, adverse reactions may increase, so the toxicity control of CART cells is

a problem that cannot be ignored.

Prospective considerations

The application of immunotherapy in the treatment of BTC has achieved initial results. Existing studies have shown that immunotherapy can improve the immune function and quality of life of patients with advanced BTC, and have some survival benefits to a certain extent. However, current research is mostly limited to small samples and lack of large sample, high-quality prospective randomized controlled trials. With the advent of the era of precision medicine and the in-depth understanding of BTC from the molecular level, the selection of specific treatment options for BTC patients in different populations and subtypes is the key to immunotherapy in the future. The combined application of multiple immunotherapies or immunotherapy combined with chemotherapy, targeted therapy and other treatment methods are also the focus of future research.

Design of clinical trials for oncology drugs in BTC

Drug therapy is an important means of tumor treatment, and the development of new antitumor drugs is an urgent clinical requirement in the world. Among them, clinical trials are the fastest, safest and most effective way to find new antitumor drugs and provide the optimal treatment for a cancer patient. However, since there is no human data and experience before the new drug enters the clinical trial, the clinical evaluation is full of unknown risks and challenges.[43]

In recent years, the level of clinical trials on new antitumor drugs has significantly improved. We reviewed the anti-cancer drug clinical trials registered on the USA clinical trial website in 2019. There were 238 ongoing oncology phase I clinical trials in mainland China. Among them, there were 160 solid tumor trials and 78 hematological malignant tumor trials. In terms of the total number of phase I clinical trials in oncology, there were 44 in Japan and 28 in South Korea in Asia. There were 327 ongoing oncology-related phase I clinical trials in Europe in 2019, of which 62 were from Spain (ranking first), followed by 50 from France, 41 from the UK, 25 from Italy, and 19 from Germany. There were 675 tumor-related phase I clinical trials in the USA in 2019. In the context of global accelerated research and development of innovative drugs, how the design of clinical trials of BTC is a topic worthy of attention.

Application of phase zero clinical trials in clinical research of antitumor drugs

In order to guide the rapid development of innovative drugs and control the clinical risks in the development of new drugs, the European Medicines Agency (EMEA) and the FDA issued respectively in 2004 and 2006 "new exploratory research guiding principle", put forward before the traditional I stage of clinical trials in the concept of zero phase of clinical trial, and a series of meaningful results are obtained.

The phase zero clinical trial refers to a drug trial conducted by the developer using a micro-dose on a small number of healthy volunteers or patients (usually 6–15 people) before the active compound is formally entered into the clinical trial after the pre-clinical trial is completed, and the necessary relevant information is collected. The test data of drug safety and pharmacokinetics to evaluate whether the research and development drug has the possibility of further development as a new drug or biological agent. It is the intermediate link in the transition from pre-clinical trials to phase I clinical trials.[44]

The purpose of phase zero clinical trial is to obtain human pharmacokinetic data, containing protein binding, enzyme inhibition rate and the combination of target, and to adopt various means of imaging studies of human tissue distribution, so that early identification of the most valuable lead compound from a set of candidates of phase I clinical trials can be facilitated. In addition, understanding the metabolic characteristics of lead compounds in humans as early as possible is also of great significance for the selection of animals for non-clinical safety studies and improving the predictive value of animal test results.[45–47]

Analysis of the mechanism of innovative drugs

The in-depth research of translational medicine has put forward new topics for the clinical research of antitumor drugs. It is necessary to develop new clinical trial methods and effective detection technology of related targets, attach importance to the construction of clinical trial-related laboratories, and actively carry out translational medicine research, so as to draw correct conclusions on the clinical application value of these new drugs with different mechanisms of action. Therefore, only a more in-depth exploration of the molecular mechanism and pharmacological mechanism of drugs in the laboratory stage can lay the foundation for the success rate of its translational research.

Individualized clinical research design

The classification of tumors of the biliary system is complex and heterogeneous, and the sensitivity of different tumors to drugs is bound to be different. When designing a clinical trial, a specific target population should be selected. Individualized molecular therapy programs and technologies based on the expression status of multiple genes or markers and the changing laws of related proteins and metabolites are the future development direction.

Multicenter collaborative research

The establishment of a multicenter collaborative organization can accelerate the process of drug development and marketing, and ensure the quality of clinical trials.[48] Many anticancer drug clinical trial multicenter collaborative organizations have been established internationally, such as the European Organization for Cancer Therapy Research (EORTC), the Eastern Cooperative Oncology Group (ECOG), the Japanese Clinical Oncology Cooperative Organization (JCOG), the Southwest Oncology Cooperative Group (SWOG) and so on, and have achieved a series of results. These research results have significantly promoted the development of clinical oncology and have become the basis for the current clinical diagnosis and treatment guidelines. Based on multicenter collaborative research, it is bound to accelerate the research and development of innovative drugs.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Writing of the manuscript (RX, RL), and conception of the idea for the study (JW, JH, WT). All authors reviewed and approved the final version of the manuscript.

Data sharing statement

All data are available upon request.

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